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Description et comportement des communautés bactériennes de la viande de poulet conservée sous atmosphère protectrice

JURY

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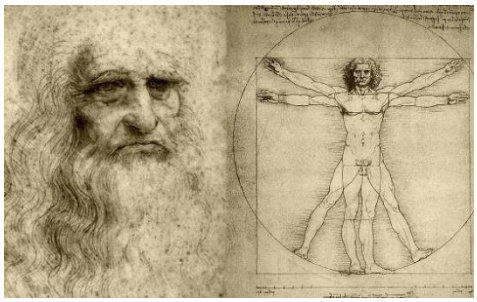
« Tout obstacle renforce la détermination. Celui qui s'est fixé un but n'en change pas. »



Léonard De Vinci

Architecte, Artiste, Ingénieur, Peintre, Philosophe, Scientifique, Sculpteur (1452 - 1519)

Remerciements

Mes premiers remerciements vont à la région Pays de la Loire pour avoir financé ce projet. Merci également à Marie-France Pilet directrice de l'unité Secalim dans laquelle j'ai effectué ce travail de thèse. Merci d'avoir été à l'écoute, toujours disponible et de m'avoir dispensé tous ces bons conseils qui m'ont permis de mener à bien ce doctorat.

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« Tout, dans la vie, n'est qu'une question de détermination et de désir. Tout n'est qu'une question d'opportunités, de rencontres et de chances à saisir. »
Extrait de L'invention de nos vies de Karine Tuil.

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« Cela semble toujours impossible jusqu'à ce qu'on y arrive. » (N. Mandela)

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« Je n'échoue jamais, je réussi ou j'apprends ».
(N. Mandela)

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Merci…

Sommaire

Abréviations

- AA Acetic Acid
- AB Agriculture Biologique
- ADN Acide Désoxyribonucléique
- ADNr Acide Désoxyribonucléique ribosomique
- AFNOR NF Association française de normalisation
- AHC Agglomerative hierarchical clustering
- ANOVA Analysis of variance
- ANR Agence Nationale de la recherche
- ARN Acide Ribonucléique
- ASC Acidified Sodium Chlorite
- BHI Brain-Heart Infusion
- bp base pair
- BPA Baird-Parker Agar
- CA Citric Acid
- CCDA Charcoal Cefoperazone Deoxycholate Agar
- CD Chlorine Dioxide;
- cDNA complementary Deoxyribonucleic Acid
- CFC Cefalotin Fucidin Cetrimide;
- CFU Colony-Forming Units
- CGAAER Conseil général de l'alimentation, de l'agriculture et des espaces ruraux
- DLA Deoxycholate Lactose Agar;
- DLC Date Limite de Consommation
- DMSO dimethyl sulfoxide
- DNA Deoxyribonucleic Acid
- EBI European Bioinformatics Institute
- EBP EcoBioPro
- edta Ethylenediaminetetraacetic acid
- ENA European Nucleotide Archive
- EU European Union
- EURL Entreprise unipersonnelle à responsabilité limitée
- FEMS Federation of European Microbiological Societies
- FISH Hybridation in situ et microscopie de fluorescence
- FROGS Find Rapidly OTUs with Galaxy Solution
- FSIS Food Safety and Inspection Service
- G Glutamal
- Gb Giga Byte
- Hab habitant
- IA Iron Agar
- ICFMH International Committee of Food Microbiology and Hygiene
- INRA Institut National de la Recherche Agronomique
- ISO Organisation Internationale de normalisation
- ITAVI Institut Technique de l'Aviculture
- kGy kilo gray
- KO Potassium Oleate
- LA Lactic Acid
- LAB Lactic Acid Bacteria
- LSD Least Significant difference
- LSV Laboratoire de la Santé des Végétaux
- LUNAM L'Université Nantes Angers Le Mans
- MALDI TOF MS Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry
- MAP Modified atmosphere packaging
- Mb Mega bytes
- MG-RAST Metagenomic Rapid Annotations using Subsystems Technology
- MRS de Man Rogosa & Sharpe;

- MTEC Millions de Tonnes Equivalent Carcasses
- NA not available
- NaCl Chlorure de sodium
- NCBI National Center for Biotechnology Information
- NGS Next generation sequencing
- O_2 dioxygen
- OECD/FAO Organization for Economic Co-operation and Development/Food and Agriculture Organization
- OTU Operational Taxonomic Units
- PA Peroxy Acids
- PAC BIO
- PCA Plate Count Agar
- PCoA Principal Coordinates Analysis
- PCR Polymerase Chain Reaction
- PCR DGGE Polymerase Chain Reaction coupled with Denaturing Gradient Gel Electrophoresis
- PCR TTGE Polymerase Chain Reaction coupled with Temperature Gradient Gel Electrophoresis
- pH potentiel hydrogène
- QALY quality-adjusted life years
- qPCR quantitative PCR
- RAPD PCR Random amplified polymorphism DNA-PCR
- RDP Ribosomal Database Project database
- REA-PFGE PCR Restriction Endonuclease Analysis - Pulsed-Field Gel electrophoresis PCR
- REP PCR Repetitive Element palindromic PCR
- RFI Recherche-Formation-Innovation
- rpm revolution per minute
- RV Rappaport de Vassiliadis
- SDS PAGE Electrophorèse en Gel de Polyacrylamide contenant du dodécysulfate de sodium
- SRA Sequence Read Archive
- STAA Streptomycin Thallous Acetate Agar
- Taq Pol *Thermus aquaticus* Polymerase
- Tec Tonnes Equivalent Carcasses
- TS Tryptone Salt solution
- TSA Tryptone Soy Agar
- TSP TriSodium Phosphate
- TTI time temperature indicators
- TVC total viable count
- UBD Use-By Date
- UE Union Européenne
- UFC Unité Formant Colonie
- UMR Unité Mixte de Recherche
- USA United States of America
- VBNC Viable But Non Cultivable
- VRBG Violet Red Bile Glucose agar
- VRBL Violet Red Bile Lactose agar
- XLD Xylose Lysine Deoxycholate agar

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Introduction

Le travail effectué au cours de ce doctorat s'est déroulé au sein de l'Unité Mixte de Recherche (INRA-Oniris) 1014 SECALIM (Sécurité des Aliments et Microbiologie) à Nantes. Les recherches menées dans l'unité se focalisent sur la caractérisation et la maîtrise du risque microbien dans les produits carnés et produits de la mer. Dans ce cadre de recherche, le projet « Pstrat », financé par la Région des Pays de la Loire (2012-2017) et porté par Monique Zagorec, vise à acquérir des connaissances sur les comportements microbiens dans les aliments par méthode de séquençage à haut débit, afin de donner les pistes pour maîtriser les flores bactériennes indésirables qui y résident.

En effet, les aliments peuvent héberger une flore endogène pouvant comprendre des bactéries pathogènes ou altérantes qui influencent la qualité du produit. La connaissance des écosystèmes est donc indispensable dans le domaine de la maîtrise de la qualité et de la sécurité des aliments. Une des difficultés majeures de l'étude des écosystèmes microbiens alimentaires est qu'ils peuvent évoluer quantitativement et qualitativement très rapidement entre le moment de la production et la date limite de consommation (DLC). De plus, les communautés bactériennes présentes sur les aliments sont extrêmement variables d'un lot à l'autre ou en fonction du procédé de conservation, ce qui rend les études difficilement reproductibles et comparables.

Ce projet a contribué à renforcer la dynamique scientifique de l'unité par l'acquisition collective des méthodes en « omiques », en particulier la métagénomique pour une vision sans a priori « d'écologie microbienne synthétique ». Dans le cadre de ce projet, le recrutement de Benoit Remenant, post-doctorant bio-analyste, a permis d'acquérir des méthodes d'analyses nécessaires à l'utilisation de ces données « omiques ». D'autre part, dans le but de comprendre les fonctions exprimées par ces écosystèmes microbiens une analyse métatranscriptomique a été réalisée dans le cadre d'une collaboration avec le laboratoire « Food Hygiene and Environmental Health » de l'Université d'Helsinki. Le partage de protocoles expérimentaux et l'apprentissage de méthodes d'analyses avec l'équipe de Johanna Bjortkröth ont été possibles grâce à deux mobilités d'une durée totale de 3 mois financées par la DARESE (INRA - Direction de l'Action Régionale, de l'Enseignement Supérieur et de l'Europe) et l'ICFMH (International Committee on Food Microbiology and Hygiene) dans le cadre de mon parcours à l'EIR-A (Ecole Internationale de Recherche Agreenium).

Les différentes étapes de cette étude sont présentées sur le schéma récapitulatif des travaux menés au cours de ce doctorat (Figure 1).

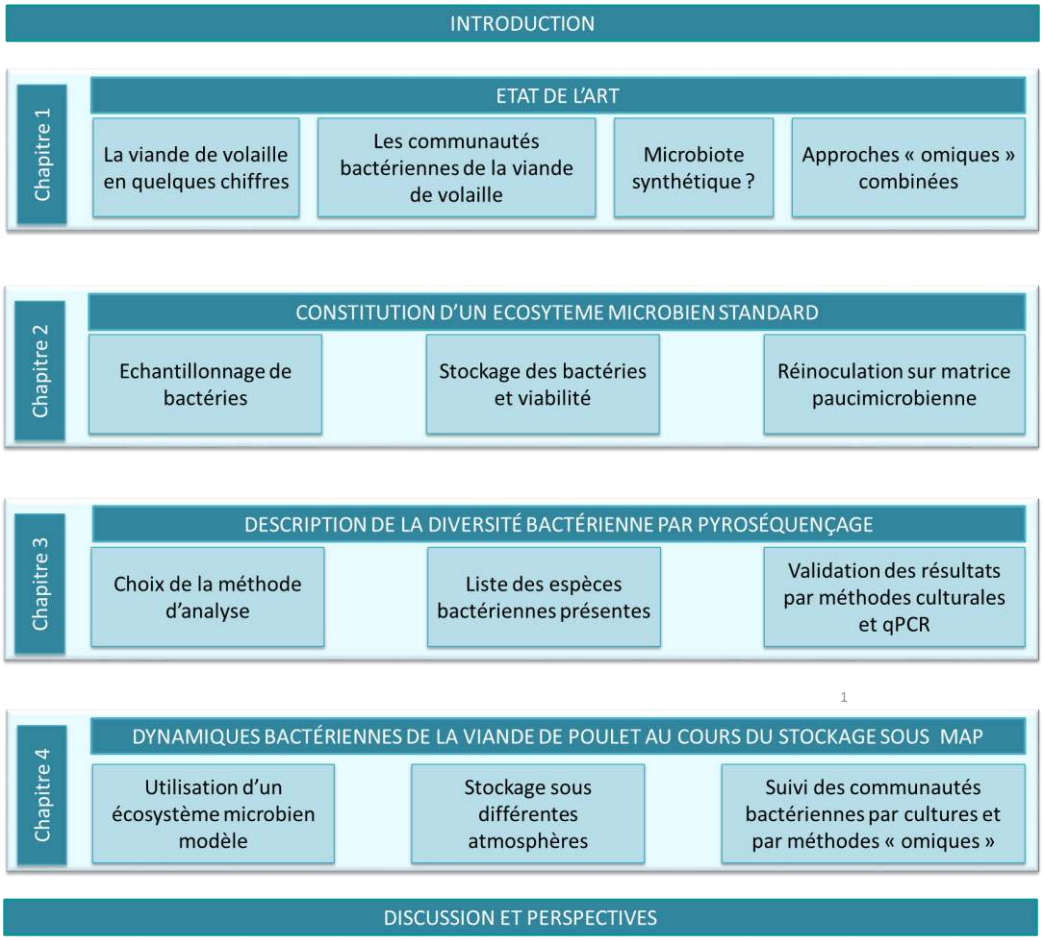

Figure 1 Schéma récapitulatif des travaux menés au cours du doctorat

Le chapitre 1 comprend une synthèse bibliographique reprenant les principaux chiffres de production et de consommation de viande de volaille ainsi qu'une analyse bibliographique préparée dans le cadre d'un projet effectué avec le pôle de compétitivité Valorial. Ce projet a abouti à une synthèse bibliographique sur les communautés bactériennes de la viande de volaille soumise dans la revue *Food Microbiology*. Les modèles d'étude décrits pour étudier des écosystèmes microbiens sont également récapitulés avant d'évoquer les méthodes utilisées en écologie microbienne.

Au vu de l'état de l'art, les contaminations présentes sur la viande de poulet sont variables suivant les découpes, les saisons, les lots, etc. Notre stratégie présentée dans le chapitre 2 a donc été de reconstruire un écosystème microbien standard que l'on a souhaité le plus proche possible de la réalité. Pour cela nous avons décidé de collecter des bactéries naturellement présentes sur la viande de poulet afin de les utiliser comme *inoculum* sur de la viande pauci microbienne. Cette première étape a nécessité la mise au point d'un protocole d'échantillonnage mais aussi un protocole d'isolement et

de stockage des bactéries. En effet, il nous a fallu collecter suffisamment de bactéries vivantes afin de pouvoir les stocker (c'est pourquoi nous avons choisi les cuisses de poulet qui sont les plus contaminées) et les ré-inoculer. Il nous a aussi fallu obtenir une suspension bactérienne la plus pure possible pour l'extraction des acides nucléiques et l'amplification d'ADN. Nous avons également vérifié que les bactéries étaient capables de survire à la congélation, et de se redévelopper sur la viande de poulet sans nécessiter une étape de pré-culture et que ce développement se faisait bien au dépend de la flore endogène de la viande. Ces travaux sont publiés dans la revue *International Journal of Food Microbiology*.

Dans le chapitre 3 nous avons décrit les communautés bactériennes présentes naturellement sur la viande de poulet avant la DLC. Pour cela nous avons comparé les résultats obtenus par méthodes culturales sur différents milieux sélectifs et les résultats d'analyses des données de pyroséquençage. Ceci nous a permis de faire un état des lieux le plus exhaustif possible des communautés bactériennes présentes sur la viande. Ce travail a fait l'objet d'une publication soumise dans *Food Microbiology.*

Une fois les bactéries connues et disposant d'un écosystème microbien standard nous avons cherché à connaître l'influence des conditions de stockage (atmosphère) sur la composition des microbiotes et les fonctions qu'ils expriment. Ces résultats sont présentés dans le chapitre 3. Nous avons utilisé deux microbiotes de notre collection pour réaliser des challenges tests sur de la viande de poulet pauci microbienne stockée sous 3 atmosphères différentes. Un suivi cinétique des bactéries a été effectué par méthodes culturales classiques durant le temps de stockage de la viande (9 jours) et nous avons également extrait les acides nucléiques (ADN et ARN). Ces acides nucléiques ont alors été utilisés dans une étude métagénomique et métatranscriptomique afin d'identifier les fonctions présentes et les fonctions effectivement exprimées par les communautés microbiennes. Un contrôle par métabarcoding/métagénétique a également été effectué afin de vérifier les espèces bactériennes présentes et confirmer les résultats obtenus par métagénomique. Un article scientifique issu de cette étude est en cours de préparation.

Pour conclure, une discussion générale et les perspectives du projet sont détaillées en fin de document.

Chapitre 1 Synthèse bibliographique

1.1- La viande de poulet en quelques chiffres

1.1.1- Production et consommation de la viande de volaille

La production de viande dans le monde était de 318 millions de tonnes pour l'année 2015 et ce chiffre ne cesse d'augmenter (OECD/FAO, 2016). La viande de volaille est la deuxième viande la plus produite dans le monde derrière la viande de porc. En 2015 d'après la *Food and Agriculture Organisation* (OECD/FAO, 2016), la production mondiale de viande de volaille a été estimée à 112,1 millions de tonnes en 2015 soit une hausse de 1,4% par rapport à l'année 2014 et ce autant dans les pays développés que ceux en développement (Figure 2).

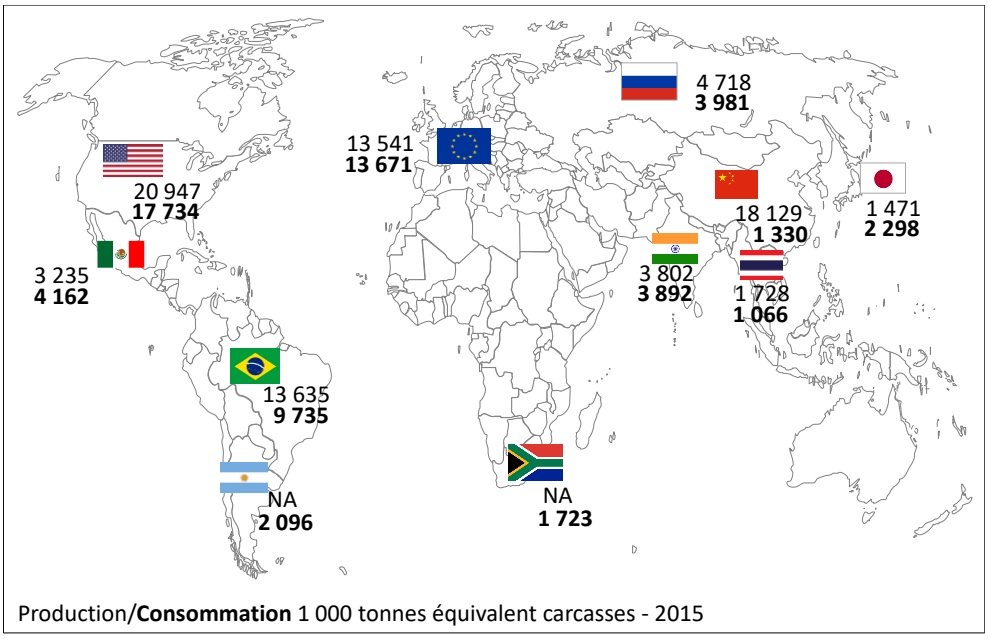

Figure 2 Production/consommation de viande de volailles par pays dans le monde en 2015.
Carte élaborée à partir des chiffres de l'Agreste[1]

La FAO prédit une augmentation annuelle de la production de viande de volaille de 1,8% de 2015 à 2024 contre 1,3% toutes viandes confondues. La viande de volaille serait alors la 1e production de viande dans le monde avec une estimation de 134,5 millions de tonnes en 2023. Dans ce contexte l'Union Européenne (UE) est le 3e producteur de viande de volaille (équivalent à la production du Brésil)

[1] http://agreste.agriculture.gouv.fr/publications/chiffres-et-donnees/

derrière les Etats-Unis et la Chine. L'UE se place aussi à la 3e position en termes de consommation de viande de volailles avec en moyenne, 36 kg équivalent carcasses par an et par habitant (Figure 3).

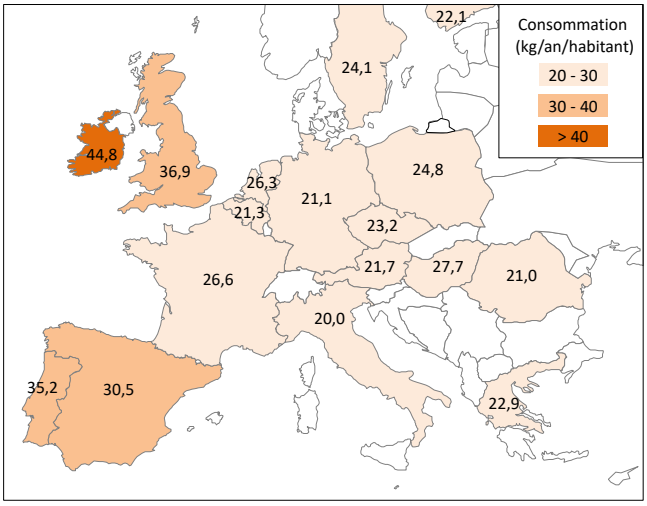

Figure 3 Consommation de viande de volaille en Europe en 2015.
Carte élaborée à partir des chiffres de l'Agreste[1]

1.1.2- Production et consommation de la viande de volaille en France

En 2015, la production était de 1,8 MTEC (millions de tonnes équivalent carcasses) et une consommation de 1,7 MTEC ce qui représente un solde financier de 99 millions d'euros (Figure 4). On exporte environ 650 000 TEC (dont la moitié vers UE et l'autre moitié vers des pays tiers) sous forme de viande congelée et on importe 559 000 TEC (la quasi-totalité en provenance de l'UE sous forme de viande fraiche et congelée). L'import/export représentent chacun environ 1.2 milliard d'euros et concerne majoritairement la viande de poulet.

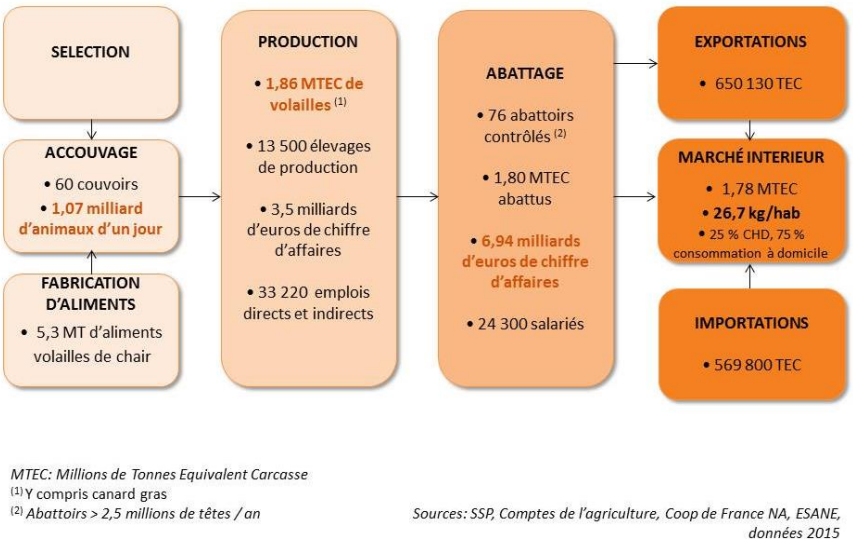

Figure 4 Filière volaille de chair en France pour l'année 2015.
Source (ITAVI, 2016)

En France la majorité des volailles abattues sont des poulets de chair. Le poulet est également la viande de volaille la plus consommée (60%). La production de volaille en France est majoritairement produite (65%) en Bretagne et Pays de la Loire (Figure 5).

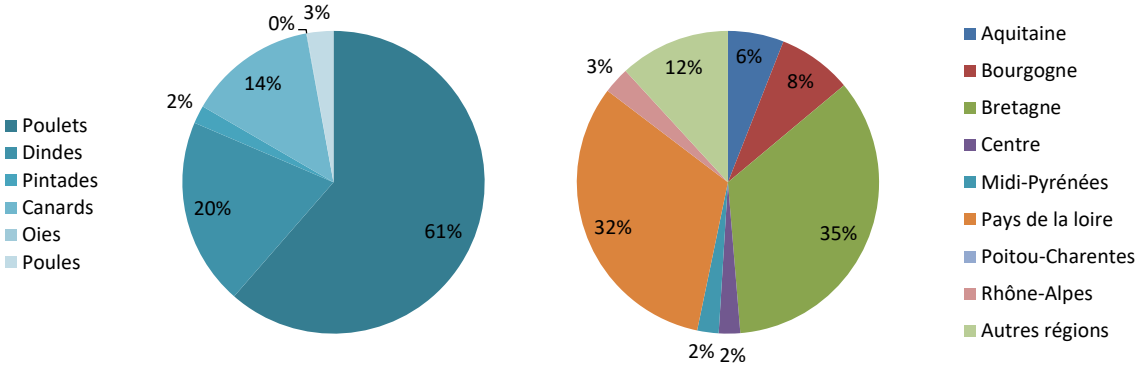

Figure 5 Volailles abattues en France en 2015.
Graphiques élaborés à partir des chiffres de l'Agreste

En 2015, en France la consommation de viande de volaille était de 26,7 kg/hab, représentée majoritairement par de la viande de poulet vendue en frais et principalement sous forme de découpes avec presque 17 kg/hab contre environ 5 kg de dinde et 3 kg de canard par habitant (ITAVI, 2016).

1.1.3- Impact environnemental de la viande de volaille

Avec l'objectif de nourrir les 9 milliards d'hommes en 2050, l'agriculture doit faire face à de nouveaux enjeux économiques et environnementaux. En effet, l'élevage intensif doit permettre une meilleure productivité tout en respectant les contraintes écologiques et environnementales (développement durable). Dans ce contexte, la viande de volaille présente un coût de production raisonné par rapport à la production de viande de bœuf par exemple. Il faut 4 kg de céréales pour produire 1 kg de viande de poulet contre 6 kg pour 1 kg de viande de porc et 12 kg pour 1 kg de viande de bœuf (Tableau 1). De plus, l'élevage de volaille nécessite une surface au sol moins importante (53 m² nécessaire pour la production d'1 kg de viande) que les autres élevages : bœuf + fourrage 323 m², poisson 207 m², porc 55 m². Enfin, dans un contexte de développement durable, la production de viande de bœuf est reconnue comme étant très émettrice de CO_2 et de méthane.

Tableau 1 Quelques éléments de comparaison des élevages bovins porcins et de volailles

	Equivalent carbone pour 1 kg de viande[1]	Besoin en eau pour 1 kg de viande[2]	Surface de sol pour 1 kg de viande[3]	Céréales pour 1 kg de viande[4]
Bœuf	27 kg	15 500 L	323 m²	12 kg
Porc	5,1 kg	4 800 L	55 m²	6 kg
Poulet	3,7 kg	3 900 L	53 m²	4 kg

[1] chiffres-carbone.fr
[2] waterfootprint.org
[3] www.wwf.fr
[4](Dutuit & Gorenflot, 2008)

Les conditions d'élevage de la volaille permettent plusieurs cycles de production dans l'année contrairement au bœuf par exemple où plusieurs mois sont nécessaires pour produire de la viande de veau et plusieurs années pour de la viande de bœuf. En France, la durée d'élevage varie suivant le mode de production de 35 jours pour un poulet standard à 81 jours pour un poulet « bio » ou « label rouge » (Tableau 2).

Tableau 2 Récapitulatif des conditions d'élevage de volaille suivant les modes de production.
Source : CIWF France = Organisation non gouvernementale internationale pour le respect du bien-être animal en élevage

Mode de production	Poulet standard	Poulet certifié	Poulet Label Rouge	Poulet Agriculture Biologique
Lignée de poulet	Croissance rapide	Croissance intermédiaire	Rustique à croissance lente	Rustique à croissance lente
Age d'abattage	35/40 jours	56 jours	81 jours minimum	81 jours minimum
Taille du poulailler	Pas de norme (jusqu'à 2000 m²)	Pas de norme (jusqu'à 2000 m²)	400 m² maximum	2 x 200 m² maximum
Densité dans le poulailler par m²	20/25 poulets	20/25 poulets	11 poulets	11 poulets
Espace en plein air	Aucun, élevage en claustration	Aucun, élevage en claustration	2 m² /poulet appellation "plein air" - 4m² /poulet appellation "en liberté"	4 m² par poulet
Eclairage	Artificiel	Artificiel	Lumière naturelle	Lumière naturelle
Alimentation	Pas d'exigence	Pas d'exigence	100 % végétaux, minéraux, vitamines dont 75% de céréales	100 % végétaux, minéraux, vitamines 90% de produits AB, dont 65% de céréales

1.1.4- Intérêt pour le consommateur

La viande de volaille présente plusieurs intérêts (économique, nutritionnel, pratique, …) pour le consommateur.

Devant le recul de la consommation de viande par les ménages (-4.9% pour le porc en 2014) les ventes de viande de volaille restent en constante augmentation (+0.4%) (ITAVI, 2016). Le prix d'achat de la viande de volaille est d'environ de 9 € par kg, équivalent au prix du porc, mais plus accessible que celui du bœuf (Figure 6).

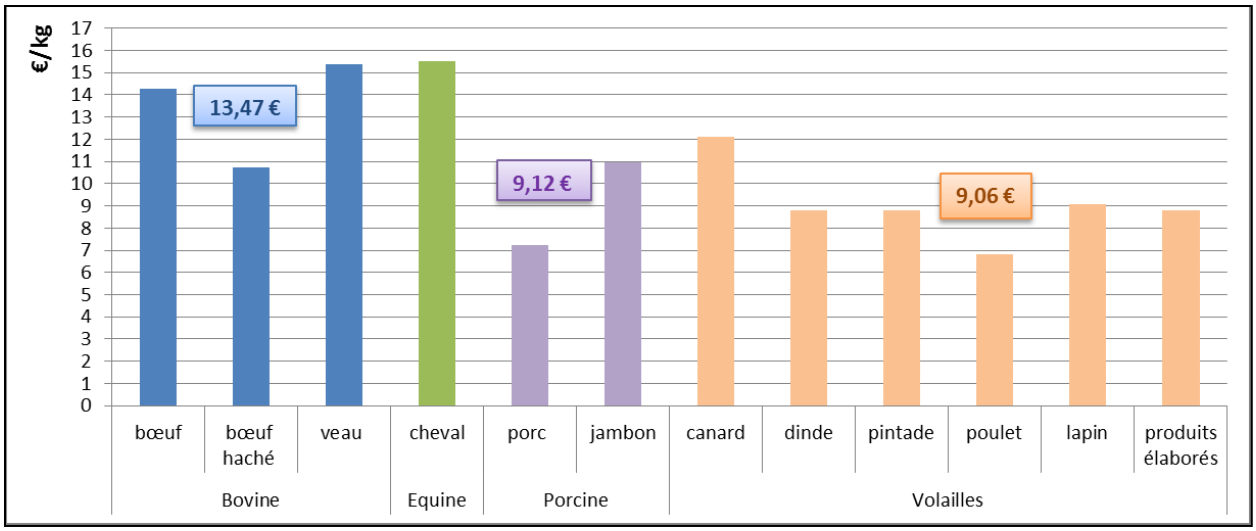

Figure 6 Prix d'achats moyens des viandes par les ménages en 2014.
Graphiques élaborés à partir des chiffres de l'Agreste

La viande volaille est considérée comme une viande blanche ayant une bonne qualité nutritionnelle et diététique. Elle est naturellement maigre et les lipides sont surtout contenus dans la peau qu'il est aisé de retirer pour limiter l'apport en graisse (14 % et 4 % de lipides pour le poulet et la dinde, respectivement). La viande de volaille est également riche en protéines et contient tous les acides aminés, vitamines et minéraux nécessaires à la nutrition humaine. Cependant dans certaines études et auprès des industriels de la filière, les filets de poulet sont considérés comme de la viande blanche alors que les cuisses de poulet sont considérées comme une viande rouge en raisons du niveau de contamination bactériennes (Baston et al., 2010).

La viande de volaille est consommée dans le monde entier notamment grâce à la facilité d'élevage des animaux sous la plupart des climats et à une compatibilité avec les pratiques culturelles de différents pays. En effet, la viande de volaille ne présente aucun interdit religieux comme le précise le Conseil général de l'alimentation, de l'agriculture et des espaces ruraux (CGAAER). En France, les volailles sont consommées sous forme entière (carcasses) notamment en période de fêtes (poulet entier, dinde, chapon) mais de plus en plus sous forme de découpes crues prêtes à cuire (cuisses de poulets, ailes, filets, etc) et conservées sous atmosphère protectrice. Le conditionnement sous atmosphère protectrice permet d'allonger la DLC des produits (McMillin, 2008). Pour les cuisses de poulet par exemple la DLC varie de 9 à 17 jours. Dans le cadre d'une enquête réalisée au laboratoire, nous avons constaté qu'au moins 3 atmosphères protectrices différentes sont utilisées par les industriels de la filière volaille (suivant les produits et suivants les industriels) : 50% CO_2/50% N_2 (viandes « blanches »), 70% O_2/30% $C0_2$ (viandes « rouges ») et 40% N_2/25% CO_2/35% O_2 (viandes marinées) (Macé et al., 2014). Nous avons observé des proportions variables de CO_2 et O_2 dans les barquettes de découpes du commerce corroborant les pratiques (Rouger et al., 2017).

1.1.5- Choix du modèle d'étude : la viande de poulet

Bien que très consommée et présentant de nombreux avantages pour le consommateur, la viande de volaille est naturellement contaminée par la bactérie pathogène *Campylobacter*, et est considérée comme la principale source de campylobactérioses. En Europe en 2015, 46,7 % des carcasses de poulet ont été répertoriées comme contaminées (EFSA, 2016). *Campylobacter* est le 1e agent pathogène responsable de gastroentérites bactériennes en Europe avec 229 213 cas recensés derrière *Salmonella* (94 625 cas recensés) (EFSA, 2016). Cette zoonose entraine des coûts de santé importants (EFSA, 2016, Saint-Cyr et al., 2016) qu'il est difficile d'estimer au vu des symptômes le plus souvent bénins (gastro-entérite) et en raison du délai entre l'apparition des symptômes et la consommation d'aliments contaminés qui rend difficile l'établissement du lien maladie/aliment incriminé. De nombreuses études visent à comprendre le comportement de *Campylobacter* afin de trouver des moyens de réduire la prévalence de ce pathogène le plus possible en amont dans la chaine de production.

Hormis les pathogènes, des bactéries altérantes sont également présentes sur la viande de volaille. Comme pour toutes les denrées hautement périssables les pertes et gaspillages liés à la contamination microbiologique de l'aliment existent aussi pour la viande de poulet. Il y a peu de données rapportées sur les pertes (avant la commercialisation) et les gaspillages (après commercialisation) mais on estime qu'1/3 des denrées, toutes confondues, sont perdues ou gaspillées. Gustavsson *et al*., (2011) précise les proportions des pertes et gaspillages pour les toutes les viandes (Figure 7).

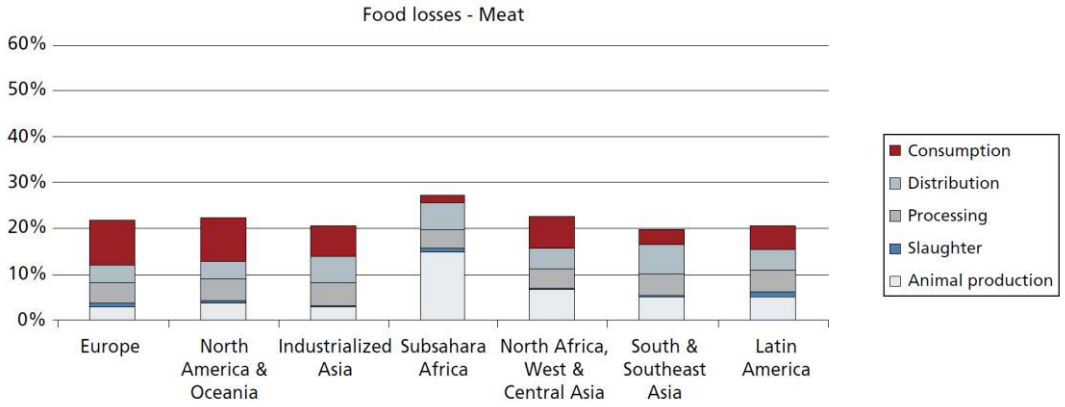

Figure 7 Proportion de la production initiale de viande perdues ou gaspillées à différents stade de la chaine de production et de consommation selon les zones géographiques
(Gustavsson et al., 2011).

Il est aisé de comprendre l'enjeu économique : connaitre les contaminations microbiologiques (pathogènes ou altérants) et leurs devenirs durant la conservation permettra de mettre en œuvre des moyens de maitrise de la qualité des aliments.

Du fait de l'important bassin de production en région Pays de la Loire, le projet financé par la région vise à étudier les contaminations bactériennes de la viande de volaille. La part occupée par la production et la consommation de la viande de poulet a orienté notre choix pour cette matrice. Avant de chercher à comprendre comment les contaminations peuvent être maitrisées au cours de la conservation de la viande, il est intéressant de connaitre et de décrire ces contaminations.

1.2- Revue bibliographique

1.2.1- Préambule

Dans le cadre d'un projet financé par le pôle de compétitivité Valorial, une revue bibliographique a été réalisée pour faire un état de l'art des communautés bactériennes décrites à ce jour sur la viande de volaille. Ce travail a donné lieu à la rédaction d'un article de synthèse soumis dans la revue *Food Microbiology* (Reference: FM_2017_316).

Les points suivants sont abordés dans la revue :

- Les réservoirs de contamination de la viande de poulet et les différentes étapes d'abattage et de transformation de la viande sont des sources potentielles de contamination
- Les méthodes de détection et de quantification des bactéries de la viande
- Les communautés bactériennes de la viande de poulet à T_0, à l'altération
- Les pathogènes présents sur la viande de volaille.

1.2.2- Endogenous contaminations occurring on poultry meat: A review

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Abstract

With the constant increase in poultry meat consumption worldwide and the large variety of poultry meat products and consumer demand, ensuring the microbial safety of poultry carcasses and cuts is essential. In the present review, we address the bacterial contamination of poultry meat from the slaughtering steps to the use-by-date of the products. The different contamination sources are listed and the methods used to identify bacterial contaminants, as well as their limitations, are reviewed. The culture-dependent techniques for detecting and counting bacterial contaminants and the

subsequent identification of isolates through molecular methods are presented. The overall approaches based on next generation sequencing, which have led to a more detailed description of bacterial contaminants of poultry meat, are also listed. Taking into account the diversity and limitations of the methods reported in the literature, we present a critical view of the contaminants occurring on poultry meat cuts and their behavior toward sanitizing treatments and the various storage conditions in use. A list of the main pathogenic bacteria of concern for the consumer and those responsible for spoilage and waste of poultry meat is established. This review also highlights the need to continue to explore poultry meat bacterial communities.

Keywords maximum of 6 keywords

Chicken meat, bacteria, slaughter, spoilage, pathogen

Highlights 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

- Bacterial contamination occurred on poultry during slaughter and transformation process.
- Methods used to describe bacterial contaminations increased with NGS technologies
- Bacterial contaminations of poultry are poorly known according to cultural methods.

Introduction

Poultry meat consumption is steadily increasing worldwide and reached 28.6 kg per year per capita in 2015 (OECD, 2016). The developed western countries, particularly the United States of America (USA), are the largest consumers with 47.7 kg per inhabitant in 2015. The same increase is observed in the European Union (EU) and in countries of the Organization for Economic Co-operation and Development (OECD). Similarly, poultry meat consumption has doubled in France over the past 30 years and has become the second most consumed meat since 2012, reaching more than 26 kg per capita in 2014 (close to the consumption reported for the EU and OECD) after pork meat (32.5 kg per capita). Among poultry meat products, chicken carcasses, cuts, and processed products are the most consumed (~75% of total poultry meat) followed by turkey (~25%) and, to a lesser extent, duck (France Agrimer, 2015). In France, 60% of the chicken meat is sold as fresh cuts (France Agrimer, 2015), often stored under various modified atmosphere packagings (MAPs) (Rouger et al., 2017). Vacuum packaging, the use of modified atmospheres, chilling, or marinades are different practices for ensuring microbial quality during the storage of poultry cuts, and depend on consumer habits and countries (see, as examples, Cunningham and Cox, 1987; Nieminen et al., 2012a; 2012b; Rouger et al., 2017).
Therefore, ensuring the microbial safety of poultry meat products is an important issue in this context of increasing consumption and production, with various consumer habits and needs. In fact, during and after slaughtering, the bacteria from animal microbiota, the slaughterhouse environment, and

equipment contaminate carcasses, their subsequent cuts, and processed meat products. Some of these bacterial contaminants can grow or survive during food processing and storage. The resulting bacterial communities present on poultry meat can include pathogenic species such as *Salmonella* and *Campylobacter*, the two main pathogens responsible for human gastroenteritis due to poultry meat consumption. Both pathogens are hosted by poultry and can therefore contaminate meat. Since 2005, *Campylobacter* has been the most commonly reported gastrointestinal bacterial pathogen in humans in the EU, where the numbers of reported confirmed cases in 2015 were 229,213 for human campylobacteriosis and 94,625 for human salmonellosis (EFSA, 2016). In the USA, among 14 foodborne pathogens, *Salmonella* and *Campylobacter* are responsible for the greatest loss of QALYs (quality-adjusted life years), which take into account economic cost, hospital treatment, morbidity, and mortality (Hoffman et al., 2012). Poultry consumption has also been shown to be the first cause of foodborne outbreaks in the USA between 1998 and 2012 (Chai et al., 2017). Other emerging pathogens, such as *Aeromonas* sp., may also be considered (Praveen et al., 2016). In addition to foodborne pathogens, bacteria responsible for spoilage may lead to large economic losses. Their growth and metabolic activity during shelf life leading to color, odor, taste, or texture defects are responsible for waste and losses of food products and have therefore an important impact on the economy of the poultry meat production sector.

Most of the literature dealing with the microbial contamination of poultry meat is based on cultural methods using various selective media. A majority of reports is dedicated to detecting the presence of pathogens (mainly *Salmonella* and *Campylobacter*) and sometimes to studying their behavior under different decontamination, transformation, or storage conditions. Poultry meat contamination by spoilage bacteria has been less studied and is often limited to their enumeration by counting CFUs (Colony Forming Units) on different, more or less specific, media. Challenge tests, based on the inoculation of individual strains or strain cocktails on meat cuts, have been used to investigate the growth ability of bacteria under various treatments. Finally, a few studies have recently used high throughput sequencing technologies to describe poultry meat contaminants, leading to a more precise description of bacterial species (Nieminen et al., 2012a; 2012b; Line et al., 2013; Mormile et al., 2013, Chaillou et al., 2015).

The aim of this review is to describe the state of the art about the knowledge available on the bacterial communities present in fresh poultry meat. The sources of contamination will be listed and the diversity of bacterial communities contaminating poultry meat will be presented, with an emphasis on the limitations of the methods used for describing poultry meat microbiota. Reports will also be presented on the bacterial growth dynamics throughout the production process, from the slaughterhouse to the end products, and depending on the storage conditions or various treatments.

Sources of contamination

Muscles are sterile in healthy living birds although various microbiotas are hosted in the digestive tract, lungs, skin, feathers, etc.. In slaughterhouses, the surfaces, air (aerosols), and liquids also encompass bacteria. Therefore, carcasses and cuts after animal killing can be contaminated by animal and slaughterhouse environment microbiota. Figure 8 summarizes the different steps in poultry slaughtering and the associated contamination routes. Although there are some differences between the practices in large-scale commercial slaughterhouses and small-scale slaughtering facilities, the main steps of poultry slaughtering are similar (FAO, 1996). Compared to the slaughtering process of mammals, the main differences to be noted for poultry slaughtering are: *i*) the use of a water bath (hot or chilled) at different stages of the process; *ii*) the feather removal step, which can be mechanical and is performed differently from removing the skin of mammals; *iii*) the small size of birds (compared to cattle or sheep, for example) which has consequences on the manipulation of carcasses and the mechanization of some processes. As a result, the nature and origin of bacterial contaminants occurring on poultry meat is different from those on meat of mammalian origin.

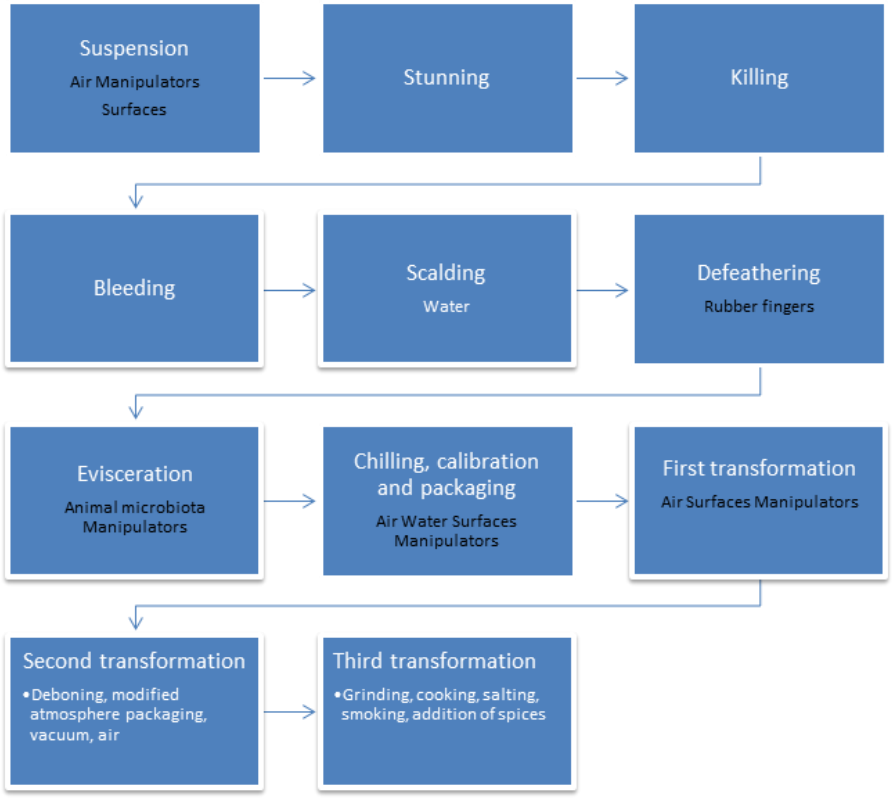

Figure 8 Steps in poultry slaughtering and the associated contamination routes.

As shown in Figure 8, after transport, the birds are suspended from the conveyor and then stunned and killed. After bleeding, the birds are scalded in hot water at a temperature ranging from 50°C to 60°C to loosen the feathers. Subsequently, the feathers are mechanically abraded from the scalded

birds. In large-scale slaughterhouses, feathers are removed using rotating rubber fingers and then the carcasses receive a spray wash prior to evisceration. Evisceration can be carried out by mechanical aspiration or manually after the carcasses have been cut open. At this stage, the gizzard, heart, and liver are also retrieved. Next, the carcasses are chilled, either by immersion in cold water or by air chilling. Subsequent transformation steps include cutting, deboning, grinding, and the use of various treatments for meat product storage such as marinating or addition of different ingredients (salt, spices) in processed products such as sausages.

During these successive steps, bacterial contamination of carcasses may occur from equipment surfaces, water, and animal microbiota. Psychrotrophic lactic acid bacteria (LAB) from the air and the environment can contaminate broiler meat (Vihavainen et al., 2007). The skin of poultry carcasses and cuts is directly in contact with air and equipment surfaces and is therefore easily contaminated. On fresh meat, bacteria are present on the surface rather than in the meat (Luber, 2009). However, in processed products such as marinated ones, bacteria can migrate into the muscles (Warsow et al., 2008).

Bacterial contamination by equipment surfaces can take place early in the process. For example, the rubber fingers used for feather removal or conveyor belts can be sources of bacterial contamination (Arnold, 2007; Arnold and Yates, 2009; Veluz et al., 2012). During the subsequent processing steps (deboning, cutting, mincing, mixing) for meat-based foodstuff production, manipulators, air and equipment surfaces are the main sources of contamination. In fact, transformation operations increase the surface area of meat in contact with working surfaces and air (Álvarez-Astorga et al., 2002). Consequently, the level of mesophilic and psychrotrophic bacteria is higher in transformed products than on primary cuts (Álvarez-Astorga et al., 2002).

The water baths used during the process have a washing effect that diminishes the bacterial loads, but can also promote cross-contamination between carcasses (Göksoy et al., 2004; Russell, 2008). Nevertheless, the high temperatures (50°C to 60°C) of the hot water used for scalding contribute to stopping bacterial growth, particularly that of pathogens whose optimal growth temperature is lower (42-43°C, 35-43°C, and 30-37°C for *Campylobacter*, *Salmonella*, and *Listeria monocytogenes*, respectively) (James et al., 2006). This helps to diminish the bacterial counts present on skin. However, high temperatures dilate feather follicles and relax poultry skin. Further processing steps may therefore lead to bacteria transfer from feathers to skin and follicles, previously dilated by the hot water, and to entrapping bacteria after the cooling of plucked carcasses. Cold water used for chilling carcasses after evisceration can act as a cross-contamination vehicle between carcasses, but also has a decontaminating effect by rinsing the surface of carcasses, particularly when chlorine is added to the water as in the USA (Demirok et al., 2013). Although cold water and air chilling procedures have

different effects on diminishing *Salmonella* and *Campylobacter* counts, no difference has been observed in the impact of the two procedures on the shelf life of cuts (Demirok et al., 2013).

The evisceration step, because of the microbiota present at high counts in the digestive tract, is a critical point of carcass contamination. The gastrointestinal tract of birds hosts many bacteria, including some that can be potentially dangerous for the consumer such as *Campylobacter* sp. or *Salmonella*. In fact, *Campylobacter* living in the intestinal tubes of birds are asymptomatic (Vandamme et al., 2005; Wassenaar and Newell, 2006). There is a correlation between the number of *Campylobacter* in the ceca and the contamination level found on carcasses (Hue et al., 2011; Pacholewicz et al., 2016). An average contamination level of 8.05 log CFU/g of ceca and 2.39 log CFU/g of carcasses has been measured (Hue et al., 2011). Poultry gut microbiota has been studied in detail, in particular to correlate animal feeding, health, and gut microbiota (see Waite and Taylor, 2014; Mohd Shaufi et al., 2015; Ranjitkar et al., 2016 as recent examples). However, to our knowledge, no study has yet been performed to establish a link between the composition of animal microbiota and that of the meat produced from these animals, although it has been reported that bacteria present in meat products originate at least partly from the animal digestive tract (Chaillou et al., 2015).

The evolution of the level of bacterial contamination throughout the slaughtering process has been described (Göksoy et al., 2004; Hinton et al., 2004). The contamination level of carcasses by *Pseudomonas* and H_2S-producing bacteria decreased by about 2 logs after evisceration and chilling by immersion in cold water. After 14 days of storage at refrigerated temperature, these bacterial populations reached more than 9-12 $\log_{10}$ CFU per ml of carcass rinses, while *Brochothrix thermosphacta* was detected only during storage reaching more than 6-12 $\log_{10}$ CFU per ml of carcass rinses (Hinton et al. (2004). Similar results were observed by bacterial enumeration performed on neck skin (Göksoy et al., 2004). This shows the washing effect at different steps, as well as the subsequent bacterial development that can occur during the storage period. After initial contamination, some bacteria can persist during meat product storage. As an example, isolates of *Chromobacterium violaceum*, a bacterium known to occur in water and soil, could be recovered from killed animals before the scalding step and also after 10 days of storage of carcasses at refrigerated temperature (Hinton et al., 2004).

Microbiological methods used to identify bacteria from poultry meat

Numerous scientific studies have been devoted to the microbiology of poultry meat. A large majority focused on detecting, counting, and/or identifying bacteria present on carcasses and on various poultry cuts by using cultural methods. Near-infrared hyperspectral imaging and spectroscopic transforms have also been proposed as a non-invasive and fast method for counting total viable

counts, *Pseudomonas* counts, and *Enterobacteriaceae* counts directly on meat samples (Feng and Sun, 2013a; 2013b; Feng et al., 2013). The large diversity of practices makes it difficult to compare the results reported by this rich literature. On the other hand, such data may provide information to assess the relevance of the microbiological criteria applied by poultry meat producers to ensure the safety of their products. Because the bacterial contamination of poultry meat occurs more frequently on the skin or the surface of cuts, several methods for recovering bacteria can be used. Table 1 reports examples of the three main methods recorded in the literature.

Table 1 Examples of the three most reported methods for bacterial recovery from poultry meat samples

Method	Principle	References
Stomaching/blending	A piece of deboned meat including muscle and/or skin is added to a liquid solution, then mixed, and the resulting mixture is filtered to remove meat residues.	Arnaut-Rollier et al., 1999a; Álvarez-Astorga et al., 2002; Capita et al., 2002a; Hinton et al., 2003; Karama et al., 2003; Goksoy et al., 2004; González-Miret et al., 2006; Patsias et al., 2006; Balamatsia et al., 2007; Chaiba et al., 2007; Chouliara et al., 2007; Cohen et al., 2007; Nieminen et al., 2012a; Herbert et al., 2013; Säde et al., 2013
Rinsing	A piece of meat including muscle and/or skin is added to a liquid solution.	Hinton et al., 2004; Zhang et al., 2012
Swabbing	A meat or equipment surface is scrubbed to collect bacteria with a swab. The swab is diluted in a solution to place bacteria in suspension.	Gill et al., 2005; James et al., 2006

The stomaching method, often used by food microbiologists, enables a smooth mechanical separation of bacteria from the meat matrix. Rinsing and contact methods, like swabbing or membrane adhesion, are used for recovering bacteria from the meat surface. These methods have been compared and classified according to their destructive or non-destructive effect (Capita et al., 2004). For stomaching, a piece of meat including muscle and/or skin is added to a liquid solution, then mixed, and the resulting mixture is filtered to remove meat residues. This enables the collection of almost all bacteria. Some authors also include an additional step using an ultrasonic bath or a pulsifier, which combines high frequency waves and strong stirring to resuspend the bacteria in the solution and improve their separation from meat (Lynch et al., 2010). The use of a pulsifier rather than a stomacher is less destructive for meat. Consequently, fewer meat residues, which could interfere with subsequent analyses, are present in the suspension (Lynch et al., 2010; Al-Nehlawi et al., 2013; Bolton et al., 2014). The swabbing method, performed to collect bacteria from a surface with a swab, is of particular

interest to harvest bacteria unevenly distributed on the carcasses. This method is appropriate for the detection of low-incidence bacteria. However, the results obtained with swabbing are less reproducible because the bacteria are not necessarily detached from the meat surface (Gill and Badoni, 2005). The last and least destructive method is rinsing. Meat samples are rinsed in a dilution solution causing no damage to the carcasses or cuts and enabling the collection of whole bacterial communities present on the surface. The bacterial recovery yields of the stomaching and rinsing methods are similar (Gill et al., 2005). For routine contamination tests performed in production plants, stomaching (or blending), rinsing and the use of sponges or swabs are part of the recommended procedures.

The bacterial suspensions obtained after stomaching, rinsing or swabbing are usually spread on agar media for enumeration. Alternatively, detection kits can be used, such as the Iso Gird membrane filter for the detection of coliforms. Such membranes are analyzed by plating the bacteria immediately after sampling. This method is efficient for low microbial contamination levels as membrane overloading should be avoided (Álvarez-Astorga et al., 2002). The most commonly used media for the microbiological analysis of poultry meat are summarized in Table 2. After incubation at optimal temperature for periods ranging from a few hours to several days, bacterial counts are enumerated as CFU/ml, CFU/g, or CFU/cm^2. The conditions of incubation and the use of media are variable to modulate their selectivity. So, for some media, standardized conditions are found in the ISO description. The incubation temperature influences the bacterial population: the incubation of PCA plates for 72 hours at 30°C or 55°C is used to select mesophilic or thermophilic bacteria, respectively. For psychrotrophic microorganisms, the incubation conditions can reach 10 days at 6.5°C. In some specific cases, for low-abundance bacteria like some pathogens, an enrichment step in liquid broth (Table 2) is performed prior to plating on agar media. When very selective media are used, such as those for the detection of important pathogens, the bacterial population can be directly determined. There are also some media to enumerate specific families or genera such as lactic acid bacteria (LAB), *Enterobacteriaceae* or Pseudomonads (see Table 2). In some cases, the colonies require further identification by various methods. As examples, the various methods described above have been reported to isolate poultry meat pathogens or have focused on only one genus with subsequent identification of isolates (Arnaut-Rollier et al., 1999a; 1999b; Capita et al., 2002a; Okolocha and Ellerbroek, 2005; Chaiba et al., 2007; Akbar and Anal, 2013). The same methods have also been used to investigate the behavior of microbial contaminants or after challenge test inoculation of sterile meat matrices regarding various storage or decontamination conditions (Lemay et al., 2002; del Río et al., 2006; 2007a; 2007b; Katzav et al., 2008; Warsow et al., 2008; Alonso-Hernando et al., 2012a; Juck et al., 2012; Alonso-Hernando et al., 2013).

Table 2 Most commonly used media for microbiological analysis of poultry meat

Targeted bacteria	Broth for the enrichment step	Medium used for plating
Total viable count	NA	PCA
LAB	NA	MRS
Pseudomonas	NA	CFC
Brochothrix thermosphacta	NA	STAA
Enterobacteria	NA	VRBG
Salmonella	RV	XLD
Campylobacter	Preston or Bolton	Skirrow, Karmali, or CCDA
H_2S-producing bacteria	NA	IA
Coliforms	NA	VRBL, DLA
Clostridium perfringens	NA	TSA
Staphylococcus aureus	NA	BPA

RV: Rappaport de Vassiliadis; PCA: Plate Count Agar; MRS: de Man Rogosa & Sharpe; CFC: Cefalotin Fucidin Cetrimide; STAA: Streptomycin Thallous Acetate Agar; VRBG: Violet Red Bile Glucose agar; XLD: Xylose Lysine Deoxycholate agar; CCDA: Charcoal Cefoperazone Deoxycholate Agar; IA: Iron Agar; VRBL: Violet Red Bile Lactose agar; DLA: Deoxycholate Lactose Agar; TSA: Tryptone Soy Agar; BPA: Baird-Parker Agar.

NA: not available

Microbiological tests have also been developed for the routine assessment of the microbial quality of poultry meat products or to determine their shelf life. These tests are mainly based on bacterial enumeration and require different steps to collect bacteria in sufficient amounts, to identify and/or enumerate them, and to check if the results meet the regulation safety criteria.

In the USA, both *Salmonella* and *Campylobacter* must be controlled in poultry and several antimicrobials can be used post-slaughtering to control them in poultry meat (FSIS, 2014; 2015). In the EU, *Salmonella* detection on poultry meat products is mandatory, as described in the hygiene criteria of CE regulation N° 1441/2007. As an example of the procedure for determining the shelf life of poultry meat products, the French regulation AFNOR NF V 01-003 recommends a sampling of 5 pieces of meat from the same slaughtering batch (muscle and skin). Five analyses must be performed at day 0 and day 5 after a storage period corresponding to 1/3rd of the shelf life at 4°C and 2/3^{rds} of the shelf life at 8°C. Microbial results expressed in CFU must match the criteria summarized in Table 3. The shelf life of the products must be assessed periodically, at least annually, and 60% of the results must be below the target value, and 100% must be below the tolerance value (10 times the target).

Table 3 Target values and acceptable values for 3 types of bacterial populations depending on the products and their storage conditions

	Storage conditions	Target value (/g)	Acceptable value (/g)
Pseudomonas	Under air +/- plastic wrap	10^7	10^8
LAB	Vacuum/ modified atmosphere	10^7	10^8
	Cooked products	3×10^5	3×10^6
Total viable count	Cooked products	3×10^5	3×10^6

For such analyses to estimate the microbiological and sensory shelf life of poultry meat, the limitations of the bacterial indicators used (mesophilic and psychrotrophic bacteria and *Enterobacteriaceae*) have been shown (Smolander et al., 2004). Moreover, the limitation of the selectivity of some media has been reported. As an example, isolates further identified as belonging to *Aeromonas*, *Acinetobacter*, *Myroides*, or *Shewanella* genera came from CFC medium described as selective for *Pseudomonas* (Hinton et al., 2004). In addition, for the food-processing industry, the delay required to obtain the results of microbiological analyses could be a critical point because of the need to maintain profitability and productivity. The CE regulation n° 2073/2005 noted that the food industry should use faster and more efficient methods of analysis, but this consideration is no longer present in the modified regulation (CE) n° 1441/2007. Special care is required for monitoring *Campylobacter* and the various methods that can be used have been recently reviewed (Josefsen et al., 2015; Macé et al., 2015).

Methods used to characterize bacterial contaminants from poultry meat after isolation

Such methods can be employed either to verify the identification of colonies or for deeper analyses aimed at typing or comparing isolates. Some are based on the major protein content. Matrix-Assisted-Laser-Desorption-Ionization-Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) is a fast and accurate method that has been used to identify food isolates, although it is mostly dedicated to foodborne pathogen identification (Kern et al., 2013 and references therein). Nevertheless, by simultaneously analyzing several thousands of colonies picked from poultry meat samples, the growth dynamics of various bacterial species under different MAP were compared (Höll et al., 2016). Another protein-based analytical method, SDS-polyacrylamide gel electrophoresis of proteins (SDS-PAGE), has also been reported as an efficient approach for typing isolates (Doulgeraki et al., 2012).

The other commonly used methods are based on DNA molecular techniques (see Doulgeraki et al., 2012, for a review). The PCR (polymerase chain reaction) is a fast, specific, sensitive and accurate method. Based on primers that can be specific for a kingdom (bacteria), a family (for instance firmicutes), a genus (*Campylobacter*) or a species (*Brochothrix thermosphacta*), it is used to amplify a

specific region of the chromosome. Depending on the conditions used for the PCR reaction (choice of the primers, stringency of the melting temperature, number of amplification cycles), various regions of DNA can be amplified. PCR can be used simply to verify the identity of a clone by the presence or absence of amplification from chromosomal DNA, or for further analysis of the PCR fragment after amplification. The 16S ribosomal RNA (rRNA) gene is mainly targeted for such analyses. In most cases, DNA sequencing of the 16S rRNA gene (or part of it) is one of the methods of choice to identify an isolate at the species level. Such a procedure was used to characterize *Enterobacteriaceae* present on poultry cuts (Säde et al., 2013). The terminal limitation fragment length polymorphism (T-RFLP) technique is based on the comparison of electrophoretic migration profiles obtained after an enzymatic digestion of the PCR fragments. As an example, the combination of T-RFLP and 16S rRNA gene sequencing led to the identification of the spoilage bacteria in marinated poultry meat as belonging to the species *Leuconostoc gelidum, Lactobacillus sakei* and *Lactobacillus curvatus* (Björkroth, 2005). Random PCR amplification can also be used and the profiles obtained can be compared between various isolates together with reference strains used as a control. Random amplified polymorphism DNA-PCR (RAPD-PCR) is based on short and nonspecific primers that hybridize randomly on DNA and provide strain-specific profiles. Primer hybridization can also take place on repeated palindromic sequences (rep-PCR) and the profiles obtained can help intra- and inter-species differentiation (Doulgeraki et al., 2012). These methods can be used alone or in combination for typing isolates and estimating intra-species diversity.

There are other molecular methods, based on the enzymatic digestion of chromosomal DNA (REA PFGE: Restriction Endonuclease Analysis - Pulsed-Field Gel electrophoresis), which can be useful to differentiate strains on the basis of their migration profiles.

To identify isolates, the PCR can also be coupled with electrophoresis of the amplified DNA under various denaturing conditions: PCR-DGGE (Denaturing Gradient Gel Electrophoresis) (see Ercolini, 2004, for a review) and PCR-TTGE (Temporal Temperature Gel Electrophoresis) (Martin-Platero et al., 2008). The DNA fragment can be amplified by PCR with universal primers targeting various bacterial species of families. The sequence and base composition of the amplified PCR fragment is species-dependent. Consequently, the migration properties under denaturing conditions depend on the sequence, providing a unique profile that is compared with those obtained for known bacteria, used as references. Bands obtained after migration can be sequenced to confirm the bacterial species. However, the length of the PCR fragments used for PCR-DGGE or PCR-TTGE (usually about 300 - 400 bp) is sometimes too short for a correct identification. Moreover, there may be different migration profiles within a species and different species may present bands with a similar migration profile, rendering the identification of clones inaccurate. Lastly, a polyphasic approach using several methods to ensure the correct identification of isolates has been suggested (Kort et al., 2005; Rahkila et al.,

2011).These electrophoresis methods combined with PCR are also currently used to describe bacterial communities directly from meat without a prior step of cultivation, as suggested by Zhang et al. (2012).

Methods used to identify bacterial communities directly from poultry meat samples

The use of a cultural step to describe the bacteria present in food products gives a reductive vision of the complex microbial ecosystems they host (Ercolini, 2004). For instance, it has been estimated in fermented food that besides the well-known and cultivable bacterial species, 25 to 50% of the bacteria are not cultivable with the media commonly used in laboratory conditions (Juste et al., 2008). Several hypotheses can explain these limitations, particularly the selectivity of the media or the incubation conditions used such as temperature or atmosphere (Doulgeraki et al., 2012). Furthermore, some bacterial species cannot yet be cultivated because no known selective media have been developed for them (Doulgeraki et al., 2012). As an example, one of the major bacterial populations encountered on spoiled cod fillet has been identified as an uncultured *Fusobacteriaceae* and has not yet been searched for by plating methods on such food products (Chaillou et al., 2015). The development of molecular methods during recent decades and of next generation sequencing (NGS) methods more recently has led to new possibilities for detecting, identifying and quantifying bacteria without a culture step as a prerequisite (for reviews, see Juste et al., 2008, and Doulgeraki et al., 2012). DNA extracted directly from complex matrices without prior microbial cultivation can be used as a basis for researching the composition of the microbial communities hosted by these matrices. The design of bacterial DNA extraction procedures directly from food matrices, including poultry meat products, has been reported (for examples, see Diaz-Sanchez et al., 2013; Chaillou et al., 2015; Rouger et al., 2017). Once the DNA is extracted, various methods can be used including some of those described above.

PCR amplification can be performed to detect the presence of various bacteria using primers specifically designed for targeting a species, a genus, or a family. Nevertheless, for some pathogenic bacteria present at low levels the detection by such a method still requires an enrichment step to increase the detection threshold, as is the case for *Campylobacter* in poultry products (Katsav et al., 2008). Real-time quantitative PCR (q-PCR) is used to quantify various species from bacterial DNA prepared from meat samples. A method has been designed for DNA extraction and q-PCR quantification of *Salmonella enterica* from poultry meat (Agrimonti et al., 2013). A linear correlation between the q-PCR quantification and bacterial enumeration by cultural methods was obtained. However, for both PCR and q-PCR, the DNA from dead bacterial cells can also be amplified and may introduce a bias in the detection or quantification. On the other hand, such methods can detect or quantify non-cultivated bacteria. In addition, food matrix residues (particularly, lipid residues) can inhibit the PCR amplification (Rossen et al., 1992; Abu al-Soud and Rådström, 2000; Lubeck et al.,

2003). The two main advantages of such methods are: i) their specificity, compared to the less specific cultural methods, particularly for the detection and quantification of pathogenic bacteria; and ii) the short time needed to obtain results, compared to the delay required to incubate plates and verify colony identity. Nevertheless, as nucleic acids from dead cells may also be amplified, all methods based on PCR amplification also generate biases.

To identify bacteria present in food ecosystems, hybridization to DNA microarrays or FISH (Fluorescence In Situ Hybridization) techniques have also been reported (Juste et al., 2008). These two methods require primers specific to the bacteria to be identified (Diaz-Sanchez et al., 2013). Because of their specificity, the methods mentioned above are not suitable to describe the microbial communities composing complex ecosystems as a whole. Other methods, based on a first step of DNA extraction followed by PCR amplification and subsequent analysis, have emerged recently aimed at an overall description of microbial (essentially bacterial) species of various ecosystems, including food products.

The method based on PCR-DGGE described above has also been performed after amplification on whole DNA extracted from food. Even in the absence of identification, the PCR-DGGE migration profiles, obtained from DNA extracted from food, can be used to compare different food samples or to follow the dynamics of the bacterial communities during storage (Villani et al., 2007). Data obtained by PCR-DGGE and 16S rRNA gene barcoded pyrosequencing on the same DNA samples extracted from seafood products have been compared (Roh et al., 2010; Chaillou et al., 2015). The results did not correlate for a quantitative comparison but enabled pyrosequencing observations to be partially confirmed.

The most exhaustive method for describing the microbial ecology of complex ecosystems, including that of meat products, is based on high throughput sequencing. Since 2005, following the pyrosequencing development that revolutionized the access to bacterial genome sequences (Margulies et al., 2005), many techniques have emerged and are still in constant evolution. A large (or even huge) number of sequencing reads can be obtained in a short time, from only a small quantity of DNA, with no need of cloning steps and for a reasonable price. There are two main approaches. The most commonly reported one is based on the sequencing of a short fragment, obtained by PCR amplification of a region that is common to the microbial communities, but with sequence differences that enable the different populations to be distinguished (metabarcoding) (Taberlet et al., 2012). The different variable regions of the bacterial 16S-rRNA gene are the most commonly reported targets for this approach. The second approach, which is now emerging, aims to sequence the total DNA extracted from a sample (metagenomics) or the cDNA obtained from total RNA (metatranscriptomics).

With metabarcoding, the microbial species present in an ecosystem are determined by comparison with sequence databases, and their relative quantification is possible. This approach has been mainly

used in environmental ecology and to describe the microbiota of the digestive tract of many animals. It emerged only recently in food science, with reports still mostly restricted to bacterial 16S rRNA gene pyrosequencing. With such a method, depending on the number of reads obtained and the diversity of the samples, the depth can reach 10^4 - 10^5 reads (*i.e.* within a bacterial population of 10^x, those present up to 10^{x-4} or 10^{x-5} will be detected). Identification of the bacteria through the partial 16S rRNA gene sequence can reach not only the genus level but also the species level. Identification accuracy depends on the quality of the sequence database used to assign sequence reads to operational taxonomic units (OTU) and on the 16S rRNA gene variable regions amplified prior to sequencing. This method can also generate errors, resulting from wrong PCR amplifications or from contamination by the food matrix DNA (mitochondrial DNA of the animals from which the food is produced or chloroplast DNA from spices). In fact, the number of reads finally assigned to chloroplasts could reach more than half of the total reads obtained from poultry sausage (Chaillou et al., 2015). These were attributed to the spices added to the sausage formula. Nevertheless, this method is useful for a more accurate assessment of the diversity of food ecosystems. Yet only a few studies have used it to characterize the microbiota present on poultry carcasses or processed poultry meat products (Nieminen et al., 2012a; 2012b; Mormile et al., 2013; Chaillou et al., 2015).

With metagenomics, the whole DNA sequence is determined to assess what is there and which functions are potentially present. To date, only one article has reported this method for poultry meat (Nieminen et al., 2012b). Such a method does not only focus on bacteria and may reveal the presence of other microorganisms such as yeasts, archeae, or viruses. None of those was found in poultry meat (Nieminen et al., 2012b), except the virome of chicken skin assessed by metagenomics (Denesvre et al., 2015). These authors also noticed that, depending on the samples, 50 to 80% of the reads actually came from meat cells as they could be aligned to the *Gallus gallus* genome (Nieminen et al., 2012b; Denesvre et al., 2015). The metatranscriptomics approach aims to reveal and quantify in a relative way the genes expressed by the microbial community of the analyzed ecosystem. Only very few metatranscriptomics analyses have been reported on food samples and, to our knowledge, none dealing with poultry meat.

Variability of bacterial communities regarding different matrices and processes

Despite the various methods used and their limitations, we have combined the data reported in the literature to draw a picture of the composition of the bacterial communities occurring on poultry meat depending on different variables. We chose to select variations depending on the meat matrix or on the storage/transformation process. The bacterial communities present on poultry carcasses and cuts and their dynamics depend on different factors: the storage temperature, the gas composition used

for MAP, the composition of marinades or various chemical treatments that can be applied to control bacteria. A number of studies were selected to illustrate the diversity of the methods used.

Variability of bacterial contaminants regarding meat matrix and origin

Most of the literature focuses on chicken meat and, to a lesser extent, turkey meat. A comparative study of the microbiological quality of poultry meat in Morocco showed that turkey meat was more contaminated (5.4 - 7.4 log CFU/g total aerobic counts) than chicken meat (4.5 - 6.6 log CFU/g) (Cohen et al., 2007). Nevertheless, for several pathogens (*Escherichia coli*, *Staphylococcus aureus*, and *Clostridium perfringens*) the contamination level was similar in chicken and turkey meat. The difference might result from the different farming conditions and/or intrinsic differences between these two birds. These authors also noticed that the traditional slaughtering process increases contamination by microbial communities. This correlates with another observation, which reported a higher contamination level of skins of chicken carcasses from traditional markets and artisanal slaughterhouses (Chaiba et al., 2007). This study, also carried out in Morocco, showed higher counts of mesophilic and psychrotrophic bacteria, total and fecal coliforms, and *S. aureus* on artisanal products than on carcasses purchased from supermarkets.

The contamination level regarding different cuts or raw *vs.* transformed products has also been evaluated. Al Alvarez-Astorga et al. (2002) enumerated the mesophilic bacteria from various poultry cuts (thighs, wings, giblets, hamburgers, and sausages). These were higher in processed products (hamburgers, sausages) with approximately 7 log CFU/g, than in the fresh cuts (thighs, wings) with approximately 5.7 log CFU/g. This may result from the temperature during the transformation process (10°C) and from the mixing steps that increase the surface area of meat in contact with surfaces and air, both favorable to bacterial growth and to the possibility of increased contamination.

Variability of bacterial contaminants regarding storage temperature

The importance of temperature for bacterial growth can be assessed at different critical points between the slaughtering and the consumption of the product, in particular:

- during carcass handling (the temperature in the processing plants is usually about 10°C);
- during the storage of meat products (with an estimation of a rupture in the cold chain between the time of sale and the consumer's fridge, whose temperature is estimated to be higher than 4°C).

Tuncer and Sireli (2008) studied the effect of chilling carcasses using chilled air or a cold water bath on their microbial communities. Refrigeration by chilled air slows down the development of the total viable count (approximately 1 log) and causes a rapid decrease in temperature. This inhibits the multiplication of *Salmonella* and *Campylobacter* and so chilled-air cooling would be more efficient.

However, it is necessary to take into account the fact that *Listeria* can grow at this storage temperature.

Smolander et al. (2004) showed that the product shelf life can be increased by storage at low temperature and the absence of a break in the cold chain. The shelf life can even be doubled when the temperature is lowered to 3.4°C compared to storage at 8.3°C. Low temperatures delay the growth of *Enterobacteriaceae*, which can produce sulfuric compounds and organoleptic deterioration of the meat quality. On the other hand, the growth of psychrotrophic bacteria is enhanced. Actually, at 4°C and 7°C, the total viable counts develop faster (Tuncer et al., 2008) than at 0°C. Consequently, the threshold of 10^7 CFU/cm² is reached earlier in the storage period when the temperature is higher. In addition, Zhang et al. (2012) showed that microbial communities develop faster at 10°C (9.7 log CFU/cm² of TVC) than at 4°C (6.4 log CFU/cm² of TVC). Storage at 4°C is damaging for *B. thermosphacta* and *S. putrefaciens* growth after 7, 10 or 14 days whereas *Aeromonas hydrophila* and *Aeromonas sobria* are psychrotrophic bacteria that can develop at low temperature (Hinton et al., 2004). Smolander et al. (2004) also pointed out that the shelf life of products cannot be lengthened too much by storage at 0°C, because pathogenic agents such as *Listeria* can multiply at these temperatures. These authors suggested that the use of time temperature indicators ("TTI") could enable the assessment of chicken meat quality.

Variability of bacterial contaminant regarding gas composition of packaging

Balamatsia et al. (2007) and Chouliara et al. (2007) compared the effect of different atmospheres used for packaging poultry meat (Table 4). *B. thermosphacta* and *Enterobacteriaceae* counts were not significantly affected by the type of packaging but were detected as bacteria responsible for spoilage (Chouliara et al., 2007). The use of vacuum packaging and some MAP extended the shelf life of chicken cuts by about 2-3 days (30% CO_2 - 65% N_2 - 5% O_2, MAP1, Table 4) and by more than 9 days (65% CO_2 - 30% N_2 - 5% O_2, MAP2, Table 4) (Balamatsia et al., 2007). CO_2 has a bacteriostatic effect, which inhibits the growth of aerobic microorganisms such as *Pseudomonas* spp. that are considered putative spoilage organisms. A MAP containing more CO_2 (70% CO_2 - 30% N_2) was more effective than one containing less (30% CO_2 – 70% N_2) (Chouliara et al., 2007). However, LAB species can grow in the presence of CO_2, which explains why this bacterial community can become dominant in products stored under CO_2-enriched MAP. These atmospheres produced a decrease of about 1-1.5 log CFU/g of total viable counts in the meat cuts and consequently increased the product shelf life by 2-3 days (Balamatsia et al., 2007; Chouliara et al., 2007).

Replacing nitrogen by argon in the composition of MAP (proportion from 15% to 82%) was tested (Herbert et al., 2013). No strong difference was observed, with only *B. thermosphacta* appearing to be significantly affected by a high proportion of Ar in the gas mixture. Nevertheless, the various

proportions of Ar or $N_2/CO_2/O_2$ in the gas mixtures tested shaped differently the growth dynamics and the ratio of different populations (LAB, *B. thermosphacta*, *Pseudomonas* spp., and *Enterobacteriaceae*). The growth of mesophilic LAB was favored by anaerobic conditions or high quantities of CO_2 or both. At low Ar or N_2 concentration (15%), the dominant microbial communities were composed of *Pseudomonas* spp., *Enterobacteriaceae*, and *B. thermosphacta* with dominance of the latter increasing during storage (Herbert et al., 2013). These authors noted the ability of *Pseudomonas* spp., considered aerobic bacteria, to grow with only residual amounts of O_2.

Table 4 Time period to reach spoilage (i.e. 7 log CFU/g of total viable counts) depending on packaging conditions.

Data are taken from Balamatsia et al., (2007) (upper part) and Chouliara et al., (2007) (lower part).

Bacterial counts at T_0 (Log UFC/g)		**Time (days) to reach spoilage (>7 log CFU/g)**					
		Air	Vacuum	MAP1	MAP2	MAP3	MAP4
Total viable count	4.9	5	7	11	15	ND	ND
LAB	3.9	5	12	NA	NA	ND	ND
Pseudomonas	4.2	7-11	NA	NA	NA	ND	ND
Total viable count	4.3	5-6	ND	ND	ND	11-12	14-15
Pseudomonas	3.4	7	ND	ND	ND	14-15	16-17
LAB	3.7	9	ND	ND	ND	13-14	15-16
B. thermosphacta	3.0	8-9	ND	ND	ND	15	13-14

NA: not achieved (threshold: 7 log CFU/g not achieved during the storage period studied)

MAP1 30% CO_2 - 65% N_2 - 5% O_2 MAP3 30% CO_2 - 70% N_2

MAP2 65% CO_2 - 30% N_2 - 5% O_2 MAP4 70% CO_2 - 30% N_2

Patsias et al. (2006) compared the effect of MAP on precooked chicken breasts. Three atmospheres were tested: 30% CO_2 - 70% N_2, 60% CO_2 - 40% N_2, and 90% CO_2 - 10% N_2. The presence of CO_2, alone or in combination with N_2, affected the growth of aerobic spoilage bacteria (for example *Pseudomonas* spp.) and favored the development of facultative anaerobic populations (LAB). The shelf life was extended by 4 days with the 30% CO_2 - 70% N_2 mixture, and by more than 6 days with mixtures composed of 60% CO_2 - 40% N_2 and 90% CO_2 - 10% N_2.

Another study (Al-Nehlawi et al., 2013) showed that a pretreatment of 3 hours with 100% CO_2 prior to packaging under 70% CO_2 - 15% O_2 - 15% N_2 improved the microbiological quality of the meat of raw chicken drumsticks and prolonged shelf life. The *Pseudomonas* counts, as well as the total aerobic counts, were significantly lower after 7 and 12 days of storage when a CO_2 pretreatment was applied. Such treatment had no additional effect on coliforms, which were undetectable after 7 days of storage

under MAP, whether or not a CO_2 pretreatment was applied. Such an effect on the shelf life resulted from a better availability of CO_2 in the headspace during storage because of the dissolution of CO_2 in meat after the pretreatment.

Variability of bacterial contaminants in marinated chicken and with various additives

The definition of marinade varies according to the country (Björkroth, 2005; Yusop et al., 2010). Marinades may be composed of a mixture of oil or salt and phosphates (in France and Spain, for instance) or a sauce with oil, organic acids, or spices, essential oil and thickener (Finland, China, and Italy). In all cases, marinades are associated with storage under different MAP.

Chouliara et al. (2007) compared the effect of adding oregano essential oil at 0.1% or 1% alone or in combination with MAP on the microbiological quality of chicken cuts. The addition of 0.1% oregano essential oil increased the shelf life by 3-4 days while the increase provided by the gas mixture (70% CO_2 - 30% N_2) was only 2-3 days. The combination of a marinade with oregano essential oil and storage under MAP showed that the two treatments could be added as the shelf life reached more than 20 days with a decrease in the total viable count of 2-3 log CFU/g.

In Finland, the consumption of marinated poultry products packaged under MAP is common and the effect of the marinade on their microbial safety has been well documented. The Finnish marinade can be complex as it is composed of acetic acid, honey, glucose, maltodextrin, NaCl, phosphate, rape seed oil, spices (sweet pepper, curry, black pepper, garlic and turmeric), thickener (guar gum and xanthan gum), and yeast extract (Nieminen et al., 2012b). Such marinades may influence the LAB population by favoring the growth of specific species, particularly because of the source of carbohydrates they provide (Björkroth, 2005). The MAP commonly used in Finland is composed of 65% N_2 and 35% CO_2. The marinade favors a LAB psychrotrophic population, not detected in the unmarinated products (Björkroth, 2005); especially *Leuconostoc gasicomitatum*, also detected in spoiled meat and seafood products (Chaillou et al., 2005) and in some vegetables associated with marinated fish products (Lyhs et al., 2003). This bacterial species, unable to survive in the digestive tract of the animal, certainly originates from the environment and is adapted to the cold because it can persist throughout the transformation process (Björkroth, 2005). As the combination of MAP and marinade favors the emergence of this group of bacteria, it is necessary to understand their mechanism of adaptation to monitor them in such products. It should be noted that the marinade had no effect on *Campylobacter*.

In a study combining the identification of isolates, as well as 16S rDNA gene pyrosequencing and metagenomics an overview of the effect of marinades on broiler fillet strip microbiology was reported (Nieminen et al., 2012a). Samples stored at 6°C under MAP (65% N_2 - 35% CO_2) with and without marinade were compared. The combination of cultural and molecular methods confirmed that among LAB, marinade favored *Leuconostoc* and particularly *L. gasicomitatum*, and decreased *B.*

thermosphacta, *Clostridium* spp., and *Enterobacteriaceae*. Among LAB belonging to the genus *Carnobacterium*, *C. divergens* was present in higher amounts than *C. maltaromaticum*, although both species seemed sensitive to marinade, certainly because of the presence of acetic acid.

Variability of bacterial contaminant regarding sanitizing treatments

The effect of several sanitizing treatments tested on artificially contaminated products has also been assessed. These treatments are summarized in Table 5.

Table 5 Examples of chemical treatments tested and experimental designs

Type of experiment / Reference	TSP	ASC	CA	PA	CD	LA	AA	K_3PO_4	KO	G
Strain Isolation										
(Alonso-Hernando et al., 2010)	x	x	x							
(del Río et al., 2008)	x	x	x							
(Alonso-Hernando et al., 2009)	x	x	x	x						
Artificial contamination										
(Alonso-Hernando et al., 2012a)	x	x	x	x	x					
(Alonso-Hernando et al., 2013)	x	x	x	x	x					
(del Río et al., 2007a)	x	x	x	x						
(del Río et al., 2006)	x									
(del Río et al., 2007a)	x	x	x	x						
Natural contamination										
(del Río et al., 2007b)	x	x	x	x						
(Hinton et al., 2003)								x	x	
(Okolocha et al., 2005)	x					x				x
(Bolton et al., 2014)	x	x	x	x		x				
(Capita et al., 2013)	x	x	x				x			

TSP: TriSodium Phosphate; ASC: Acidified Sodium Chlorite; CA: Citric Acid; PA: Peroxy Acids; CD: Chlorine Dioxide; LA: Lactic Acid; AA: Acetic Acid; KO: Potassium Oleate; G: Glutamal.

In laboratory conditions (*in vitro*), the effect of 3 treatments on the lag phase and on the maximum growth rate was measured on several pathogenic (*Salmonella enterica* serotype Enteritidis, *L. monocytogenes*) and spoilage (*Pseudomonas fluorescens* and *B. thermosphacta*) bacteria (del Río et al., 2008). Acidified sodium chlorite was the most effective at decreasing the growth of all tested bacteria, whereas trisodium phosphate and citric acid were more effective against Gram-negative and Gram-positive bacteria, respectively. However, the effectiveness varied with the concentrations used. For example, at low concentrations trisodium phosphate increased the growth rate of *S. enterica* and *L. monocytogenes*. As well as the consequence of the strong effect of citric acid toward *B. thermosphacta*, the possible increased growth of pathogens was questioned. Thus, the authors questioned the potential danger to consumers of some treatments, by increasing the proportion of pathogenic bacteria with regard to the spoilage ones. Alonso-Hernando et al. (2012a) reached the

same conclusion about the dangerous effects of treatments favoring pathogenic bacteria as an indirect consequence of inhibiting spoilage bacteria.

In addition, the acid stress response of *L. monocytogenes* after exposure to acidic poultry meat decontaminants may even enhance its survival of a subsequent exposure to stronger acidity such as that encountered during gastric transit (Alonso-Hernando et al., 2009). This adaptation to acidic conditions involves membrane fluidity in *L. monocytogenes* and *S. enterica* and suggests that other decontaminants should be preferred rather than sub-inhibitory concentrations of citric acid or peroxy acids (Alonso-Hernando et al., 2010). Other studies have been carried out under laboratory conditions to investigate the effectiveness of treatments against pathogenic bacteria (Alonso-Hernando et al., 2013; del Río et al., 2006; 2007a). In summary, these studies showed that trisodium phosphate and citric acid were effective against Gram-positive pathogenic bacteria and peroxy acids and acidified sodium chlorite against Gram-negative bacteria. However, the observation of significant reductions in the microbial level immediately after treatment resulted from trials that were not performed in real meat conditions.

Naturally contaminated meat matrices have also been used (Bolton et al., 2014; Capita et al., 2013). In these conditions, all decontaminants tested (trisodium phosphate, lactic and citric acids, peroxy acids, acidified sodium chlorite) reduced the total viable counts, *Enterobacteriaceae*, *Pseudomonas,* and LAB counts. The most effective concentrations reported were 14% for trisodium phosphate and 5% for citric acid. Trisodium phosphate, citric acid, acidified sodium chlorite, and peroxy acids were considered interesting treatments for extending the shelf life and improving the safety of products (del Río et al., 2007b).

The effectiveness of chemical decontaminants and physical treatments (like steam, hot water, and electricity) during or after the slaughtering process has been reviewed (Loretz et al., 2010). These authors emphasized that besides the relative effectiveness of treatments toward a variety of bacterial species, these must be considered as part of an integral food safety system. In that sense, some authors also completed the analysis of treatments of carcasses against pathogens with a sensory analysis performed by trained panelists on the cooked carcasses (Okolocha and Ellerbroek, 2005). Since then, several other authors have also included the analysis of the sensory impact of decontamination treatments (see Samant et al., 2015, for a recent review).

The impact of other physical decontamination processes on the microbiology of poultry meat has also been investigated. High hydrostatic pressure associated with the addition of nisin or glucono-delta-lactone was effective at decreasing the counts of psychrotrophic bacteria and, to a lesser extent, mesophilic bacteria (Yuste et al., 1998). Gamma irradiation associated with storage under different MAP was also effective at reducing LAB, *B. thermosphacta*, *Pseudomonas,* and *Enterobacteriaceae* (Chouliara et al., 2008). Nevertheless, although such physical treatments have proven their ability to

reduce the microbial load, they may have indirect effects on the sensory attributes of meat (color, texture). In addition, the perception by consumers of such practices can be controversial and their use is regulated differently depending on the country (see Ahn et al., 2013; Garriga and Aymerich, 2009; EC regulation No. 258/97).

The major bacterial contaminants of poultry meat

Bacterial contaminants

As shown above, a wide range of studies has been dedicated to the detection or enumeration of various bacterial families and species present on poultry meat. The influence of various storage processes on microbial growth dynamics during the shelf life of products has also been widely investigated. The microbial communities present during the product manufacture and then after a few days of storage have been estimated. To illustrate the diversity of the bacteria targeted, we list the results (enumerations in log CFU/g) of several studies carried out by cultural methods on chicken meat (Table 6), resulting in a global inventory of the microbiota that can be encountered. Total viable counts represent various bacterial species, increasing during storage, and varying considerably between samples. As an example, we have previously shown that total viable counts from chicken legs sampled after storage at 4°C for 2/3^{rds} of their shelf life varied from 3 to 8 log CFU/g (Rouger et al., 2017).

Table 6 Values reported for various contaminants occurring on poultry meat.
Data were collected from: A (Al-Nehlawi et al., 2013); B (Balamatsia et al., 2007); C (Chaiba et al., 2007); D (Capita et al., 2002a); E (Chouliara et al., 2007); F (Capita et al., 2013); and G (del Río et al., 2007b). Values are expressed in log CFU/g.

	A	B	C	D	E	F	G
Total viable count	5	4.9	ND	4.88-5.41	4.28	5.66	ND
Psychrotrophic bacteria	ND	ND	4.02-4.48	ND	ND	ND	4
Mesophilic bacteria	ND	ND	4.74-6.18	ND	ND	ND	5
LAB	ND	3.9	ND	ND	3.66	ND	3.5
Pseudomonas	3.5	4.2	ND	ND	3.38	ND	4.5
Enterobacteriaceae	ND	ND	ND	2.58-3.53	ND	ND	3
B. thermosphacta	ND	ND	ND	ND	ND	ND	4
E. coli	2	ND	0.70-2.34	2.60-3.63	ND	ND	ND
Coliforms	2.2	ND	3.54-4.64	ND	ND	ND	3
S. aureus	ND	ND	0.68-2.43	ND	ND	ND	ND

ND: not determined

Pseudomonads, often recorded in poultry meat, are mainly represented by the species *Pseudomonas fragi*, *Pseudomonas lundensis*, and *Pseudomonas fluorescens* (Arnaut-Rollier et al., 1999a; 1999b). Among *Enterobacteriaceae*, the main genera are *Hafnia* (*Hafnia alvei*, *Hafnia paralvei*), *Serratia* (*Serratia fonticola*, *Serratia grimesii*, *Serratia liquefaciens*, *Serratia proteamaculans* and *Serratia quinivorans*) and *Rahnella*, *Yersinia*, and *Buttiauxella* (Säde et al., 2013). Several new *Enterococcus* species such as *Enterococcus viikkiensis and Enterococcus saigonensis* have also described in poultry meat products (Rakila et al., 2011; Harada et al., 2016). Among the various reports found in the literature, some targeted more specifically spoilage bacteria whereas others focused on pathogens.

Spoilage bacteria

Once bacteria contaminate meat and constitute the initial microbiota, the storage conditions and the various treatments applied shape the fate of this microbiota. The storage temperature as well as the nature and concentration of the gas used in gas mixtures for packaging are selective for some bacterial populations. Storage at low temperature favors the growth of psychrotrophic and psychrophilic bacteria while CO_2 has an inhibitory effect on *Pseudomonas* spp. Some species can survive throughout the process such as *S. putrefaciens*, frequently found on carcasses during the slaughtering process and still present after 14 days of storage under air (Hinton et al., 2004). During storage, the bacterial load increases but the microbiota diversity decreases compared with that initially present (Chaillou et al., 2015; Höll et al., 2016). Microbial spoilage occurs as a consequence of the growth and metabolic activities of spoiling bacteria. In most studies, the bacteria that dominate spoiled food have been considered those responsible for spoilage and, in some studies, the criterion of microbiological acceptability (total viable counts reaching 7 log CFU/g) has been used to define spoilage. Examples of bacteria enumerations in spoiled chicken meat products are listed in Table 7. *B. thermosphacta*, LAB, *Enterobacteriaceae* and *Pseudomonas* spp. are considered potential spoilers of poultry meat. However, from these examples, it is clear that these potential spoilage bacteria were not systematically the dominant ones (columns A, B, and D, Table 7). This suggests either that the presence of bacterial species causing spoilage was not detected by the methods used in these studies or that spoilage may be caused by subdominant species. Table 7 also illustrates the extreme variability in the microbial communities present in spoiled poultry meat and the difficulty of clearly identifying the spoilage bacteria. Therefore, the definition of poultry meat spoilage bacteria must be considered carefully.

Table 7 Enumeration of bacteria from spoiled chicken meat.
Bacterial counts are expressed as (log CFU/g), except in C (log CFU/cm2). Data were collected from: A (Zhang et al., 2012); B (Capita et al., 2013); C (Chouliara et al., 2007); D (Al-Nehlawi et al., 2013); E (Balamatsia et al., 2007) and F (Björkroth, 2005).

	A	B[$$]	C	D	E	F[$]
Storage duration (days)	4	5	9	11	15	Until spoiled
Storage temperature	10°C	7°C	4°C	3°C	4°C	6°C
Storage packaging	Air	Air	Air	70% CO_2, 15% O_2, 15% N_2	Air	65% N_2, 35% CO_2
Total viable count	9.5*	8.27	7.55	6.5	8	9.0
LAB	8*	ND	7.02	ND	7	9.1
Enterobacteriaceae	8*	ND	ND	ND	6	7.6
B. thermosphacta	ND	ND	7.23	ND	6	ND
Pseudomonas	6*	ND	7.21	5	6	ND
Coliforms	ND	ND	ND	3.7	ND	ND

ND: not determined
[$] marinated poultry
[$$] bacterial count determination after rinsing with water

A list of bacteria present in different meat products and their occurrence depending on the packaging atmosphere used for storage has been established by Doulgeraki et al. (2012). Some of them were reported as poultry meat spoilage microorganisms. *B. thermosphacta*, *P. fluorescens,* and *S. putrefaciens* are among the spoilage bacterial species most cited in spoiled chicken meat products (Hinton et al., 2004; Russell, 2008; Zhang et al., 2012). The spoilage potential of *Aeromonas salmonicida*, *P. fluorescens*,

P. fragi and *S. liquefaciens* has also been evaluated by challenge tests and sensory evaluation (Wang et al., 2017). A. *hydrophila* and *A. sobria* have been reported as psychrotrophic bacteria that could cause spoilage in addition to being potentially pathogenic for humans (Hinton et al., 2004). Molecular identification of colonies isolated from marinated spoiled poultry meat showed the involvement of several LAB species, in particular *Leuconostoc gelidum* subsp. *gasicomitatum* and *Lactobacillus oligofermentans* (Koort et al., 2005; Björkroth, 2005; Nieminen et al., 2012). Further investigation based on sensory analyses and genome or metabolic activity characterization of these LAB species confirmed their role in spoilage (Rahkila et al., 2012; Jääskeläinen et al., 2013). MALDI-TOF MS was also applied to colonies isolated from chicken breasts stored under 2 different MAPs and at 2 different temperatures in order to identify spoilage bacteria (Höll et al., 2016). *B. thermosphacta*, *H. alvei* and bacteria belonging to the genera *Carnobacterium, Janthinobacterium, Pseudomonas*, and *Serratia* were identified in the dominant microbiota. However, in this study, spoilage was considered to occur when total viable counts reached 7 log CFU/g, with no indication about sensory deterioration (Höll et al., 2016). Most of the species cited above, highlighted by isolation, correlate with the genera detected

by sequencing after PCR-DGGE of DNA extracted from broiler chicken carcasses following storage under different conditions (Zhang et al., 2012).

Pathogens

Numerous articles have investigated the prevalence of various pathogens in poultry meat. Among these, *Campylobacter* and *Salmonella* make up a large majority of the reports. These two human pathogens can be present at high loads in the gastrointestinal tract of birds but, after contamination of poultry meat, it is important to detect their presence even at a very low level. Therefore, some studies have focused on establishing correlations between the occurrence in animals and in meat (Hue et al., 2011). The emergence of antimicrobial resistance among foodborne pathogens is also extensively recorded (for a recent review, see Grant et al., 2016). In addition, the impact of breeding or farming on the prevalence and antibio-resistance in *Campylobacter* has been addressed (Economou et al., 2015). Methods for fast and accurate detection and identification of *Campylobacter* have been proposed (Fontanot et al., 2014, and references therein). Nevertheless, the data obtained by different methods should be carefully interpreted. As an example, the *Campylobacter* proportion enumerated in poultry feces determined either by high-throughput sequencing or by plating on various *Campylobacter* selective media gave quite different values (Oakley et al., 2012). Both *C. jejuni* and *C. coli* can be isolated from poultry meat (Hue et al., 2011), but also from human clinical cases that may result from contaminated food consumption (Wassenaar and Newell, 2006). No clear correlation could be established between the presence of *Campylobacter* in poultry meat and the level of bacterial contamination of chicken or turkey cuts (Fontanot et al., 2014). *Salmonella enterica* is among the most tracked human pathogen with the serovar Enteritidis being mainly associated with poultry meat and with outbreaks (Jackson et al., 2013). Other foodborne human pathogens present in various meat products have also been investigated such as *Listeria monocytogenes* (Capita et al., 2001; Gudbjörnsdóttir et al., 2004; Van Nierop et al., 2005; Cohen et al., 2007; Alonso-Hernando et al., 2012b). *Listeria* spp. prevalence in poultry meat is noticeable with *Listeria innocua* as the dominant species followed by *L. monocytogenes* and several other *Listeria* species (*Listeria welshimeri*, *Listeria grayi*, and *Listeria ivanovii*). The prevalence of *Staphylococcus aureus* on poultry meat products has been addressed although most of the literature has focused on antibiotic resistance and typing of the isolates (Capita et al., 2002b; Waters et al., 2011; Akbar and Anal, 2013; Krupa et al., 2014). Although there are a few reports on the detection of *Clostridium perfringens* on poultry meat (for example, see Cohen et al., 2007) most of the literature focuses on assessing and modeling its growth on meat after spore germination following the slaughtering process (Juneja et al., 2013; Mohr et al., 2015; Huang, 2016). Lastly, the emergence of *Aeromonas* from poultry meat products as a vector of human infection has also been reported (Praveen et al., 2016). Among *Aeromonas* spp. detected on poultry carcasses,

A. caviae, *A. hydrophila*, *A. salmonicida-masoucida*, and *A. schuberti* have been reported to survive after 14 days of product storage (Hinton et al., 2004).

Conclusion

The poultry meat sector tends to provide ready to eat products, which are safe for the consumer and have a long shelf life. Thus, the impact of various treatments (temperature, chemical treatment, marinade, or preservation processes) in reducing pathogens has been investigated. Many studies have also been conducted to test such treatments for extending the shelf life and avoiding spoilage.

The large number of publications dedicated to poultry meat microbiology and the variety of the results highlight the wide diversity of the microbiological status of poultry meat products. The bacterial loads can vary by several log CFU/g for similar cuts, stored under similar conditions. To date, the microbial ecology of poultry meat products has been considered mainly through cultural methods, which can introduce a bias because of the relative selectivity of the media used. In particular, poorly selective media targeting large families of bacteria such as LAB or *Enterobacteriaceae* have been used, leading to a poor characterization of the bacterial species present. The studies aimed at assessing the spoilage and/or shelf life of the products have used various criteria that make it difficult to describe clearly which bacteria can spoil poultry meat under which conditions, except for marinated poultry. In fact, marinades providing sugar and acetic acid lead to a pressure selection on the bacterial diversity, including bacteria responsible for spoilage, with the identification of the bacterial functions involved in spoilage appearance. Concerning pathogens, most of the efforts have focused on tracking them while only a few describe their behavior in the meat matrix and consider the meat microbiota. In fact, two approaches can be distinguished: one focusing on only one or a few species, mostly pathogenic, with little attention paid to the microbiota because of the low contamination level of pathogens regarding that of total counts; and one focusing on a wider range of microbes, but assessing microbiota with techniques that induce a bias in the identification or that are generalist because of the media used. A third approach, already used for investigating complex environments, has recently appeared in food microbiology and tends to study the microbiota by non-cultural methods. The advantage of the latter is a better description of the bacterial species present on poultry meat, regardless of the detection of pathogens that are often present at a lower level. Finally, although the gastrointestinal tract of birds and slaughtering facilities have been identified as the main reservoirs for the origin of poultry meat contaminants, there is a lack of knowledge about the flux of microbiota from the animals to the end products. The few studies about the transmission from animal to meat have mainly focused on pathogens.

The combination of high throughput sequencing approaches with highly selective cultural methods throughout the production chain will be necessary to assess the nature and origin of meat contaminants and their dynamics during processing and storage.

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1.2.3- Ce qu'il faut retenir de la revue

La viande est inévitablement contaminée lors des étapes d'abattage et de transformation (plumaison, éviscération, découpe…). Les méthodes culturales sont le plus souvent utilisées pour décrire ces contaminations. Bien que largement utilisées dans l'industrie pour surveiller le niveau de contamination et la présence de *Salmonella* (critère de sécurité de la viande), ces méthodes, dont les biais sont maintenant identifiés, ne sont pas exhaustives pour la description des contaminations bactériennes. D'autres méthodes, en particulier de biologie moléculaire, existent bien que peu utilisées au niveau industriel. Ces méthodes souvent plus rapides permettent de s'affranchir des biais liés à la non-cultivabilité des bactéries. Dans la littérature, il est noté que suivant les découpes choisies (présence de la peau ou non), suivant les saisons ou encore suivant les procédés de transformation (volaille entière, différentes découpes) la charge bactérienne est variable. Ainsi, si l'on souhaite étudier l'influence de paramètres de conservation sur les contaminants, une réflexion s'impose pour la mise au point d'un protocole standard permettant des expériences reproductibles pour s'affranchir de cette variabilité.

1.3- Microbiotes standards

1.3.1- Pourquoi utiliser un microbiote standard ?

La définition de l'écologie microbienne énoncée par Thomas Brock est l'étude du comportement et des activités des microorganismes dans leur environnement naturel (Brock, 1978). Dans le cadre de notre étude des communautés bactériennes de viande de poulet, nous avons constaté que les contaminations microbiennes suivant les lots peuvent être très variables. Outre le fait de décrire les

communautés bactériennes présentes nous souhaitions comprendre leurs dynamiques au cours du stockage avec l'idée de pouvoir un jour les maitriser plus efficacement. Une étude de Møller et al. (2013) a comparé la croissance de *Salmonella* inoculée sur de la viande stérile ou sur de la viande naturellement contaminée. Des modèles mathématiques de prédiction de la croissance de cette bactérie pathogène ont été développés et les auteurs ont noté que *Salmonella* semble moins se développer en présence du microbiote naturel de la viande. Il est donc intéressant de tenir compte de l'ensemble des contaminants.

Dans ce contexte, l'utilisation d'un microbiote standard va permettre d'améliorer grandement la répétabilité des expérimentations et va fournir des données reproductibles, s'affranchissant ainsi de la variabilité entre les lots. Ce microbiote sera une approximation de la réalité mais sera utile pour établir des hypothèses et répondre à des questions en conditions réelles de conservation de la viande. Un dessin de l'humoriste vétérinaire Kastet (Figure 9) résume ce propos montrant la complexité du microbiote intestinal de l'homme par rapport à la vision que l'on peut avoir dans des conditions de laboratoire.

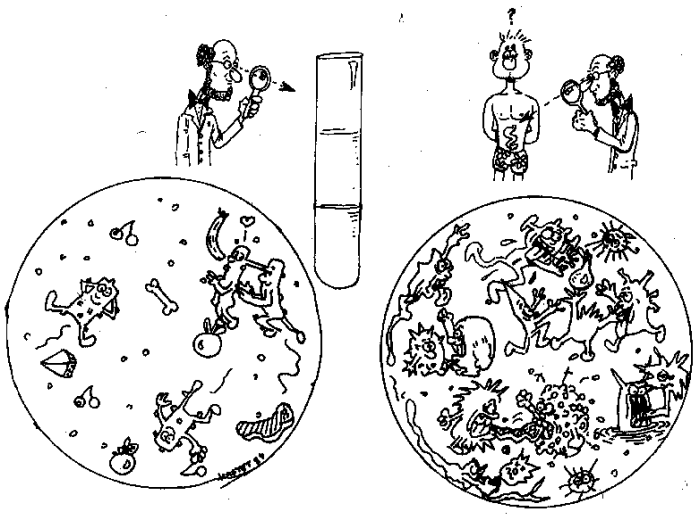

Figure 9 Dessin de l'humoriste vétérinaire Kastet représentant la complexité du microbiote intestinal de l'homme[2].

Un modèle d'étude visant à mimer la viande de poulet a été mis au point par Birk et al. (2004). Ce « jus de poulet » se rapproche au mieux de l'aliment pour étudier le comportement de *Campylobacter*. L'équipe note la simplification des expériences en milieux de culture mais comme pour tous les écosystèmes bactériens, la proportion des différents contaminants que l'on obtient par méthode culturale ne reproduit pas la réalité et la complexité de l'écosystème. Il nous revient alors de

[2] (Corpet & Brugère, Revue de Médecine Vétérinaire, 1995, 146, 2, 73-92)

développer un écosystème standard qui se rapprocherait au plus près de la viande de poulet naturellement contaminée.

1.3.2- Les pratiques utilisées en écologie microbienne

Dans le domaine des sciences de l'environnement (sol, océan, etc…), l'écologie microbienne a connu un essor depuis une trentaine d'année. L'utilisation de dispositifs expérimentaux appelés microcosmes permet de réunir plusieurs espèces en interactions dans un système de taille réduite afin d'étudier les interactions biotiques. En 1980, les 1^{e} échantillons d'ADN sont extraits à partir de sol. Nesme et al. (2016) font une revue sur les méthodes utilisées dans ce domaine : le 1^{e} séquençage par métagénomique a eu lieu en 2005 et la 1^{e} étude en métatranscriptomique a lieu l'année suivante. Par exemple lors de prélèvements d'eau ou de sol, il est possible de récolter une grande quantité d'une même matrice. Suivant les questions biologiques que l'on se pose, cela peut permettre de s'affranchir de la variabilité liée à l'échantillon. Si l'on souhaite étudier une dynamique des communautés bactériennes au cours d'une cinétique cela est faisable par exemple à l'échelle d'un océan en fixant le même point de prélèvement. Mais la situation est plus compliquée en science des aliments. Pour cela les chercheurs utilisent un même lot, par exemple un même lot de viande (même abattoir, même jour,…) et peuvent stocker les échantillons. Il faut donc s'assurer que les variations observées sont dues aux conditions expérimentales et non à la variabilité du lot.

Une des solutions pour s'affranchir de la variabilité est l'utilisation d'un modèle d'étude représentatif de l'écosystème à observer. L'étude de l'écosystème fromager illustre cette approche. En effet, le fromage est un aliment fermenté qui a donné lieu à de nombreuses études. Le fromage peut être réalisé à partir de différent consortia microbiens. Callon et al. (2011) ont inoculé du lait avec des consortia plus ou moins simplifiés pour fabriquer des fromages et montrer leurs effets anti-listeria. Ainsi l'écosystème microbien peut être simplifié. L'inventaire des espèces bactériennes et des levures et moisissures des écosystèmes fromagers a été réalisé au cours d'une thèse (Cholet, 2006). Devant la complexité de l'écosystème (Monnet et al., 2016) une étude de métatranscriptomique a été réalisée *in situ* sur un fromage Reblochon fait avec quelques souches bactériennes et de levures. Ce reblochon est produit avec deux bactéries lactiques *Streptococcus thermophilus* et *Lactobacillus delbrueckii sp.,* une bactérie d'affinage, *Brevibacterium aurantiacum* et deux espèces de levures *Debaryomyces hansenii* et *Geotrichum candidum.* Ce consortium d'inoculation permettait de diminuer la complexité du microbiote du fromage et d'étudier les comportements et les activités de quelques espèces majoritaires, déjà décrites dans la littérature et dont les génomes sont séquencés.

L'inoculation simplifiée est une solution bien adaptée pour traiter des produits fermentés. Cependant pour des matrices non fermentées (charge bactérienne plus diverse et moins élevée), la dynamique écosystémique est perdue et l'on étudie alors les capacités d'une ou quelques souches microbiennes seulement. Pour exemple, les études portant sur les microbiotes intestinaux complexes ont recours à des souris axéniques inoculées avec une flore intestinale. En 1874, Billroth démontre que les fœtus extraits chirurgicalement de manière stérile sont dépourvus de germe (Billroth, 1874). On parle alors d'« axénie». En laboratoire, il est assez aisé de maintenir les nouveaux nés d'animaux en environnement stérile. Ainsi sur des animaux maintenus axéniques, il est possible d'inoculer une flore connue, on parle alors d'animaux gnotobiotiques (Gnoto, en grec signifie « connu », biota évoque les « formes de vie »). L'utilisation de souris axéniques que l'on inocule avec des flores isolées de microbiote humain est un modèle d'étude utilisé pour comprendre comment le microbiote intestinal influence l'organisme (Corpet et al., 1989).

Ainsi pour aborder l'écologie microbienne on peut avoir recours à un écosystème simplifié représentatif de l'écosystème à étudier ou le constituer. Lors de l'étude d'aliments non fermentés dont la charge bactérienne est plus faible mais plus diverse que celles des aliments fermentés, il est difficile de simplifier l'écosystème microbien tout en gardant une diversité importante. La méthode la plus simple pour constituer ce microbiote standard est donc l'inoculation d'une flore connue sur une matrice stérile.

1.3.3- Challenge tests : inoculation sur des matrices pauci microbiennes

En microbiologie des aliments, des challenges tests sont souvent effectués, dans lesquels on inocule sciemment une ou plusieurs espèces bactériennes sur une matrice afin d'examiner un phénomène. Il s'agit d'une technique utilisée pour démontrer par exemple l'efficacité antimicrobienne d'une substance produite par une souche donnée, ou pour étudier le potentiel d'altération d'une ou de plusieurs espèces ou souches. Comme mentionné précédemment, les matrices alimentaires sont naturellement contaminées. Afin de s'affranchir de ce problème et suivant l'objectif de l'étude, l'inoculation se fait sur une matrice stérile (ou pauci microbienne) ou bien sur une matrice naturellement contaminée.

Pour rendre une matrice pauci-microbienne la pratique utilisée en microbiologie environnementale, est réalisée par dilution de l'échantillon pour diminuer la charge bactérienne, par exemple avec des échantillons de sol (Philippot et al., 2013). Rendre une matrice alimentaire liquide stérile est aussi possible par filtration ou stérilisation. Cependant ces méthodes sont peu adaptées à la matrice viande (solide et crue). Pour des matrices solides telles que la viande, Juck et al. (2012) ont utilisé un traitement thermique couplé à un traitement par hautes pressions. L'objectif de cette étude était de

déterminer la pression d'inactivation des agents pathogènes dans un modèle alimentaire. Si les agents pathogènes sont bien détruits, la structure et la composition de la matrice est également modifiée. Dans ce type d'approche un biais sur la croissance des bactéries sera observé. Le traitement thermique impose de travailler sur une matrice cuite.

L'ionisation est la méthode la plus utilisée en microbiologie des aliments (Joffraud et al., 1998, Warsow et al., 2008, Fall et al., 2012). L'ionisation par rayons X ou γ permet de prolonger la durée de conservation et d'inactiver les bactéries. Dans la littérature, différentes doses appliquées ont été rapportées: une dose de 11,95 kGy pour ioniser de la viande de dinde (Warsow et al., 2008); 1,5 à 3 kGy pour du saumon (Joffraud et al., 1998) ou encore 3,76 kGy pour des crevettes cuites décortiquées (Fall et al., 2012). Cette méthode présente toutefois des limites : elle peut générer des molécules comme des formes réactives de l'oxygène, pouvant avoir un effet antagoniste ou inhibiteur sur les bactéries ré-inoculées ou sur les enzymes comme la Taq Polymérase (Consortium du projet ANR ECOBIOPRO, résultats non publiés).

La découpe stérile peut être utilisée pour certaines matrices. En effet, l'intérieur du muscle, juste après l'abattage, est stérile. Ainsi en effectuant une découpe, à l'aide d'ustensiles stériles, suivi d'un traitement rapide à l'éthanol on peut alors obtenir une matrice pauci-microbienne comme décrit par Jorgensen et al. (2001) avec du saumon.

Nous comprenons donc qu'il est possible de constituer une matrice dite standard (microbiote connu) afin d'étudier l'écologie microbienne de la viande de poulet. Pour cela, quels sont les outils pour étudier communautés bactériennes dans leur globalité ?

1.4- Méthodes utilisées en écologie microbienne / Approches omiques combinées

1.4.1- Limites des milieux de cultures pour l'écologie microbienne

Plusieurs études ont montré les limites des méthodes cultures-dépendantes pour identifier les bactéries (Martin-Platero et al., 2008, Jaffrès et al., 2009). En effet, comme l'évoquent Juste et al. (2008) sur des matrices fermentées simples, de 25 à 50% de la communauté bactérienne n'est pas cultivable par les méthodes utilisées en laboratoire. L'existence d'un état viable non cultivable (VBNC) est controversé mais pourrait expliquer les différences parfois observées entre les résultats obtenus par méthodes moléculaires et culturales (Stokell & Steck, 2001). D'autres hypothèses peuvent expliquer les limites des méthodes culturales comme notamment la sélectivité des milieux ou encore

les conditions d'incubation. Ercolini (2004) a également évoqué les limites de ces méthodes culturales en expliquant le manque de connaissances sur le développement bactérien dans son habitat naturel. En effet, il est difficile de reproduire les conditions de l'environnement sur un milieu de culture universel. Les milieux de culture sont utiles lorsque l'on étudie une espèce bactérienne en particulier avec un milieu propre à l'espèce étudiée (Basu et al., 2015). En revanche pour des communautés complexes, il existe des milieux plus ou moins sélectifs permettant le dénombrement et la détection de certaines espèces, pathogènes notamment. Juste et al. (2008) montrent que les techniques moléculaires permettent de montrer la diversité d'un écosystème, d'identifier les bactéries qui le composent et enfin de les quantifier.

Il existe de nombreuses méthodes indépendantes de la culture pour identifier les espèces bactériennes parmi lesquelles, l'hybridation *in situ* et microscopie de fluorescence (FISH) ou encore la PCR couplée à la TTGE (Temporal temperature gradient gel electrophoresis) ou à la DGGE (Denaturing Gradient Gel Electrophoresis) mais aussi le séquençage à haut débit. Ces méthodes sont listées dans la revue précédemment présentée. Nous nous concentrerons dans la suite de cette synthèse bibliographique sur les méthodes de séquençage à haut débit utilisées dans ce projet.

1.4.2- Le pyroséquençage

Depuis les premières méthodes décrites en 1977 par Maxam et Gilbert et par Sanger et al. (Maxam & Gilbert, 1977, Sanger et al., 1977), les méthodes de séquençage ont largement évolué du séquençage d'un gène, d'un génome complet jusqu'à permettre aujourd'hui le séquençage d'un microbiote.

Le pyroséquençage est une des premières techniques dite « innovante » de séquençage à haut débit décrite par Margulies et al. (2005). Cette équipe développe une technique de séquençage à très haut débit, on parle de séquençage de nouvelle génération NGS. Ils décrivent la technologie 454 (développée par Roche) utilisée pour décrire des écosystèmes alimentaires. Comme travaux pionniers dans le domaine, on peut citer Humblot & Guyot (2009), Jung et al. (2011), Sakamoto et al. (2011), Park et al. (2012). Elle permet de séquencer à partir de molécules d'ADN uniques et de traiter, en une seule fois, plus de 20 millions de bases nucléotidiques par cycle de quatre heures, ce qui correspond à plus de 100 fois la capacité des instruments reposant sur les techniques de type Sanger. Le pyroséquençage permet alors le séquençage rapide (5 jours pour un génome microbien) et révolutionnaire par rapport à la méthode Sanger et à moindre coût. Cette méthode est dite « semi quantitative » car la proportion d'une séquence par rapport à une autre (et donc d'une espèce bactérienne par rapport à une autre) peut être évaluée sans toutefois apporter d'éléments précis sur la proportion des individus au départ.

Humblot et Guyot (2009) ont utilisé pour la première fois le pyroséquençage de l'ADN ribosomique (ADNr) 16S pour déchiffrer le microbiome d'un aliment fermenté. Néanmoins, à cette époque-là seules 200 pb du gène de l'ARNr 16S pouvaient être séquencées, et parce que les espèces bactériennes impliquées dans le processus de fermentation étaient phylogénétiquement proches, l'assignation taxonomique n'a été possible que jusqu'au niveau du genre. Mais ce problème a également été rencontré dans d'autres méthodes couramment utilisées telles que la PCR-DGGE suivie par le séquençage des bandes.

1.4.3- Evolution des techniques de séquençage

Les techniques de séquençage à haut débit évoluent rapidement. Goodwin et al. (2016) décrivent les différentes technologies utilisées maintenant en routine (Pacific BioSciences, Illumina, SoliD, …) avec les caractéristiques de chacune (Tableau 3).

Tableau 3 Comparaison des techniques de séquençage haut débit en fonction de la longueur des lectures et du nombre de lectures par cycle de séquençage.
D'après Alberti et Labadie, Journée Transcriptomique Génoscope juin 2014, Glenn (2011) et Goodwin (2016).

Technologie de séquençage		**Longueur maximum des lectures**	**Nb de séquences (millions)**	**Données générées**	**Durée du séquençage**
Roche 454	GS Flex +	800 pb	1	800 Mb	1 jour
Illumina	HiSeq	2x250pb	3000	10-1800 Gb	Quelques jours
	MiSeq	2x300pb	15 -25	0.3-15 Gb	Quelques heures
	NextSeq	2x150 pb	130-400	16-120 Gb	Quelques heures
PacBio	RSII	30 kb	0.05	275-375 Mb	Quelques heures
Life technologie	SOLiD	75 pb	1400	25-100 Gb	Quelques jours
	Ion PGM	400pb	0.5-5	30 Mb -2 Gb	Quelques heures
	IonProton	200 pb	60-80	10 Gb	Quelques heures

Les différents avantages et inconvénients de ces technologies de séquençage sont listés dans le Tableau 4.

L'évolution des technologies de séquençage est très rapide. En octobre 2013, la technologie 454 de Roche est arrêtée. En parallèle, Illumina est la technologie la plus couramment utilisée dans la littérature (HiSeq en 2010, MiSeq en 2012 et NextSeq en 2013). Fondée en 1998, la société Illumina développe son propre service de séquençage en 2009. Aujourd'hui on estime à 90% des séquences

d'ADN produites sur des machines Illumina[3]. De son côté, Ion Torrent la technologie de séquençage haut débit de Life technologies se développe aussi (SOLiD en 2007, IonPGM en 2010 et IonProton en 2012).

Tableau 4 Avantages et inconvénients des différentes technologies de séquençage à haut débit.
Source Alberti et Labadie, Journée Transcriptomique Génoscope juin 2014

	AVANTAGES	INCONVENIENTS
454 Roche	-Séquençage de longs fragments (> 800 bases)	-Préparation des banques -Taux erreur élevé (homo-polymères) -Coût élevé (systèmes enzymatiques) -Débit limité
Illumina	-Débit élevé -Taux erreur <2% -Coût -Large domaine d'applications	-Lectures courtes (haut débit) -Fréquence des évolutions techniques et logicielles -Taille des fichiers générés -Faible complexité (haut débit)
SOLiD	-Débit (150 Gb/sem) -Coût -Taux erreur <1%	-Préparation des banques -Lectures courtes -Complexité d'analyse
Ion PGM Ion Proton	-Pas de système optique mais un détecteur électronique -Utilisation de nucléotides non marqués -Capacités dépendantes des puces	-Préparation des banques -Difficulté à séquencer les homopolymères -Coût -Taux d'erreur (~10%)
PacBio	-Pas d'amplification -Rapidité (3 bases/sec) -Longueur de lecture (5 Kb en moyenne)	-Taux d'erreur élevé (~15%) -Photo-inactivation de la polymérase -Coût

Les technologies de séquençage peuvent être comparées selon différents critères : le coût, le débit mais aussi le taux d'erreur et la procédure de préparation des échantillons. Les coûts de séquençage ont largement diminué à ce jour tandis que les débits de séquençage ne cessent d'augmenter (5000$/Mb et 1Mb / jour en 2005 contre 0.03 $/ Mb et 160Gb /jour en 2013). L'équipe de Liu et al. (2012) a comparé des systèmes de séquençage tandis que Ross et al. (2013) ont mesuré les biais de séquence. Lors du séquençage, des erreurs peuvent survenir. Nakamura et al. (2011) listent les erreurs retrouvées dans les séquenceurs Illumina. Les technologies Illumina semblent générer moins d'erreurs (0.01 % à 1 %) que les technologies Ion Torrent (0.1 % à 10 %) par exemple (Ross et al., 2013).

1.4.3- Biais liés à ces méthodes de séquençage

Comme pour les méthodes culturales, les techniques moléculaires présentent elles aussi de nombreux biais qui peuvent impacter l'analyse de la diversité microbienne d'un échantillon. Head et al. (1998) font le point sur dix années d'études moléculaires ainsi que sur les biais de l'ensemble de ces

[3] www.technologyreview.com/s/531091/emtech-illumina-says-228000-human-genomes-will-be-sequenced-this-year/

techniques de biologie moléculaire. Dans ce paragraphe nous nous intéresserons aux biais couramment décrits en lien avec la procédure de séquençage haut débit.

La procédure de traitement d'un échantillon classiquement utilisée est présentée Figure 10. Wintzingerode et al. (1997) font un inventaire des biais majeurs rencontrés à chacune des étapes d'amplification et de séquençage. Pour chaque étape clé, voici quelques remarques ou questions à soulever.

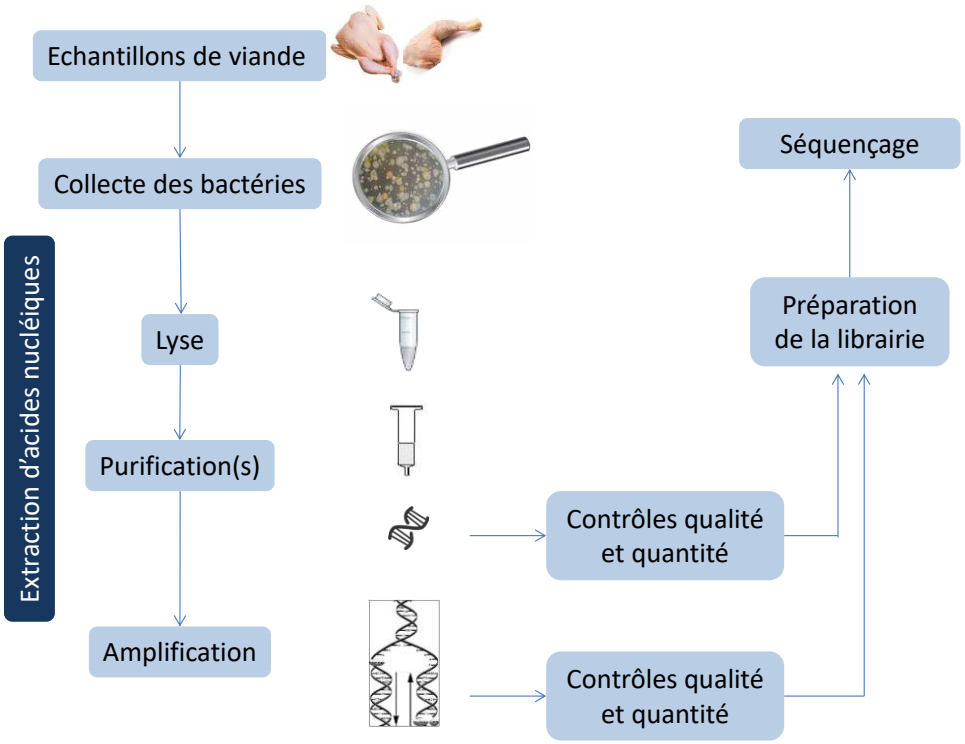

Figure 10 Procédure de traitement d'un échantillon en vue de l'analyse de la diversité bactérienne.

Suivant la question de recherche, l'échantillonnage peut varier. En effet, suivant si l'on cherche à décrire les communautés bactériennes ou si l'on souhaite réaliser une cinétique, le prélèvement des bactéries ne sera pas le même. L'échantillon doit être représentatif de l'environnement étudié. C'est pourquoi il faut aussi s'assurer que l'échantillon soit suffisamment contaminé, permettant une extraction d'acides nucléiques en quantité suffisante. La question de la protection (lyse, multiplication) des bactéries durant le transport ou le moment de collecte peut être importante. Par exemple la procédure pour filtrer 200 L d'eau ou prélever 10 g de matrice ne nécessite pas les mêmes précautions. Les acides nucléiques peuvent être extraits directement à partir de la matrice étudiée mais des résidus pouvant inhiber ou limiter les étapes suivantes peuvent subsister.

Lors de la collecte des bactéries, il faut s'assurer du rendement de cette étape. En effet si les bactéries sont organisées en biofilm par exemple ou en surface d'une matrice, la méthode devra être adaptée.

La procédure de stockage des bactéries (couramment à -80°C en présence de glycérol) devra être réfléchie. Lors des travaux portant sur le séquençage de l'ADNc, il est préconisé de stopper les réactions se produisant dans le milieu, en plongeant les culots bactériens dans l'azote liquide par exemple (McCarthy et al., 2015).

Lors de l'étape de lyse, l'efficacité du traitement, qu'il soit chimique ou mécanique, doit être prise en compte. Une lyse mécanique trop forte peut engendrer la fragmentation ou la dégradation des acides nucléiques, provoquant ainsi une source importante d'erreurs lors des étapes d'amplification (formation de séquences chimériques par exemple).

De plus les ARN sont sensibles aux RNases potentiellement présentes dans le milieu et pouvant être co-extraites avec les ARN. Différentes solutions de protection (RNA protect ou RNA later) existent pour y remédier (McCarthy et al., 2015).

Différents kits de purification existent. Le plus souvent, ils sont composés de colonnes de fixation par affinité des acides nucléiques, afin de les nettoyer puis ensuite de les éluer dans un tampon adapté. Ces colonnes peuvent être saturées si la quantité d'acide nucléique est trop importante. Il faut donc s'assurer de la gamme prévue par le fabriquant du kit. La fragilité de l'ARN nécessite de travailler rapidement et sur glace afin d'inhiber au maximum les réactions enzymatiques. Même si des kits sont couramment utilisés, l'extraction d'acides nucléiques bactériens à partir de matrice alimentaire nécessite des étapes de mise au point (Pinto et al., 2007). La présence d'inhibiteur de PCR dans la matrice par exemple peut engendrer des biais lors des étapes ultérieures. Dans le sol par exemple, l'acide humique est connu comme étant un inhibiteur de PCR (Feinstein et al., 2009). Certains fabricants ont breveté des technologies d'anti-inhibiteurs présents dans leurs kits (Faber et al., 2013).

Après extraction, différentes étapes peuvent être nécessaires. Pour exemple en métatranscriptomique, les ARN extraits comportent jusqu'à 95% d'ARNr qui peuvent être retirés par déplétion. Des kits utilisés pour cette étape permettent de retirer également des potentiels ARN eucaryotes, mitochondriaux ou chlorophylliens contaminants. Ces différentes étapes peuvent influencer le rendement d'extraction. La quantité et la qualité des acides nucléiques dépendent donc de la concentration bactérienne initiale mais aussi des différents rendements de chacune des étapes.

Dans le cas d'études de diversité à partir d'un gène représentatif (16S ou gène de ménage par exemple), les acides nucléiques sont amplifiés. Nous avons vu que la présence d'inhibiteur de PCR dans la matrice peut influencer mais une contamination par de l'ADN ou de l'ARN eucaryotes peut également générer des biais lors de l'identification par exemple (Glassing et al., 2016). Des difficultés d'amplification par PCR ont été mises en évidence lors de la détection de *Salmonella* sur de la viande

naturellement contaminée ou inoculée (Bülte & Jakob, 1995). Un biais lors des amplifications par PCR peut également exister avec des bactéries dites VBNC par exemple (Ceuppens et al., 2014).

Le choix des amorces est également une étape clé. En effet, pour étudier la totalité des communautés bactériennes, des amorces permettant d'amplifier un fragment de l'ADNr 16S sont le plus souvent utilisées. Cependant le nombre de copies de ce gène (et parfois la séquence) varie suivant les espèces, ce qui entraine des biais lors de la quantification ; on parle alors de quantification semi-relative (Klappenbach et al., 2000). De plus les amorces utilisées pour le pyroséquençage sont longues (environ 30 pb) et peuvent s'hybrider entre elles et limiter le rendement de la PCR. Des amorces dites universelles peuvent permettre l'amplification de fragments plus ou moins longs entrainant alors des difficultés lors de l'amplification. Le choix de la polymérase est donc important (Abu Al-Soud & Rådström, 1998). La fidélité d'une polymérase doit permettre de ne pas générer trop d'erreurs lors de l'amplification mais elle doit également être efficace pour amplifier des fragments longs (Keohavong & Thilly, 1989, Cline et al., 1996). Des solutions comme la T4gene 32 permettent de faciliter les amplifications (Wilson, 1997, Abu Al-Soud & Rådström, 2000). C'est lors de l'étape d'amplification que peuvent être formées des séquences chimériques. Des fragments, dont la synthèse ne se serait pas terminée à temps lors d'une phase d'élongation de la PCR, peuvent servir d'amorces sur un brin d'ADN différent pour le cycle suivant. Lors de la phase d'élongation suivante, la synthèse continue sur ce deuxième ADN et le fragment ainsi obtenu est composé de deux morceaux d'ADN provenant de deux espèces bactériennes différentes. Ces fragments, s'ils sont générés lors des 1^{e} cycles de PCR peuvent être largement représentés lors du séquençage (Haas et al., 2011). La détection des séquences chimériques, lors du nettoyage des lectures obtenues, doit donc être envisagée.

Il est aussi possible de séquencer l'ADN sans étape préalable d'amplification par PCR. C'est le cas dans l'étude de Nieminen et al. (2012) qui ont réalisé à la fois le séquençage d'un fragment de l'ADNr 16S et le séquençage de l'ADN total extrait de viande de poulet marinée et stockée pendant 13 jours à 6°C. Un total de 560 000 séquences brutes a été obtenu. Les séquences provenant des cellules de poulet ont été retirées en les comparants au génome de *Gallus gallus*. Les auteurs ont montré que la marinade diminue les proportions de *B. thermosphacta*, de *Clostridium* et *d'Enterobacteriaceae* dans le microbiote des produits étudiés. Cette analyse métagénomique a révélé la présence de bactéries non associées à l'altération de produits marinés comme *Vagococcus* et *Vibrio*. Ainsi, les résultats confirment que la marinade peut prolonger la durée de vie sensorielle de la viande de poulet en retardant la croissance de bactéries associées à l'altération et en favorisant *Leuconostoc gasicomitatum.*

Le contrôle de la qualité et de la quantité d'acides nucléiques est nécessaire avant le séquençage. Plusieurs appareils permettent le dosage de la concentration d'acides nucléiques. Les plus couramment utilisés sont le spectrophotomètre NanoDrop ou le spectrofluoromètre Qubit présenté comme la meilleure méthode pour doser les acides nucléiques (Robin et al., 2016). La qualité des acides nucléiques peut être vérifiée sur des puces à ARN ou ADN à l'aide de systèmes d'électrophorèse en micro-capillaires automatisés (par exemple le Bioanalyzer d'Agilent ou l'Experion de Biorad).

Bien que la qualité des séquences dépende de la pureté de l'ADN séquencé, Tyler et al. (2016) ont montré l'importance de la méthode de préparation des librairies. L'optimisation des méthodes de préparataion des libraires Illumina a été rapportée (Oyola, *et al.*, 2012). Par exemple le kit de préparation de librairies Illumina TruSeq produit des données présentant une qualité plus uniforme qu'après utilisation de la méthode Nextera XT. Les biais principalement identifiés lors de la fragmentation des acides nucléiques ou lors d'amplifications aléatoires sont décrit dans la revue de (van Dijk et al., 2014).

Une fois les librairies préparées, le séquencage peut avoir lieu. Nous avons vu précedemment les avantages et inconvénients de chacunes de ces méthodes.

1.4.4- Bio-analyse : pipelines et utilisation de bases de données

L'identification des espèces se base sur la comparaison par rapport à des séquences d'ADN. Les logiciels d'analyse de données requièrent donc des bases de données de séquences fiables et exhaustives afin de comparer les séquences obtenues à des séquences références. Les méthodes de séquençage à haut débit ont généré une pléthore de séquences disponibles dans différentes bases de données. Ainsi il existe une multitude de séquences plus ou moins complètes et de plus ou moins bonne qualité de bactérie comme évoqué dans la littérature (Humblot & Guyot, 2009).

Les pipelines et logiciels d'analyses de données, bien qu'en constante évolution, sont de plus en plus accessibles (Mothur, Qiime, Frogs…). Ils reposent tous sur le même principe illustré Figure 11.

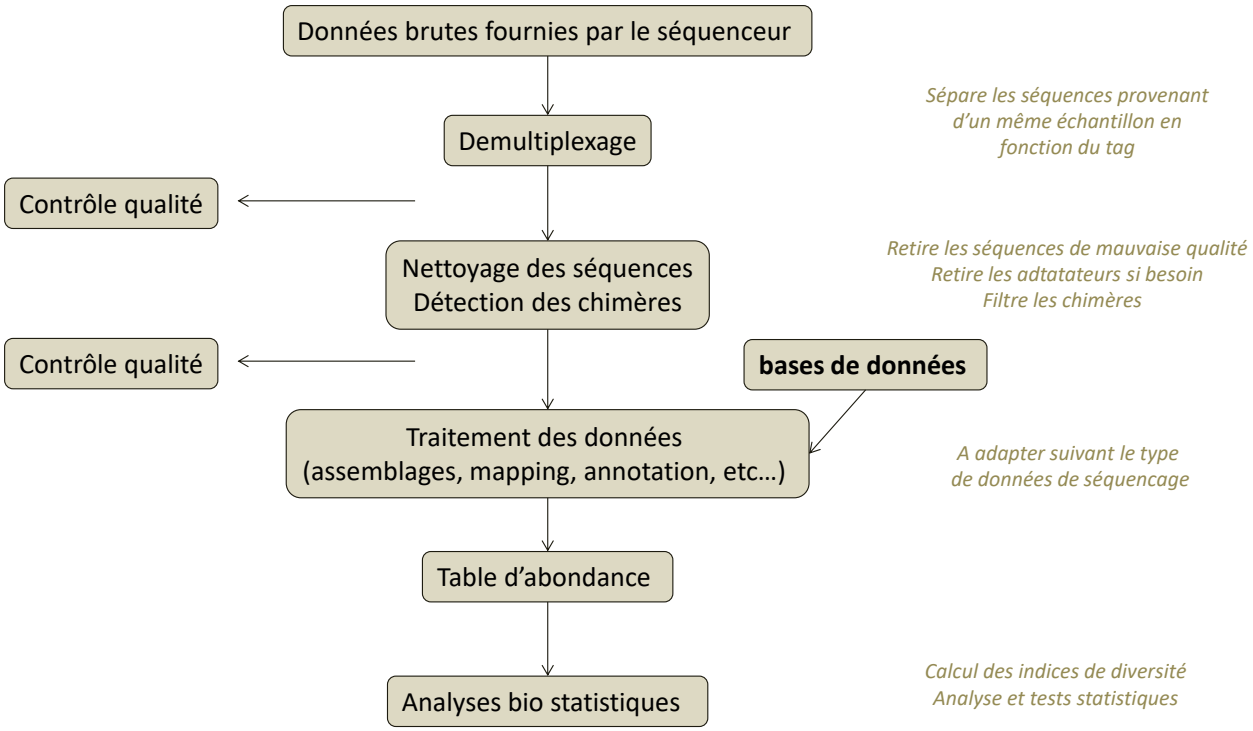

Figure 11 Procédure d'analyse des données de séquençage haut débit

Des contrôles qualité permettent de vérifier le nombre de séquences mais aussi leur qualité en sortie de séquenceur et après les étapes de nettoyage pour vérifier que ce dernier a bien été effectué.

La plupart des études visent à identifier des espèces bactériennes au sein d'écosystèmes complexes basées sur la séquence de l'ADNr. En effet cet ADN, existant chez toutes les espèces, présente des zones très conservées et d'autres spécifiques de chaque genre ou espèce. Ainsi les zones conservées peuvent alors servir pour dessiner des amorces pour amplifier par PCR la même région d'ADNr à partir d'ADN total extrait d'un écosystème. En revanche, la séquence des zones spécifiques peut servir à identifier les espèces ou les genres. Des bases de données de séquence d'ADNr ont été créées, contenant des séquences non redondantes et curées, afin de permettre d'identifier ses propres données par comparaison (Cole et al., 2005, Cole et al., 2007). Ce type de base de données classe et met à jour mensuellement toutes les données provenant des recherches scientifiques concernant les séquences d'ADNr. Une étude visant à comparer les différentes procédures d'analyse de données de métagénomique ciblée montre la difficulté à comparer les résultats de données de séquençage à haut débit de par le choix des amorces ou le nombre de copies du gène cible (souvent ADNr 16S) mais également suivant la base de données de référence utilisée (Siegwald et al., 2017). Une normalisation des données grâce à l'utilisation d'un jeu de données comme étalon interne pourrait être un bon

indicateur de comparaison (Siegwald et al., 2017). Cependant des ambiguïtés d'identification peuvent persister.

1.4.5- Les travaux publiés en écologie microbienne des aliments

Nous comprenons donc l'apport du développement de nouvelles méthodes de séquençage à l'écologie microbienne. En science des aliments, ces approches sont récentes en comparaison de l'étude des microbiotes environnementaux (eau et sol) ou animaux. De plus, les études recensées sur les aliments ont été essentiellement menées sur des produits fermentés et se sont souvent limités à décrire les communautés microbiennes (Tableau 5). En effet, les produits fermentés contiennent une charge bactérienne importante permettant l'extraction facilitée des acides nucléiques en vue d'un séquençage.

L'impact des technologies de séquençage à haut débit a permis de rendre plus accessible la caractérisation des écosystèmes microbiens des aliments. L'application de ces méthodes permet d'envisager de nouveaux enjeux industriels comme par exemple la maitrise des écosystèmes microbiens durant les procédés de fabrication et durant la conservation de l'aliment (Galimberti et al., 2015). Quelques études visant à décrire les communautés bactériennes de denrées hautement périssables (viande et produits de la mer) sont répertoriées dans le Tableau 5.

Tableau 5 Liste (non exhaustive) des études des microbiotes en science des aliments

Denrée	Méthodes de séquençage	Objectif de l'étude	Référence
PRODUITS FERMENTES			
Ben-saalga (millet fermenté)	Pyroséquençage	Description de la diversité microbienne	(Humblot & Guyot, 2009)
Produits de la mer fermentés	Pyroséquençage	Investigation des archées et des bactéries	(Roh et al., 2010)
Liqueur chinoise	Pyroséquençage	Diversité des bactéries et des levures	(Li et al., 2011)
Nukadoko (riz fermenté)	Pyroséquençage	Investigation des communautés bactériennes	(Sakamoto et al., 2011)
Kimchi (chou fermenté)	Pyroséquençage	Description des communautés microbiennes	(Jung et al., 2011, Park et al., 2012)
Fromage polonais	Pyroséquençage	Biodiversité du microbiote de l'Oscypek (fromage)	(Alegria et al., 2012)
Narezushi (poissons fermentés)	Pyroséquençage	Changement de populations durant la fermentation	(Kiyohara et al., 2012)
Grains de kéfir	Pyroséquençage	Description des communautés microbiennes	(Nalbantoglu et al., 2014)
Fromage belge	Pyroséquençage	Exploration du microbiote du fromage Herve	(Delcenserie et al., 2014)

Fromage mexicain	Pyroséquençage	Changement des communautés bactériennes durant la fabrication du fromage Poro	(Aldrete-Tapia et al., 2014)
Fromage italiens	Pyroséquençage	Caractérisation de l'usine et de l'affinage du fromage Caciocavallo pugliese	(De Pasquale et al., 2014)
Fromage français	Solid métagénomique	Activités métaboliques au cours de l'affinage du reblochon	(Dugat-Bony et al., 2015)
Chicha (maïs fermenté)	Pyroséquençage	Analyse de la diversité des bactéries lactiques	(Elizaquívela et al., 2015)
Levains de panification	Pyroséquençage	Description de la diversité bactérienne	(Lhomme et al., 2015)
Salami italien	Pyroséquençage	Diversité du microbiote pendant la fermentation naturelle	(Greppi et al., 2015)
Salami italien	Illumina MiSeq	Diversité bactérienne	(Połka et al., 2015)
Fromage kazakh	Séquençage SMRT* Pacbio	Comparaison des microbiotes de fromages belges, italiens et kazakhs	(Li et al., 2017)
Zhenjiang (vinaigre de céréales)	Illumina métagénomique	Identification des voies métaboliques des microbes responsable de l'odeur typique du vinaigre	(Wu et al., 2017)
PRODUITS NON FERMENTES			
Viande de poulet	Métagénomique 454	Effet de la marinade sur les communautés bactériennes	(Nieminen et al., 2012)
Steak de bœuf	Pyroséquençage	Origine des bactéries altérantes	(De Filippis et al., 2013)
Saucisses de porc	Pyroséquençage	Dynamique des communautés microbiennes durant réfrigération en fonction de la composition et de la température	(Benson et al., 2014)
Viande de porc	Pyroséquençage	Origine des contaminations en abattoir	(Hultman et al., 2015)
Produits carnés et produit de la mer	Pyroséquençage	Diversité bactérienne de 10 denrées à T0 et après altération	(Chaillou et al., 2015)
Viande bœuf	Pyroséquençage	Dynamique des bactéries durant la réfrigération et effet de différentes MAP	(Stoops et al., 2015)
Saucisses de porc	Pyroséquençage	Impact de la concentration en sel sur les communautés microbiennes	(Fougy et al., 2016)

** (Single Molecule, Real-Time)

Dans la plupart de ces études, le but est de décrire les communautés bactériennes présentes sur des aliments (Tableau 3) ou bien de montrer l'impact d'un procédé de conservation sur les communautés microbiennes de la viande (marinade, réfrigération, salage) (Nieminen et al., 2012, Benson et al., 2014, Fougy et al, 2016). Par exemple, Chaillou *et al.,* (2015) décrivent les communautés bactériennes de 10 denrées au moment de l'emballage et après altération. Ils ont mis en évidence la présence d'espèces bactériennes jusqu'à lors non décrites dans la littérature dans des produits de la mer. Ce sont également les premiers à avoir montré l'existence de communautés microbiennes présentes à la fois dans les produits carnées et les produits de la mer. Les sources de contaminations de la viande ne sont

pas seulement les microbiotes des animaux dont sont issus les aliments mais également l'environnement d'abattoir et de l'usine de transformation des produits et les conditions de stockage (à froid). Ces conclusions sont reprises également pour les microbiotes de viande de bœufs (De Filippis et al., 2013, Chaillou et al., 2015, Hultman et al. 2015). Les conditions de stockage (sous vide ou sous atmosphère modifiée) influencent les communautés bactériennes de la viande de porc où les communautés microbiennes sont plus diverses dans les emballages sous vide (69 OTUs vs 46 OTUs) (Fougy et al., 2016). De plus, des analyses sensorielles montrent que l'altération est réduite lorsque les communautés microbiennes sous dominantes sont plus abondantes dans une formulation enrichie en sel et stocké sous vide (Fougy et al., 2016). Ces différents exemples illustrent des questions de recherche résolues en science des aliments à l'aide de méthodes de séquençage à haut débit.

Nous remarquons également que le pyroséquençage est la méthode la plus largement utilisée. Cette méthode a permis de décrire les communautés bactériennes mais reste basée sur la comparaison de fragment d'ADNr 16S à des bases de données. Parmi les études listées dans le tableau 5, seules 3 études utilisent la métagénomique (séquençage de l'ADN sans étape d'amplification) permettant ainsi de limiter les biais liés à l'amplification PCR (Jung et al., 2011, Nieminen et al., 2012, Dugat-Bony et al., 2015). La métagénomique est un outil puissant car elle permet l'exploration de la biodiversité et donne un aperçu du potentiel fonctionnel d'un écosystème. Il est également intéressant de comprendre comment les bactéries se comportent au sein de l'écosystème. Pour cela quelques études récentes de métatranscriptomique ont été réalisées en science des aliments. Il s'agit d'études de produits fermentés préalablement décrits par pyroséquençage. Weckx et al. (2011) et Jung et al. (2013) ont étudié les fonctions bactériennes exprimées lors de la fermentation, respectivement du levain de panification et du kimchi. Ensuite sur le fromage qui a fait l'objet de nombreuses études, De Filippis et al. (2016) ont cherché à montrer les fonctions qui variaient au cours de l'affinage de fromages italiens quand Monnet et al. (2016) se sont intéressés aux interactions se produisant entre les différentes espèces de l'écosystème fromager du reblochon.

1.4.6- Intérêt des approches « omiques » combinées

Le développement des techniques de séquençage à haut débit a ouvert de nouvelles perspectives en écologie microbienne des aliments. Plusieurs revues répertorient les différentes stratégies pour analyser les communautés microbiennes (Bergholz et al., 2014, Franzosa et al., 2015). Aguiar-Pulido et al. (2016) notent qu'il est intéressant de combiner les approches « omiques » comme la métagénomique, la métatranscriptomique et la métabolomique. En science de l'environnement, domaine dans lequel les technologies de séquençage à haut débit ont été largement utilisées, ces approches combinées sont courantes (Hultman et al., 2015).

La Figure 12 inspirée de la revue de Cardenas et Tiedje (2008) résume ces nouvelles méthodes et les questions auxquelles chacune permet de répondre en écologie microbienne des aliments.

Il parait donc indispensable d'utiliser les méthodes de séquençage haut débit en plein essor, mais il convient également de valider et vérifier ces résultats par d'autres approches (qPCR, TTGE, DGGE…). Afin d'avoir une vision plus globale d'un écosystème, les méthodes de biologie moléculaire peuvent aussi être couplées à des méthodes de microbiologie culturale plus classiquement utilisées (Cholet, 2006, Corre, 2015, Fougy, 2016).

Les résultats obtenus au cours de ces travaux de doctorat sont présentés dans les chapitres suivant.

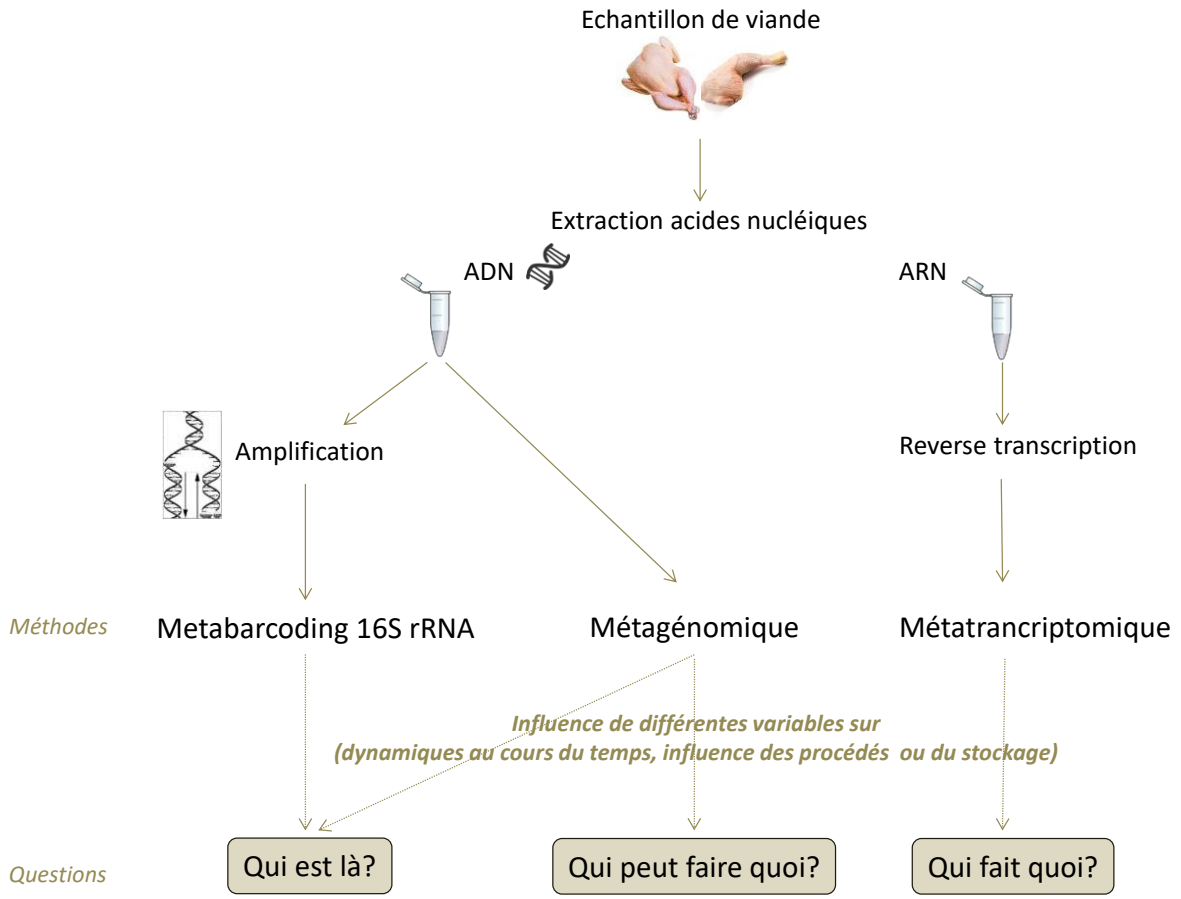

Figure 12 Méthodes de séquençage haut débit couramment utilisées pour caractériser la diversité microbienne.

Inspirée de (Cardenas & Tiedje, 2008)

Chapitre 2 Mise au point d'un microbiote standard

2.1- Préambule

Dans la littérature, nous avons pu constater que les contaminations de la viande de poulet sont variables suivant les lots et les découpes mais aussi suivant les procédés de transformation et suivant les saisons. Ces contaminations, bien qu'étudiées par méthodes culturales restent peu décrites par des méthodes moléculaires. Dans l'objectif d'étudier les microbiotes de la viande de volaille, nous devons nous affranchir de la variabilité mentionnée précédemment. Nous avons donc développé un modèle expérimental. Notre idée a été de reconstituer écosystème représentatif de viande de volaille que l'on pourrait réutiliser pour réaliser des challenges tests de manière reproductible.

Nous avons dû choisir parmi les découpes de poulet (poulet entier, cuisse avec peau, filet sans peau) lesquelles présentaient une charge bactérienne suffisamment importante pour constituer notre stock. En effet, juste après emballage, la viande est peu contaminée, puis la charge bactérienne augmente jusqu'à la DLC. Cependant à la DLC, la diversité des communautés bactériennes diminue. En effet, une ou quelques espèces de bactéries altérantes peuvent s'être largement développées. Nous avons effectué un suivi cinétique de la charge bactérienne pour choisir le moment de récolte des bactéries, afin d'avoir un niveau de contamination suffisant mais dont la diversité était suffisamment représentative.

Nous nous sommes assurés de pouvoir obtenir l'ADN de ces communautés bactériennes afin d'envisager l'optimisation des méthodes NGS ensuite. Un protocole de séparation des bactéries de la matrice et d'extraction d'ADN bactérien à partir de matrice alimentaire viande de volaille a été mis au point.

Après avoir vérifié la viabilité des communautés bactériennes récoltées et conservées à 80°C, nous avons testé leur capacité à se redévelopper sur la viande, sans étape préalable de culture (afin de limiter les biais de sélection des espèces cultivables). Pour cela, des matrices de viande de poulet pauci microbienne ont été utilisées pour des challenges test avec les communautés microbiennes préalablement stockées. Nous avons suivi par méthodes culturales le développement de quelques

flores au cours du temps de conservation de la viande et montré la capacité de notre microbiote standard à permettre des expériences répétables avec des microbiotes contrôlés.

Ces travaux ont fait l'objet d'un article accepté en avril 2016 dans *Internationnal journal of Food Microbiology*, (dx.doi.org/10.1016/j.ijfoodmicro.2016.04.028).

2.2- A method to isolate bacterial communities and characterize ecosystems from food products: Validation and utilization in as a reproducible chicken meat model

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Abstract

Influenced by production and storage processes and by seasonal changes the diversity of meat products microbiota can be very variable. Because microbiotas influence meat quality and safety, characterizing and understanding their dynamics during processing and storage is important for proposing innovative and efficient storage conditions. Challenge tests are usually performed using meat from the same batch, inoculated at high levels with one or few strains. Such experiments do not reflect the true microbial situation, and the global ecosystem is not taken into account. Our purpose was to constitute live stocks of chicken meat microbiotas to create standard and reproducible ecosystems. We searched for the best method to collect contaminating bacterial communities from chicken cuts to store as frozen aliquots. We tested several methods to extract DNA of these stored communities for subsequent PCR amplification. We determined the best moment to collect bacteria in sufficient amounts during the product shelf life. Results showed that the rinsing method associated to the use of Mobio DNA extraction kit was the most reliable method to collect bacteria and obtain DNA for subsequent PCR amplification. Then, 23 different chicken meat microbiotas were collected using this procedure. Microbiota aliquots were stored at -80 °C without important loss of viability. Their characterization by cultural methods confirmed the large variability (richness and abundance) of bacterial communities present on chicken cuts. Four of these bacterial communities were used to estimate their ability to regrow on meat matrices. Challenge tests performed on sterile matrices showed that these microbiotas were successfully inoculated and could overgrow the natural microbiota of chicken meat. They can therefore be used for performing reproducible challenge tests

mimicking a true meat ecosystem and enabling the possibility to test the influence of various processing or storage conditions on complex meat matrices.

Introduction

Microbial diversity is shaping the ecology of very diverse ecosystems. For example, bacteria are known to be a major part of geo-chemical cycles in natural environment. Studying the microbial diversity and interactions of bacteria with the support and the other organisms is always a challenge due to the extreme variability which can occur between samples. In meat agro-food industry, contaminating bacteria originate from animal microbiota (feces, hide, skin, or feather), from production plant environment (air, equipment, surfaces) and from human manipulators (Chaillou et al., 2015). Therefore, a large diversity of species can be hosted by meat products. After initial contamination of carcasses or cuts, processing steps and storage conditions like temperature and the atmosphere used for packaging, shape the evolution of this microbiota. The microbial diversity and its dynamics during food production can influence the product shelf life and safety if spoilage bacteria are favored and pathogenic bacteria present and able to grow.

In poultry meat, the total viable counts reported in the literature and expressed as colony forming units per gram (CFU/g) ranges from 6.5 to 9 log depending on authors, and on storage conditions and poultry cuts (Björkroth, 2005, Balamatsia et al., 2007, Chouliara et al., 2007, Zhang et al., 2012, Al-Nehlawi et al., 2013, Capita et al., 2013). This suggests that a great quantitative variability of bacterial contamination hosted by poultry meat exists. *Pseudomonas* sp., *Enterobacteriaceae*, *Brochothrix thermosphacta*, and lactic acid bacteria such as *Carnobacterium* sp. and lactotobacilli are among the most often bacterial contaminants reported by authors. A large majority of the published results are focused on pathogenic bacteria whereas spoilage microorganisms were rarely investigated. Indeed, *Salmonella* and *Campylobacter* prevalence, or characteristics of *Staphylococcus aureus* isolates from poultry cuts have been reported from several countries (see as examples (Atanassova & Ring, 1999, Capita et al., 2001, Capita et al., 2002b, Capita et al., 2007). In addition, only few studies dealing with the whole microbiota of poultry meat have been reported ((Hinton Jr & Ingram, 2003, del Río et al., 2007b, Nieminen et al., 2012). Many articles focused only one bacterial species and did not consider the natural bacterial contaminants, despite their impact on the bacterial dynamics. For instance on pork meat, the conclusions drawn by using *Salmonella* growth predictive models were different when sterile or naturally contaminated meats were used, the natural microbiota of meat reducing *Salmonella* growth (Møller et al., 2013). This example shows the importance to consider food matrices as a global ecosystem hosting complex microbial communities (Fleet, 1999).

Several studies aiming at understanding the mechanisms of bacterial adaptation to food environment have been reported. In general, the approaches used are based on challenge tests in which bacteria (commonly one or a few strains) are inoculated at empirical levels, which do not always reflect the conditions that occur in commercialized and consumed products. As an example, the effect of modified atmosphere packaging on the growth of *Campylobacter* was studied on chicken breast fillet by inoculating meat at 10^4 to 10^5 CFU/g with a five-strain cocktail (Meredith et al., 2014). Although informative the results obtained in such conditions, do not reflect the real situation of the products that can be proposed on the market as the concentration of *Campylobacter* in naturally contaminated products is difficult to estimate (Rohonczy et al., 2013). Indeed, most often only prevalence of *Campylobacter* is reported (see Economou et al., 2015 as example) and only few reports about the contamination level are available, as it varies along the food chain and is batch-dependent (Gruntar et al., 2015).

Poultry meat samples constitute very heterogeneous matrices depending on the type of cuts. The unavoidable bacterial contamination occurs mostly at the surface and on the skin of the cuts during the different steps of the slaughtering process (Luber, 2009). The poultry meat worldwide production is in constant increase each year reaching 106.8 million tons in 2013. In connection with the human population growth, the needs for meat production also increase especially in developing countries. According to the FAO, increased consumption is mainly due to an attractive price-quality ratio and to health and nutrition benefits of poultry meat. On the other hand, chicken meat attractivity increases because producers develop retails and ready-to-eat products, fast and easy to prepare, fitting with to consumers demand. It is therefore necessary to guaranty the safety of poultry meat to face this increasing demand.

The effects of different treatments have been studied in order to develop strategies for fighting human pathogens or spoilage species. Among those the use of modified atmosphere packaging, alone (Al-Nehlawi et al., 2013, Meredith et al., 2014) or combined to protective cultures (Melero et al., 2012) or essential oils (Chouliara et al., 2007) as well as decontamination with various chemicals (Okolocha & Ellerbroek, 2005, del Río et al., 2007b, Alonso-Hernando et al., 2012a, Capita et al., 2013) are the most documented. The effects of other treatments such as irradiation (Szczawińska et al., 1991) or marinades (Nieminen et al., 2012) have also been described. To overcome variability, microbiologists usually inoculate food or matrices from one batch in order to obtain reproducible matrices. In microbial ecology studies aiming to elucidate bacterial interactions, with the food matrix and/or other micro-organisms, the challenge is i) to define reproducible and reliable experimental conditions to lead to biological interpretation, or ii) to multiply sampling or experiments to obtain statistical significance of the results. In the present study we designed a method to collect poultry meat bacterial

communities in order to develop an accurate model useful to reproducibly investigate the effect of various meat processing and storage conditions on the evolution of meat microbiota.

Table 8 Description of the 23 chicken leg samples used for microbiota collection.

Samples	Shef-life [a] (days)	French Department Origin/ Slaughter house [b]	Free-range	Appellations label / Descriptions	O_2-CO_2 (%) [c]
A	11	44/1	X	Label Rouge/ Yellow	53.0-23.4
B	NA	85/1	-	-/-	53.0-18.0
C[d]	11	44/1	X	Label Rouge/ White	45.1-24.7
D	11	56/1	-	-/-	48.1-22.2
E	17	85/2	-	-/ White	36.8-21.2
F[d]	NA	85/1	X	Label Rouge/ Black	44.7-21.6
G	NA	72/1	X	Label Rouge/-	3.3-22.6
H	12	53/1	-	-/-	7.6-15.4
I	12	72/2	-	-/-	53.9-24.4
J [d e]	12	72/2	-	-/-	19.0-0.0
K	14	44/1	X	Label Rouge/ White	53.3-21.7
L[d]	NA	85/1	-	-/-	44.4-19.0
M	12	85/3	X	Organic/-	0.6-13.7
N[d]	NA	72/1	X	Organic/-	1.9-22.7
O	NA	85/1	X	Organic/-	34.6-16.3
P	13	85/1	X	Label Rouge/ Black	41.4-20.3
Q	12	53/1	-	-/-	0.8-23.3
R	11	56/1	-	-/-	45.9-24.7
S[d]	13	85/3	X	Organic/-	0.4-18.2
T	NA	85/1	X	Label Rouge/ Black	42.8-20.6
U	9	85/1	X	Label Rouge/-	22.0-17.6
V	11	53/2	X	Label Rouge/-	2.1-20.7
W	11	61/1	-	Halal/-	32.1-17.8

[a] estimated shelf life calculated from the indicated UBD and date of production
[b] the different slaughterhouses were numbered
[c] CO_2 and O_2 percentages measured in the headspace when bacteria were collected
[d] Bacteria were collected at UBD
[e] unclosed (damaged) package
NA not available
- not documented

In France chicken legs are a popular meal and are often sold as portions of 2, 4, 6 or more legs packaged under various modified atmospheres. In addition, a large choice of meat is proposed, issued from various farming practices (including organic, free-range, "label rouge" farming), and performed on various genetic backgrounds (white, yellow and black races). We took into account this large diversity of producing conditions in our sampling procedure and collected microbiota from chicken meat to constitute a livestock that could be characterized and used to re inoculate fresh matrices to create a standard ecosystem.

Materials and methods

Chicken meat samples

Chicken cuts (portions of 2 legs or 1kg - *i.e.* 4-6 - breast fillets) stored under modified atmosphere were collected from local supermarkets on the day of arrival, *i.e.* 1-2 days after slaughtering, and stored at 4°C until experiments. Gas composition of the meat packages was measured just before collecting bacteria as described by Melero et al (2012)using a digital O_2/CO_2 analyzer (Oxybaby, WITT Gasetechnik GmbH & Co KG, Germany).

For the constitution of life stocks representing diverse bacterial communities naturally present on poultry meat 23 packs of two chicken legs (coined here A to W) from various origins and labels were used. The characteristics of the 23 samples are summarized in Table 8. After rinsing one leg for 5 min in 200 mL TS, bacteria were collected by centrifugation, the pellet was resuspended in 85 mL of TS and 1 mL-aliquots were stored at -80 °C for further studies. Bacteria were enumerated before and after various freezing periods at -80 °C (1 to 28 weeks depending on batches).

Bacteria collecting

The experimental design to set up a reliable method for collecting and store the bacterial communities from meat samples is summarized Figure 13. Four different treatments were tested to recover bacteria from meat (stomaching, rinsing, swabbing, and scrapping). Collected bacteria were resuspended in sterile TS then stored at -80 °C as 1 mL aliquots with 15% (v/v) glycerol and the efficiency of each treatment was estimated by CFU counting at each step (Figure 13).

Stomaching

Fifty grams of meat were aseptically transferred into a sterile stomacher bag, with 200 mL TS (8.5 g/L NaCl, 1 g/L tryptone in distilled water) containing 1% Tween 80. Meat samples were then homogenized for 2 min in a stomacher (Masticator, IUL Instruments, England). The homogenate was filtered through the bag filter and centrifuged through a filter (F) or a column (C) from Nucleospin Plant II Midi kit

(Macherey Nagel, EURL, France) at 8 000x*g* during 10 min at room temperature. These filters bind cell fragments whereas columns bind eukaryote DNA from the matrix. Unlysed bacteria were therefore collected in the pellet and resuspended into 3.3 mL TS. Alternatively, 30 mL of blended mixture were filtered by gravity through a sterile paper filter or used for 2 successive centrifugation steps a low gravity to remove food residues: 30 mL were first centrifuged at 100x*g*, 3 min at room temperature and 25 mL of supernatant were subsequently centrifuged at 500x*g* for 5 min. Then 20 mL of filtrate or supernatant were centrifuged at 3 000x*g* 20 min at 4 °C and the bacterial pellet was resuspended into 3.3 mL TS.

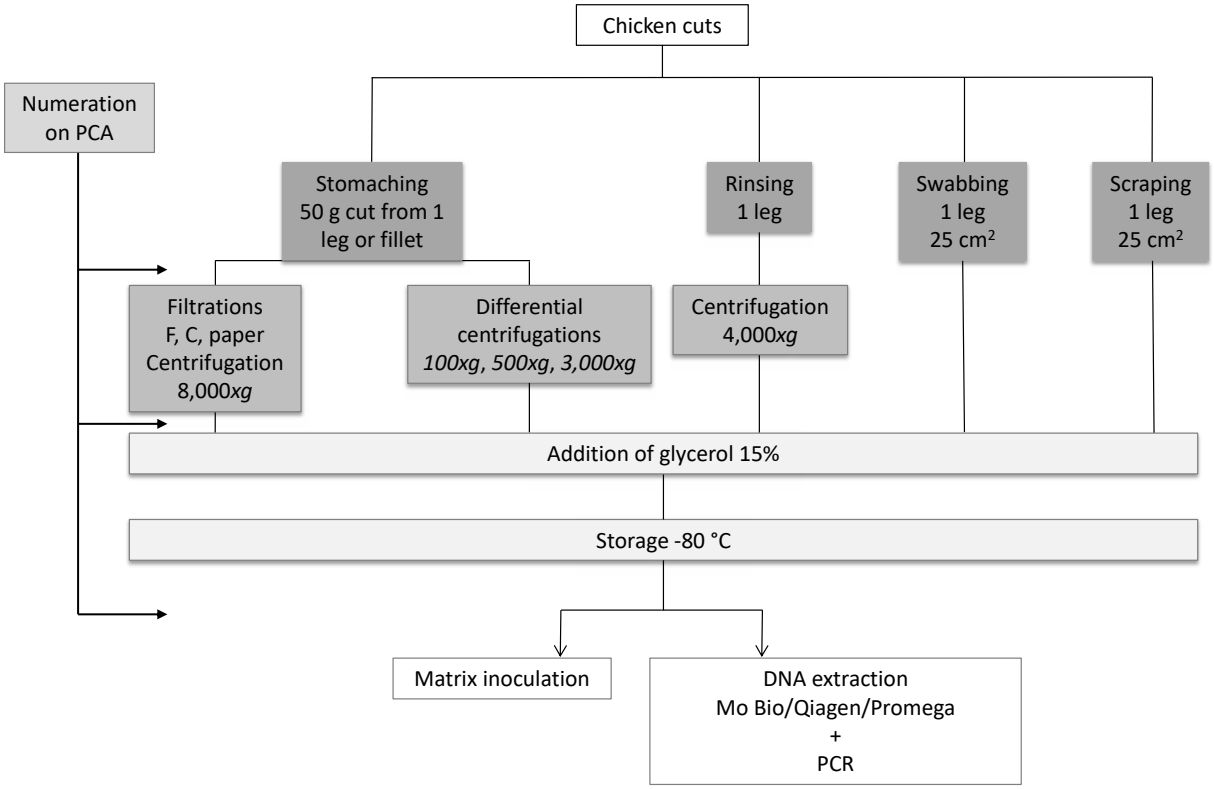

Figure 13 Experimental design to set up an efficient and reliable method to collect and analyse a viable bacterial community model characteristic of poultry cuts.

Rinsing

A whole portion of meat was added with 200 mL TS into a sterile stomacher bag. Alternatively TS containing 1 % Tween 80 or peptone water (peptone 10 g/L, sodium chloride 5 g/L, disodium phosphate 3.56 g/L, potassium dihydrogen phosphate 1.5 g/L, pH 7.2 at 25 °C) were tested. Hand-agitation was performed during 30 sec to 5 min. The liquid was filtered through the bag filter, centrifuged at 4 000x*g* for 20 min at 4 °C then the bacterial pellet was suspended into 100 mL TS.

Swabbing

A 5 cm x 5 cm zone on chicken skin was swabbed. The swab (Copan Diagnostic 155C, Italy) was vortexed with 5 mL TS containing 1% Tween 80 and the operation was repeated four times on the same zone. The volume was adjusted to 15 mL with TS containing 1% Tween 80.

Scraping

A volume of 1 mL TS containing 1% Tween 80 was sprayed with a pipet onto a 5 cm x 5 cm surface of chicken skin. Scraping with a sterile scalpel was performed and the liquid was collected into a sterile Petri dish. The operation was repeated four times on the same zone. The bacterial suspension was adjusted to 15 mL with TS.

DNA extraction

To isolate DNA from the collected bacteria, 1 mL of bacterial suspension was centrifuged at 10,000*xg*, 10 min at 4 °C. Several DNA extraction methods were tested as described below. After bacterial pellet suspension in various lysis buffers, incubation for 10 min at 56 °C to dissolve the fatty moiety of meat residues or sonication in an ultrasonic bath 3 min at 50 °C (Aerosec Industry, France) to strengthen the lysis efficiency were tested.

The Qiagen DNeasy Blood and tissue kit (Qiagen, Germany) was used as recommended by the manufacturer. Bacterial pellet was resuspended in lysis solution (Tris–HCl 20 mM, pH 8.0, EDTA 2 mM, 1.2% Triton X-100) containing 20 mg/mL lysozyme and 29 U/mL mutanolysin then incubated at 37 °C for 1 h. After addition of 0.3 g of glass beads (150 - 200 µm diameter), a mechanical lysis was performed by shaking twice 2 min in a bead beater (MM200 30 Hz, Germany) interspersed by 2 min storage on ice. Proteins and RNAs were degraded by adding 200 µl AL buffer from the kit containing proteinase K (20 mg/mL) and Rnase A (1 mg/mL) (Qiagen, Germany) then incubating 30 min at 56 °C. After centrifugation at 10,000*xg* for 3 min the supernatant was collected and DNA was precipitated by addition of 200 µl ice-cold ethanol. DNA was purified on Qiagen kit columns as recommended by the manufacturer.

When the Promega wizard genomic DNA purification kit (Promega, France) was used, bacteria were suspended in Nuclei lysis solution, provided with the kit, and incubated at 80 °C for 5 min, then 3 µL of RNAse solution from the kit were added with a further incubation for 1 h at 37 °C. A volume of 200 µL of protein precipitation solution included in the kit was added and the mixture was incubated 5 min in ice. After a centrifugation step at 13,000*xg* for 3 min, DNA was precipitated from the supernatant with 600 µL isopropanol and collected by centrifugation at 13,000*xg* for 2 min. The DNA pellet was washed

with ethanol 70%, dried and suspended with rehydration solution 1 h at 65 °C according to the Promega instruction manual.

With Mo Bio Power Food Microbial DNA isolation kit (Mo Bio laboratories, Inc., USA), 450 µL of heated lysis solution PF1 was used to suspend bacterial pellet. The suspension was transferred in Micro Bead tubes provided with the kit and mechanical lysis was performed by shaking 10 min in a MobioVortex (Genie2). Other steps were performed according to the manufacturer instruction manual with the use of Mobio colums for DNA purification.

Removing residual proteins from the final DNA solutions by extracting twice with phenol:chloroform:isoamyl-alcohol (25:24:1) and once with chloroform:isoamyl-alcohol (24:1) was also tested.

DNA quantification and PCR conditions

DNA concentrations were measured with a Qubit fluorometer (Invitrogen, CA, USA) and PCR fragments were visualized after electrophoresis on 1-1.5% (w/v) agarose gels. All PCR amplifications were performed in a PTC-100 Thermocycler (MJ Research Inc., Watertown MA, USA).

The 1,500 pb 16S rRNA gene fragment was amplified by PCR with primer pairs fd1 (5'-AGA GTT TGA TCC TGG CTC AG) and rd1 (5'-TAA GGA GGT GAT CCA GCC) (Weisburg et al., 1991). The PCR mixture (50 µL) contained 1X Taq buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3), 0.2 mM dNTP (New England Biolabs, USA), 0.4 µM each primer, 1.5 U of Taq DNA polymerase (New England Biolabs, USA) and 2.5 µL of DNA. The amplification was performed with first a denaturation step (94 °C, 10 min) followed by 35 cycles of [denaturation (94 °C, 1 min) annealing (56 °C, 1 min 15 sec) extension (72 °C, 1 min 15 sec)] and a final extension step (72 °C, 7 min).

The primers 702-F (5'-AAT TGC CTT CTT CCG TGT A) and 310-R (5'-AGT TGC GCA CAA TTA TTT TC) were used to amplify a 420 bp fragment of the *Lactobacillus sakei katA* gene as previously described (Ammor et al., 2005). *L. sakei* is a lactic acid bacterium which is usually not present on poultry meat (Najjari et al., 2008). Therefore this species easily identified by PCR targeting its *katA* gene was used as a control of DNA extraction and subsequent PCR efficiency.

Challenge tests

Samples of fresh breast chicken meat from the local supermarket were rinsed with ethanol 100% or sodium lactate sodium lactate 2% in sterile water (Loretz et al., 2010). After briefly drying on sterile filter paper, breasts were aseptically cut in 2 cm dices. One aliquot (1 mL) of the bacterial communities isolated from chicken legs and stored at -80 °C was gently defrosted, diluted in TS to obtain appropriate

cell concentration. A volume of 1 mL of appropriate dilution was inoculated per 100 g of meat dices, and the mixture was homogenized. For each challenge test, three replicates were performed. After homogenization 50 g portions were packaged under two different modified atmospheres routinely used by meat producers (50% CO_2 - 50% N_2 and 30% CO_2 - 70% O_2) and stored at 4 °C.

CFU were determined after plating serial 10-fold dilutions on various media. The total aerobic viable counts were determined after 2 days incubation at 30 °C on Plate Count Agar (PCA) (Biokar, France). Lactic acid bacteria (LAB) were counted on MRS agar medium pH 5.2 (AES, France) after 4 days incubation at 25 °C under anaerobic conditions (Anaerocult A, Merck, Germany). Numeration of *Pseudomonas* sp. and *Brochothrix thermosphacta* were determined at 25 °C on specific media: Cephalosporine Fucidine Cetrimide CFC (Biokar, France) for 2 days, and Streptomycin Sulfate Thallium Acetate Actidione agar STAA (Oxoid, France) for 3 days, respectively. Enterobacteria counts were determined on Violet Red Bile Glucose agar VRBG (Biokar, France) after 1 day incubation at 37 °C.

Statistical analyses

Results obtained from bacterial enumeration after rinsing were analyzed using Student's T-test. P values <0.05 were considered statistically significant. For comparing bacterial viability after storage at -80 °C, analysis of variance (ANOVA) and pair-comparison of treatment means were achieved by the Fisher least significant difference (LSD) test (95.0%) with the XLstat version 2014.3.07 extension, with mean uncertainty of 0.5 log CFU/g. Principal component analyses of the 23 chicken leg samples and PCR amplification from their DNA were performed with "ade4" and "ape" packages in R version 3.0.2 © 2013 The R Foundation for Statistical Computing.

Results and discussion

To collect bacterial communities naturally contaminating poultry meat, both chicken breast fillets and chicken legs were tested. However, due to a very poor initial bacterial contamination on chicken breast (data not shown), we rapidly chose to extract bacteria only from chicken legs, where bacterial contamination were higher, due to the presence of the animal skin on the product. We determined the optimal conditions to collect bacterial communities that we could store as aliquots to be reproducibly reused for inoculating meat matrices, and that could be characterized using both cultural and molecular methods. For that purpose, we determined i) the best method to separate bacteria from meat; ii) the best conditions to extract DNA from the stored communities for their analysis by molecular methods needing PCR amplification; and iii) then the best moment to collect bacteria in sufficient amount during the product shelf life. Life stocks were then constituted following these optimized methods. Their viability after cold storage and their ability to regrow on meat matrices were checked.

Bacteria separation method and DNA extraction

We tested different methods previously used to collect bacteria from meat (Capita et al., 2004, Gill & Badoni, 2005) and associated them with different DNA extraction protocols in order to set up the best method allowing to separate the most possible viable bacteria from the matrix and extract their DNA, whilst avoiding PCR inhibitors.

Stomaching is a method broadly used because it usually permits to collect bacteria with a high efficiency. However, as chicken legs are heterogeneous matrices, one difficulty is to pick exactly the same proportions of skin, fat and muscle. In addition a prior deboning of meat is required for stomaching. A subsequent step (filtration, centrifugation or decantation) is often necessary to clear bacteria from matrix residues especially when a further DNA extraction step is required. Other methods such as swabbing or rinsing methods can also be used as they are described in standard protocols for the detection of pathogens. These three methods, as well as scraping test allowed recovery of bacteria (Table 9). Then we tested various DNA extraction procedures following examples reported in the literature (Pinto et al., 2007, Pirondini et al., 2010). To check the efficiency of DNA recovery and the possible presence of PCR inhibitors, DNA samples extracted with various methods were used to amplify the 16S rRNA gene. After stomaching, DNA could not be PCR amplified whatever the method to separate bacteria from meat matrix was, and whatever the DNA extraction procedure used (Table 9, Figure 18).

After scraping, only the use of Mobio kit led to a positive PCR amplification. As well, after swabbing, only the Mobio kit utilization led to a positive PCR reaction, and an additional ultrasonic bath for DNA extraction had a negative effect on the PCR amplification. Finally a positive PCR amplification was obtained after rinsing, with both Mobio and Promega DNA extraction kits and an ultrasonic treatment did not appear to have an impact on the PCR efficiency. For subsequent steps, the rinsing method associated to the use of Mobio DNA extraction kit was chosen. In addition, we considered rinsing to be a more accurate and reproducible method to collect bacteria independently from the heterogeneity of poultry cuts. The reasons why other methods, although enabling bacteria recovery, did not lead to PCR amplification may results from the presence of PCR inhibitors issued from meat, as reported before and particularly for chicken meat (Rossen et al., 1992, Abu Al-Soud & Rådström, 2000, Lübeck et al., 2003). Different sources of contamination by a likely PCR inhibitor like glove powder, plastic tubes and matrices are known (Rossen et al., 1992, Wilson, 1997, Abu Al-Soud & Rådström, 2000). But it seems that the strongest inhibitor contaminations occur in some food matrices as reported in poultry meat in which the presence of PCR inhibitors and of DNases preventing *Salmonella* DNA extraction (Park et al., 2014). Meat and fat residues could also lead to DNA degradation or a protection of bacteria

during the lysis step of the DNA extraction. Further experiments would be required to fully understand why such broadly used methods as stomaching are efficient on most food matrices but not on chicken cuts.

Table 9 Comparison of recovery of bacteria and estimation of quality of DNA extraction using different protocols

Bacterial Isolation	Additional step for bacterial isolation	Culture numbered	DNA extraction kits	Additional step for DNA extraction	16S rRNA gene amplification
Stomacher	Nucleospin	5.7 10^4 [a]	Qiagen	none	-
			Qiagen	purification[b]	-
			Qiagen	heated [c]	-
			Promega	ultrasonic bath [d]	-
			Mobio	none	-
			Mobio	ultrasonic bath [d]	-
	Paper filtration + Nucleospin	7.2 10^4 [a]	Qiagen	none	-
	Differential centrifugations	1.0 10^5 [a]	Qiagen	none	-
Rinsing	Ø	5.9 10^7 [e]	Promega	ultrasonic bath [d]	+
			Mobio	none	+
			Mobio	ultrasonic bath [d]	+
Swabbing	Ø	3.2 10^6 [e]	Promega	ultrasonic bath [d]	-
			Mobio	none	+
			Mobio	ultrasonic bath [d]	-
Scraping	Ø	9.4 10^6 [e]	Promega	none	-
			Promega	ultrasonic bath [d]	-
			Mobio	none	+
			Mobio	ultrasonic bath [d]	+

[a] CFU/g; [b] phenol-chloroform purification (see 2.6.2); [c] heating step for 10 min at 56 °C; [d] ultrasonic bath for 3 min at 50 °C;
+/- positive or negative amplification
Chicken legs were stored at 4 °C until UBD before to apply the different extraction methods

Method optimization and validation

To tentatively improve the efficiency of bacteria recovery we tested whether several successive rinses could improve the yield of recovery. Six successive rinses of 5 min or 30 sec were performed and total viable counts in each successive rinsing solution were measured. Rinsing for 5 min was slightly more

efficient than for 30 sec (Figure 14). In both cases the first rinsing step was the most efficient as more than 90% of bacteria were collected after the first rinse and each of the subsequent rinses allowed only a negligible additional recovery. Therefore we chose to perform only one rinse but for a 5 min period. Indeed, although a 30-second-rinse is usually recommended in protocols for microbial assessment of food products, a very short time for a handed experiment and its reproducibility may be experimenter dependent.

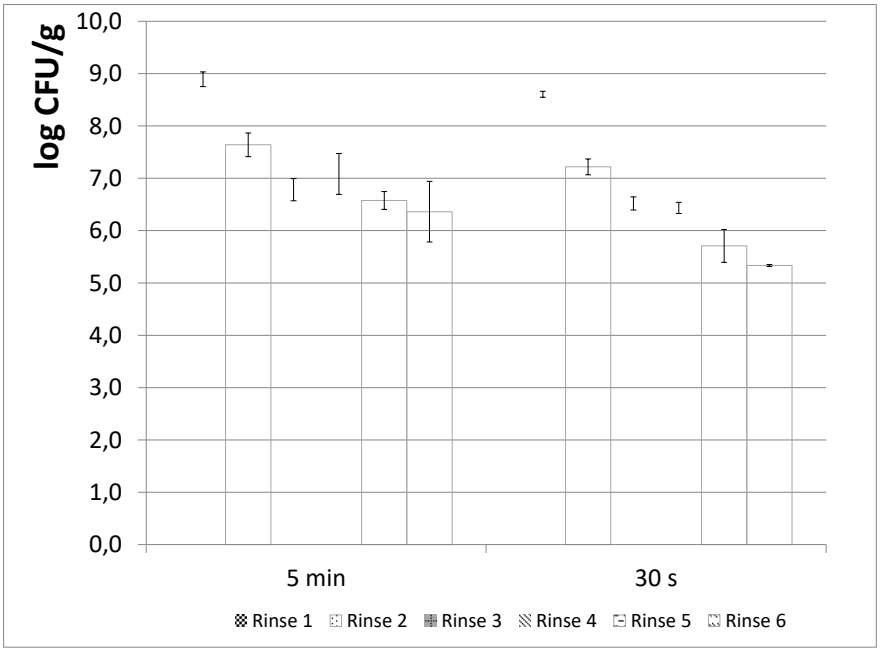

Figure 14 Efficiency of successive rinsing steps on the recovery of bacteria

Results are the mean of data obtained for 3 different chicken legs. Results are expressed as CFU/mL of rinsing solution. Asterisks show the values that are not statistically different ($P < 0.05$)

After validation of the method to collect bacteria, we checked whether it was optimal for efficient recovery of bacteria from meat and for DNA extraction and subsequent PCR amplification. For that purpose, a batch of chicken legs was inoculated with an overnight MRS culture of *L. sakei* (10^8 CFU/mL) used as a marker. The rinsing protocol and DNA extraction described above were immediately performed on this artificially contaminated meat. To check the putative presence of PCR inhibitors in the DNA extracted from bacteria collected from chicken meat, the pure culture of *L. sakei* was also added in the rinsing solution obtained from a naturally contaminated chicken leg, at 10^5, 10^6, 10^7, and 10^8 CFU/mL of rinsing solution. Then DNA was extracted and a PCR targeting the *katA* gene specific of *L. sakei* was performed. As expected, *L. sakei* was not detected in the bacteria collected from poultry meat. A clear *L. sakei* band was observed with samples issued from the chicken meat inoculated with *L. sakei,* showing that our protocol allowed indeed to collect bacteria, and to extract their DNA with a

quality that was good enough for a PCR reaction. When *L. sakei* was added in the rinsing solution before DNA extraction, the *katA* PCR amplification was also positive showing this procedure is efficient for bacteria that can be recovered in quantities ranging from 10^5 to 10^8 CFU/mL and that no strong PCR inhibitors issued from poultry meat are present. Therefore, for subsequent experiments, we chose to keep this method that appeared as the most efficient.

Determination of the optimal collection time

In order to collect the microbial communities present on poultry cuts for subsequent use to re inoculate meat matrices, a sufficient level of bacteria was required. As well, a significant bacterial diversity of the collected microbiota was necessary. Previous studies showed meat microbiota diversity decreases during storage or with spoilage occurrence (Rossen et al., 1992, Wilson, 1997, Abu Al-Soud & Rådström, 2000, De Filippis et al., 2013, Chaillou et al., 2015). Last, we estimated that microbiota stocks at a concentration of 10^7 CFU/mL would be optimal to perform challenge tests, and that 20 to 100 aliquots of 1 mL would be required for obtaining enough repeats. We needed then to collect ~10^9 CFU per bacterial community. Therefore we monitored the total viable counts on chicken legs from T_0 (time of arrival in the supermarket *i.e.* 0 – 2 days after slaughtering) to UBD (10 days after the date of arrival in the supermarket). Cuts were incubated without any protective modified atmosphere at 4 °C and 8 °C (Figure 15), and the rinsing method described above was used for bacterial determination of meat contamination.

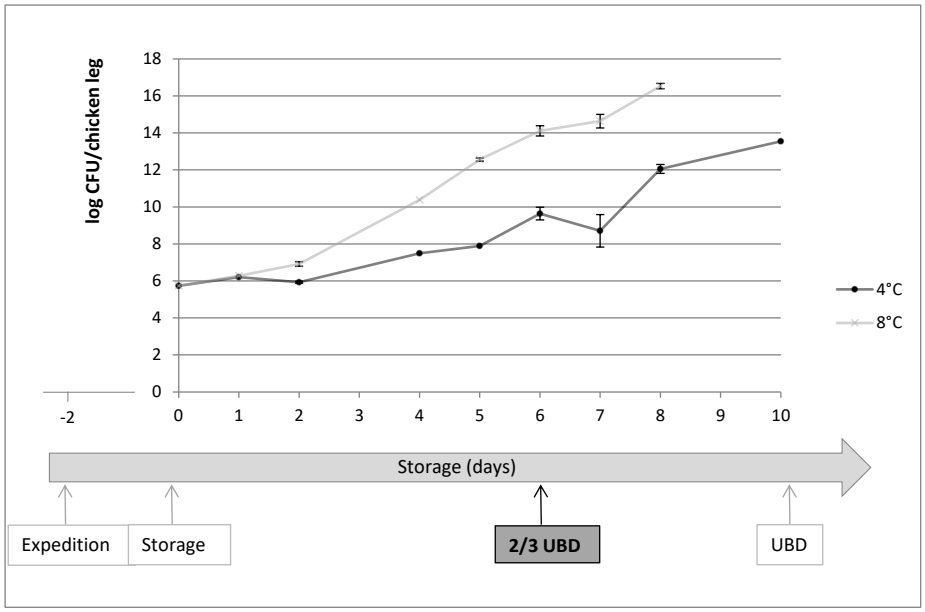

Figure 15 Total viable counts recovered per chicken leg.

At the beginning of storage, total counts were too low to collect a sufficient bacterial stock. After 8-10 days the total bacterial population collected in the rinsing solution (*i.e.* per chicken leg with an average

weight of 278.5 ± 89 g) reached ~13 log CFU after storage at 4 °C and more than 16 log CFU when cuts were stored at 8 °C. At this time, the quantity is sufficient to prepare a bacterial stock. However, at the end of the storage period we had a risk to collect microbiota with low diversity and enriched in spoilage bacteria (De Filippis et al., 2013, Chaillou et al., 2015). To have a stock with enough bacteria and still representing the diversity occurring on poultry cuts, we decided to collect bacteria from single chicken legs stored at 4 °C for 6 to 11 days, depending on the shelf-life of each batch (see table 1), a period corresponding to 2/3 of their UBD.

Constitution of a viable poultry meat microbiota collection

The results of bacterial communities recovered for 9 samples (A to I) are shown in Figure 16. Results obtained for all 23 samples are shown in supplementary Figure 17.

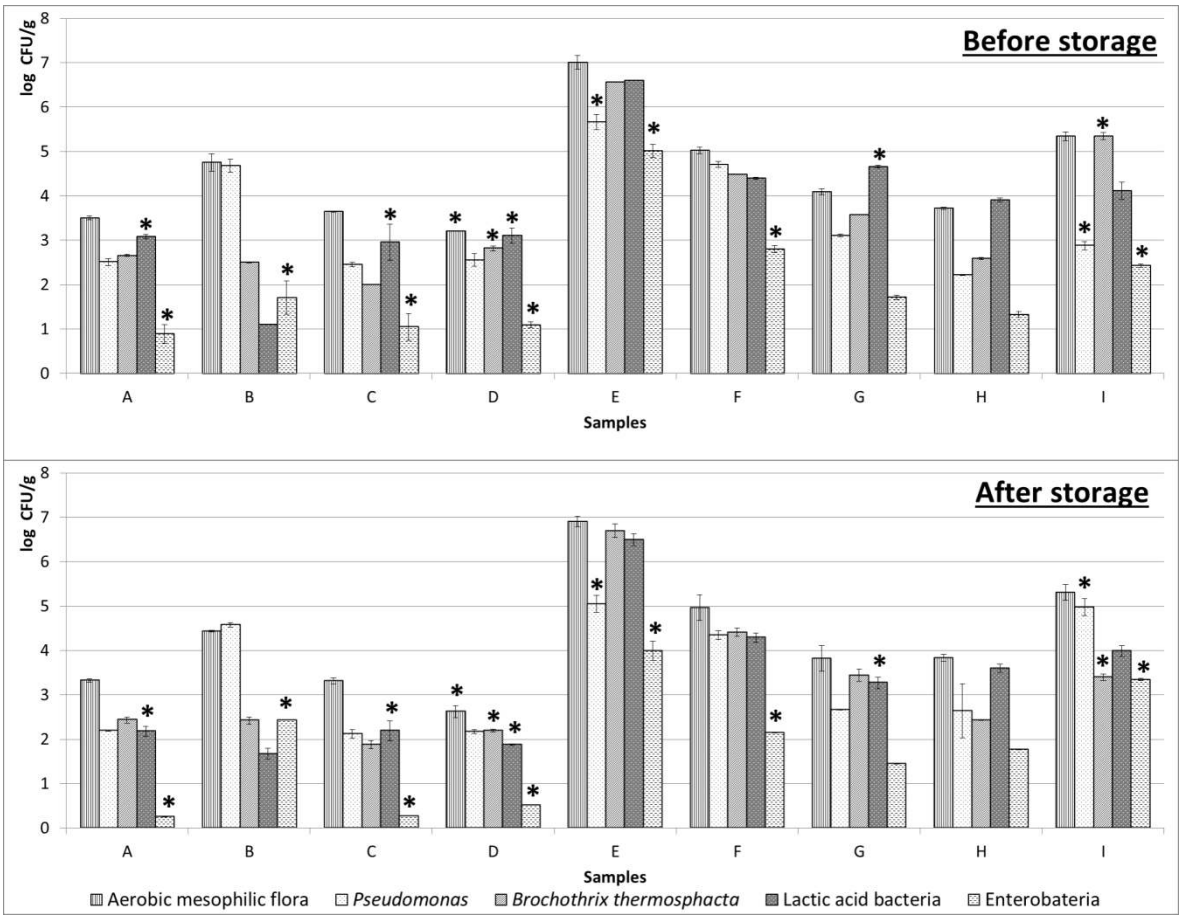

Figure 16 Composition and viability of bacterial communities from 9 samples of chicken legs before and after frozen storage at -80 °C, determined by enumeration on various specific media.

Asterisks show when the values before and after freezing are statistically different.

An important variability in both total viable counts and diversity of bacterial population is observed between samples. Total viable counts vary from ~5 log CFU/g between the less contaminated samples

(10^3 CFU/g in samples A or D) to the most contaminated ones (10^8 CFU/g for samples J or N). Bacterial diversity also differed between samples: *Pseudomonas* sp. and *B. thermosphacta* ratio differed between chicken legs. In sample B *Pseudomonas* sp. were dominant by about 2 log units when compared to the *B. thermosphacta* population whereas in samples N and Q the opposite situation was observed. This might be the consequence of the gas composition used for packaging as sample B was stored under an O_2 rich packaging whereas packaging atmosphere of samples N and Q was poor in O_2. Indeed several gas ratios were measured in the pack head-space (Table 8). One gas mixture contained high O_2 concentration apparently completed with CO_2 (samples A, B, C as example), one poor in O_2 and completed with CO_2 (and probably N_2) (like samples G, H, M) and possibly a third one with another CO_2 - N_2 - O_2 balance (like samples E, O, U). These observations may explain the different microbiotas observed.

After storage at -80 °C, bacterial population remained cultivable and the richness in aliquots was not particularly affected (Figure 16 and Figure 17). However, a significant decrease or increase of *Enterobacteriaceae* counts was observed in most samples (Figure 17), which can result from a fluctuation in counting analysis from VRBG plates. Lactic acid bacteria and *B. thermosphacta* viability was decreased in some samples (Figure 17), without modifying drastically the balance of the bacterial communities of our life stocks.

For the 23 stored bacterial stocks, we also tested our DNA extraction procedure. DNA concentration was measured and PCR amplifications were performed on 16S rRNA gene. Despite the optimization of the method and several repeats, only 10 amplifications were successful (Table 10, Figure 18). All PCR-positive reactions were obtained with DNA extracted from high cell concentration bacterial samples (>10^5 CFU/g). However, several samples issued from bacterial communities with such high bacterial concentration did not lead to a positive PCR reaction. It seems clear that is not a problem of DNA extraction or stability because samples leading to similar DNA concentrations could be either positive or negative (samples Q and R, Table 10).

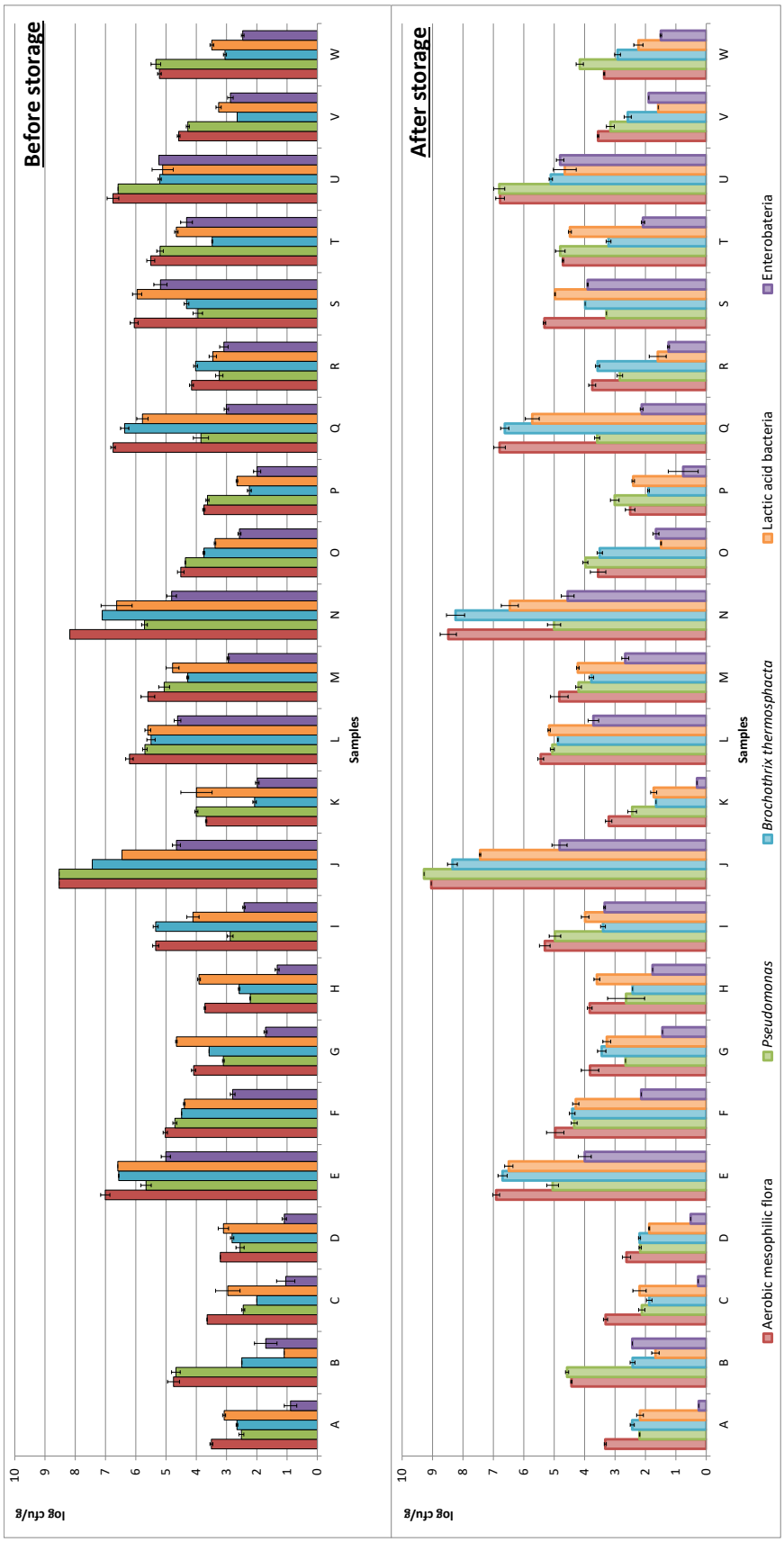

Figure 17 Supplementary Figure. Composition and viability of bacterial communities from 23 samples of chicken legs before and after frozen storage at -80 °C, determined by enumeration on various specific media.

Table 10 Concentration of DNA extracted from the bacterial stocks of the 23 samples and subsequent PCR efficiency

Samples	Bacterial counts (Log CFU/mL)	DNA concentration (µg/ml)
A	3.8±0.03	10,4
B	4.9±0.02	14,3
C	3.8±0.07	8,9
D	3.1±0.13	11,1
E*	7.5±0.11	19,6
F*	5.6±0.29	8,1
G*	4.3±0.29	8,3
H	3.9±0.08	30,8
I*	5.7±0.18	18,4
J*	9.4±0.00	95,6
K	3.6±0.10	7,2
L	5.9±0.10	10,2
M*	5.6±0.29	14,1
N*	8.8±0.27	12,1
O	4.1±0.25	26,0
P	3.0±0.16	4,8
Q*	7.3±0.19	22,8
R	4.2±0.11	23,8
S	5.7±0.04	7,9
T*	5.6±0.02	10,2
U*	7.3±0.14	29,0
V	3.9±0.02	10,1
W	3.7±0.02	27,2

*positive amplification

We also could not correlate the PCR efficiency with the nature of bacterial population, the weight of the meat sample used, the nature of the gas packaging (see Figure 18). Such differences in PCR amplification could be explained by the presence of PCR inhibitors and/ or of DNases in some samples but not in some others.

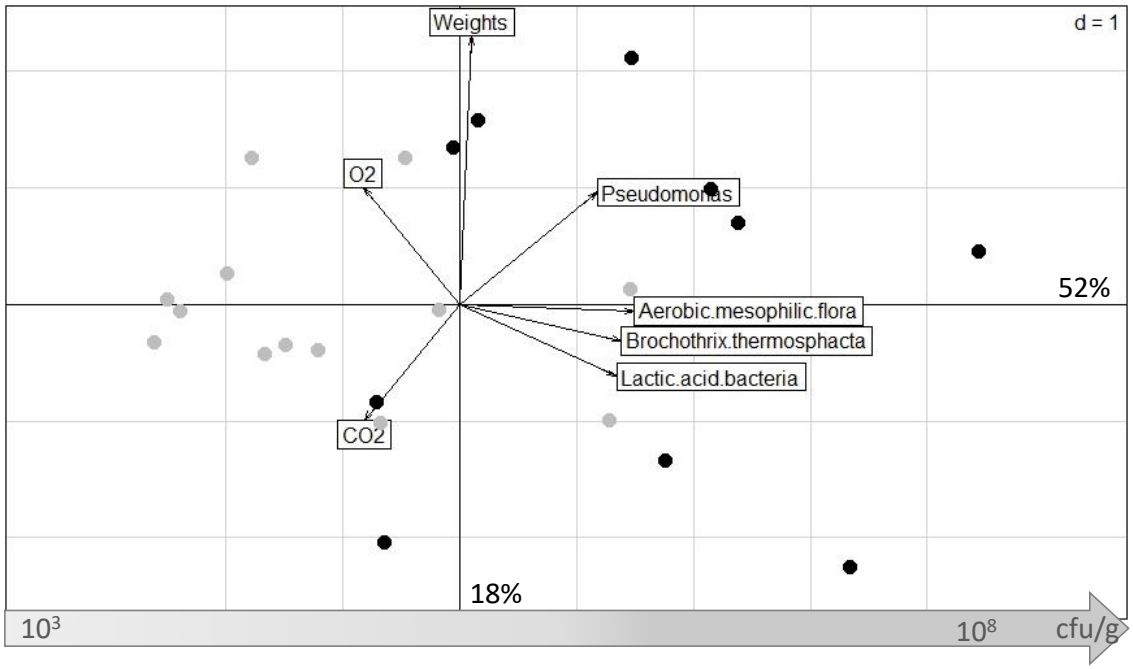

Figure 18 Principal component analysis of the 23 chicken leg microbiotas and PCR amplification from their DNA.

O2 and CO2 concentrations in the pack head space, weight of chicken legs, lactic acid bacteria, *B. thermosphacta*, *Pseudomonas* sp. and total bacterial counts are indicated. Black circles indicate samples for which PCR amplification was successful and grey ones when PCR was negative.

Challenge tests with bacterial communities

To ensure that microbiota stocks were able to recolonize a meat matrix, several challenge tests were performed. Because in our first attempts to extract bacteria from the meat matrix, we found chicken breasts were initially less contaminated than chicken legs, we choose to perform challenge tests on chicken breast. We tested two different decontamination protocols for their effect on indigenous microbiota of fresh chicken breasts from the local supermarket. We observed that ethanol or lactic acid rinsing were equivalent as both decreased indigenous microbiota of about 1 log CFU/g.

In first trials, microbiotas E, L, S, and U showing various bacterial diversities (Figure 17) were chosen for inoculating ethanol or lactate treated chicken breasts at 10^3 CFU/g. Challenge tests were performed in duplicates (microbiotas E and U) or triplicates (microbiotas L and S) and a non-inoculated control was included. Inoculation level was in the same range as indigenous microbiota (~10^3 CFU/g). Although it was clear that frozen microbiota stocks were able to contaminate meat by direct inoculation, and to multiply during meat storage the reproducibility of such challenge tests was not satisfactory (data not shown). The level of indigenous microbiota of meat was probably too high by

comparison to the inoculation level. Indeed, the importance of the level of the natural contamination on the growth of protective cultures has been previously shown in beef meat (Chaillou et al., 2014).

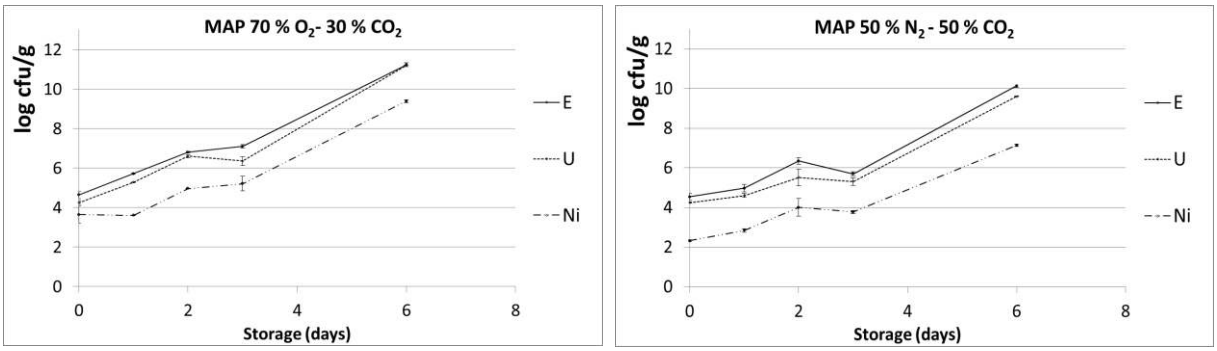

Figure 19 Challenge-tests of microbiotas E and U inoculated on chicken breast dices and incubated under two different modified atmosphere packaging.

Total aerobic mesophilic bacteria were enumerated at T_0, and then at day 1, 2, 3, and 6. A non-inoculated control (Ni) was also performed. O_2/CO_2 ratios in modified atmosphere packaging (MAP) are indicated.

For further experiments, microbiotas were inoculated at 10^5 CFU/g. Microbiotas E and U were chosen because of their different abundance of *B. thermosphacta*. Meat was then stored at 4 °C under CO_2/N_2 or CO_2/O_2 atmospheres and bacteria were enumerated on contaminated meat and on non-inoculated control at T_0 and during storage. Dynamics of total aerobic mesophilic counts is presented Figure 19. From inoculation time till day 6 both inoculated microbiotas dominated the indigenous contaminants, whatever the storage atmosphere used. Figure 20 shows *B. thermosphacta* and *Pseudomonas* sp. counts. At T_0 *B. thermosphacta* level was ~4 10^4 CFU/g and ~2 10^3 CFU/g in meat samples inoculated with microbiotas E and U, respectively. Despite this initial difference, under O_2 rich atmosphere, the final *B. thermosphacta* population reached similar levels (2.2 10^{11} CFU/g and 1.3 10^{11} CFU/g) at day 6. Conversely, at the end of storage under CO_2/N_2 atmosphere, *B. thermosphacta* counts remained about 1 log higher with microbiota E (5.7 10^7 CFU/g) than with microbiota U (6.6 10^6 CFU/g).

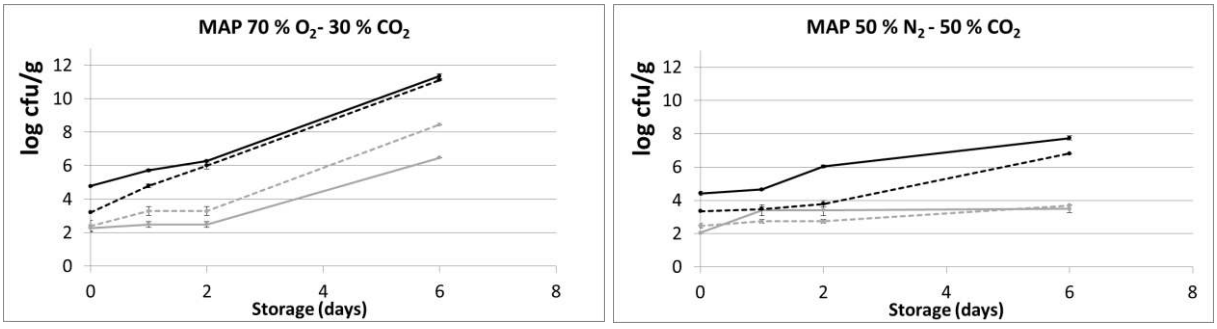

Figure 20 Kinetics of *B. thermosphacta* and *Pseudomonas* sp. reimplantation monitored on specific media after inoculation of microbiota E or U.

Pseudomonas sp. (grey lines) and *B. thermosphacta* (black lines) were enumerated at T_0, and then at day 1, 2, and 6. O_2/CO_2 ratios in modified atmosphere packaging (MAP) are indicated.

Pseudomonas sp. behavior was different: with an initial inoculation level of ~2 10^2 CFU/g with both microbiotas, in the presence of O_2 *Pseudomonas* sp. final population was 1 log higher with microbiota U than after inoculation of microbiota E (Figure 20). As expected, in the absence of O_2, *Pseudomonas* sp. did not grow. However a stable population level was observed during storage, suggesting that those bacteria could survive. Finally; the comparison of *B. thermosphacta*, *Pseudomonas* sp. and total aerobic mesophilic counts at the end of storage confirmed that packaging atmosphere has an important impact on bacterial development. The use of a ratio 50% CO_2 - 50% N_2 showed a better inhibiting activity than 30% CO_2 - 70% O_2. This cannot rely only on CO_2 as the two atmospheres we used contained high levels of this gas.

Conclusion

A high variability between poultry meat samples had been shown in this study. The contamination level as well as the nature of bacterial species contaminating chicken cuts can be drastically different depending on the batches. To ensure poultry meat microbial safety, microbial ecology studies are necessary, which are complicated by the above mentioned high variability. We propose a method enabling the collection of viable bacterial stocks that can be stored as aliquots for performing reproducible and standard challenge tests. This method, based on a rinsing step of meat, followed by bacteria concentration and freezing allowed collecting 23 different viable microbiotas. Four of those were chosen to conduct challenge tests and have successfully recolonized meat without a prior culture step, which could potentially lead to a bias in microbial diversity evaluation. We also developed a protocol for extracting bacterial DNA out of these microbiotas, for subsequent PCR amplification. Although DNA extraction was successful, PCR amplification efficiency needed a minimal amount of bacteria (>10^5 CFU/g) and the presence of PCR inhibitors was suspected in about half of the samples. Nevertheless, the use of such a method should help for the detailed characterization of meat microbiota and the study of its dynamics during different meat treatments or storage conditions dedicated to improve microbial safety, such as the use of various atmosphere packaging or decontamination treatments (Doulgeraki et al., 2012). In particular, we think that our method will be useful to study the response to storage conditions, by species occurrence and co-occurrence in order to better understand the microbial role in meat spoilage and to plan consequent improvement of meat storage systems. In fine, the results may lead to describe relevant markers (bacterial species, genes…) for the development of simple, fast, accurate and low-cost methods to be used by the agro-food industry for a better control of poultry meat safety.

Acknowledgements

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2.3- Ce qu'il faut retenir du chapitre 2

Dans le but de reconstituer un écosystème microbien de viande de volaille, nous avons tout d'abord récolté des bactéries provenant de cuisses de poulet. En effet, la peau présente sur les cuisses de poulet est fortement contaminée, ce qui nous a permis de récolter suffisamment de bactéries entre 2/3 de la DLC et la DLC du produit. Nous avons collectés les bactéries provenant de 23 lots de cuisses de poulet de marques, d'origines et d'appellations différentes. Les bactéries ont été stockées en présence de glycérol à -80°C et leur viabilité a été testé. Les bactéries sont capables de survire à la congélation et nous retrouvons les proportions globalement similaires avant et après congélation. Nous avons constaté que les espèces que nous avions recherchées par méthodes culturales sont retrouvées dans des proportions variables suivant les lots. En flore totale, cela représente de 3 à 8 log UFC/g. Nous avons constaté également les variations de la composition de l'atmosphère protectrice suivant les lots. Dans le but de réaliser différentes études par biologie moléculaire, nous avons extrait l'ADN après optimisation du protocole. L'extraction d'ADN et l'amplification PCR a été possible pour 10 des 23 lots.

Enfin les communautés bactériennes ont été ré-inoculées sur de la viande pauci microbienne et nous avons montré que sans étape de culture préalable les bactéries étaient capables de se ré implanter et de se développer sur la viande au cours de la conservation sous amphotère protectrice.

Nous avons donc mis au point une méthode permettant de collecter et de conserver des microbiotes standards de viande de poulet. Cependant bien que les méthodes culturales nous aient permis d'évaluer le niveau global de contamination et le suivi de quelques flores d'intérêt, la composition de ces communautés microbiennes isolées dans cette étude est peu exhaustive. Nous avons donc cherché, dans la suite de ces travaux de thèse, à décrire par méthode de biologie moléculaire, les communautés bactériennes à partir de l'ADN bactérien isolé des 10 lots de cuisses de poulet.

Chapitre 3 Description de la diversité bactérienne

3.1- Préambule

Dix des 23 communautés récoltées (chapitre 2) ont été analysées de manière plus approfondie par pyroséquençage de la région V1-V3 de l'ADNr 16S. Différents pipelines d'analyse des données ont été testés et les données ont été comparées aux résultats obtenus par microbiologie culturale classique et par qPCR.

Cette étude a fait l'objet d'un manuscrit soumis en janvier 2017 dans la revue *Food Microbiology* (Reference: FM_2017_102).

3.2- Diversity of bacterial communities in French chicken cuts stored under modified atmosphere packaging.

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Abstract

Poultry meat, the second most consumed meat in France, is commercialized mainly as portions of chicken cuts with various quality labels, stored under various modified atmosphere packaging (MAP), with shelf-life ranging from 9 to 17 days. We used 16S rDNA pyrosequencing to describe microbiota of chicken legs. Ten samples representing a wide diversity of labels and MAP available on the market were collected from local supermarkets and stored at 4°C. Microbiota were collected, total DNA was extracted, and V1-V3 fragment of 16S rRNA genes were amplified and sequenced. For data analysis

several pipelines were compared. The Qiime pipeline was chosen to cluster reads and we used a database previously developed for a meat and fish microbial ecology study. Variability between samples was observed and a listing of bacteria present on chicken meat was established. The structure of the bacterial communities were compared with traditional cultural methods and validated with quantitative real time PCR. *Brochothrix thermosphacta*, *Pseudomonas* sp., and *Carnobacterium* sp. were dominant and the nature of the gas used for packaging influenced the relative abundance of each suggesting a MAP gas composition dependent competition between these species. We also noticed that slaughterhouse environment may influence the nature of the contaminants.

Highlights

- Microbiota of chicken cuts is variable
- Pyrosequencing approaches have to be combined to other methods to validate results
- Slaughterhouse environment may influence the nature of the meat contaminants
- Nature of the gas shapes the relative abundance of bacteria.

Keys word: Pyrosequencing; chicken meat; spoilage; modified atmosphere packaging; microbiota.

Introduction

Richness and abundance of microbiota present in food products, and especially meats, play an important role in the shelf life of the products, their microbial safety, and therefore the consumer health. Unlike fermented food, where unwanted bacteria are controlled by the addition of bacterial starters that become dominant during the process, fresh meat contamination is more diversified. Sources of contamination are the animal and the environment microbiota, and depend on the farming and slaughtering process (Chaillou et al., 2015). Poultry meat can host very diverse microbial communities varying with seasonal changes (Cohen et al., 2007) among which spoilage bacteria (Doulgeraki et al., 2012) or pathogens such as *Campylobacter* (Gruntar et al., 2015) and Salmonella (Rasschaert et al., 2008) which must be controlled to ensure safety of the products (Álvarez-Astorga et al., 2002).

The use-by-date (UBD) of fresh poultry meat is determined as the time period during shelf life for bacterial contamination to reach around 7 log CFU.g^{-1} (Okolocha & Ellerbroek, 2005). It usually varies from 4 to 15 days depending notably on the type of gas used for packaging, *i.e.* air or modified atmosphere packaging (MAP). In France, the chicken cuts most commonly sold in supermarkets are

packed under various MAP, either enriched or devoid of O_2 and the shelf-life can reach 17 days (Rouger et al., 2017). In addition a large panel of quality labels (standard, organic, halal, free range) is available and various breeding or farming practices exist, that may influence the bacterial loads present on meat.

Most of the information dealing with fresh meat product bacterial contamination is issued from cultural methods (for a review see (Doulgeraki et al., 2012). These cultural methods use selective media for bacteria detection and quantification such as total viable counts, lactic acid bacteria, Enterobacteria, *Pseudomonas* sp., *Brochothrix* (Mead, 2004). In a previous study, we used such plating methods to determine the contamination level of chicken legs and a large variation of total aerobic counts between samples (from 3 to 8 log $CFUg^{-1}$) was observed (Rouger et al., 2017). We also noticed that the ratio between lactic acid bacteria, *Pseudomonas*, Enterobacteria, and *Brochothrix thermosphacta* loads differed within samples. However, we did not observe any correlation between these variations and meat quality labels or MAP gas composition. Nevertheless a competition between bacterial contaminants exists during poultry meat storage (Alonso-Hernando et al., 2012a) and storage conditions may influence food microbiota (Chaillou et al., 2015). With the development of high-throughput sequencing methods, the description of complex microbial communities of many environments has been revisited. Next generation sequencing (NGS) technologies are nowadays commonly used, in particular to investigate animal and environmental microbiota and In addition software and analysis pipelines are easily and freely available (Ercolini, 2013, Mayo et al., 2014). More recently, these have been also applied to food but mainly to fermented products which microbial diversity is less complex than that of fresh products.

Nevertheless few studies using sequencing approach have been reported on non-fermented meat products, most of them dedicated to beef or pork meat (Ercolini et al., 2006, Benson et al., 2014, Chaillou et al., 2015, Hultman et al., 2015). To our knowledge, only two studies using NGS focused on poultry meat, a comparison of microbiota present in marinated *vs* non marinated Finnish chicken breast (Nieminen et al., 2012) and the analysis of the contamination along the production chain in USA, from broiler chicken production to carcasses, which are rinsed in a chlorinated solution (Oakley et al., 2013).

In the present study, we describe the diversity of the microbiota of chicken legs from 10 different samples collected from French supermarkets and stored under various MAP, by a 16S rRNA gene pyrosequencing approach.

Materials and methods

16S rRNA gene pyrosequencing

DNA extraction from meat microbiota

In a previous study we collected bacterial communities from 23 chicken leg samples and stored them at -80°C with glycerol 15%, and bacterial DNA was extracted from 10 out of these communities (Rouger et al., 2017). Briefly, after thawing tubes, bacteria were collected by centrifugation at 10 000*xg* for 10 min at 4°C. DNA was extracted with Mobio Power Food Microbial DNA isolation kit with a prior step of incubation in an ultrasonic bath (see (Rouger et al., 2017).

Pyrosequencing PCR conditions

The V1-V3 region of the 16S rRNA gene (567 bp) was amplified by PCR with 27F (CGTATCGCCTCCCTCGCGCCATCAGxAGAGTTTGATCCTGGCTCAG and 534R (CTATGCGCCTTGCCAGCCCGCTCAGxATTACCGCGGCTGCTGG) with x representing the barcodes specific for each of the 10 samples (see Table 11).

The 50 µL PCR mixture was composed of 2.5 U of high fidelity Pwo DNA polymerase (Roche Diagnostics, France), 1X Pwo buffer (100 mM Tris–HCl, 250 mM KCl, 50 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, pH 8.85), 0.2 mM dNTP (New England Biolabs, USA), 0.6 µM of each primers, and 2.5 µL of the DNA solution. All PCR amplifications were performed in a PTC-100 Thermocycler (MJ Research Inc., USA). The PCR protocol encompassed an initial denaturation step (94 °C for 2 min) followed by 30 or 35 cycles comprising a denaturation step (94 °C for 30 s), primer annealing steps using a temperature gradient (60 °C for 30 s, −0.5 °C per cycle), and an extension step (72 °C for 1 min). At the end a final extension at 72 °C for 7 min was performed. Two PCR amplifications were performed per sample, with either 30 or 35 cycles.

DNA quantification and quality control

PCR fragments were visualized on 1 % (w/v) agarose gels. PCR products were purified with the QIAquick kit (Qiagen SA, France) according to the manufacturer's procedure, then concentrated in a SpeedVac system (Thermofisher scientific, France) to obtain a final volume of 30 µL purified DNA. DNA concentration was measured with a Qubit fluorimeter (Invitrogen, CA, USA), quality and quantity parameters were checked on Experion DNA 12K chips (Biorad, France) prior sequencing.

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Table 12 Primers used in this study

	Primer sequence (5'→3')	Primer name	Barcodes[a]			Fragment size (bp)	Target	Reference
			Samples	Forward	Reverse			
All bacteria	CGTATCGCCTCCCTCGCGCCATCAGxAGAGTTTG ATCCTGGCTCAG[a]	27F-MID08/534R-MID14	E	CTCGCGTGTC	CGAGAGATAC	567	16S rRNA gene V1-V3 region	Chaillou et al., 2015
		27F-MID10/534R-MID13	F	TCTCTATGCG	CATAGTAGTG			
		27F-MID43/534R-MID02	G	TCGCACTAGT	ACGCTCGACA			
		27F_MID19/534R_MID21	I	TGTACTACTC	CGTAGACTAG			
	CTATGCGCCTTGCCAGCCCGCTCAGxATTACCGC GGCTGCTGG[a]	27F_MID23/534R_MID25	J	TACTCTCGTG	TCGTCGCTCG			
		27F_MID27/534R_MID29	M	ACGCGAGTAT	ACTGTACAGT			
		27F_MID31/534R_MID33	N	AGCGTCGTCT	ATAGAGTACT			
		27F_MID35/534R_MID37	Q	CAGTAGACGT	TACACACACT			
		27F_MID39/534R_MID41	T	TACAGATCGT	TAGTGTAGAT			
		27F_MID15/534R_MID17	U	ATACGACGTA	CGTCTAGTAC			
B. thermosphacta	GGACCAGAGGTTATCGAAACATTAACTG	QSF03-BTH-F				148	rpoC	Fougy et al, 2016
	TAATACCAGCAGCAGGAATTGCTT	QSF03-BTH-R						
C. divergens	CCGTCAGGGGATGAGCAGTTAC	CB1				340	16S rRNA gene	Scarpellini et al., 2002
	ACATTCGGAAACGGATGCTAAT	CB2R						
Pseudomonas spp.	ACTTTAAGTTGGGAGGAAGGG	Pse435F				251	16S rRNA gene	Bergmark et al., 2012
	ACACAGGAAATTCCACCACCC	Pse449R						
Shewanella spp.	CGCGATTGGATGAACCTAG	She211f				116	16S rRNA gene	Todorova and Costello, 2006
	GGCTTTGCAACCCTCTGTA	She1259						
Campylobacter spp.	ATCTAATGGCTTAACCATTAAAC	MD16S1				857	16S rRNA gene	Linton et al., 1997
	GGACGGTAACTAGTTTAGTATT	MD16S2						

[a]: x represent the barcodes specific for each of the 10 samples

Sequencing and data analysis

For each sample, the DNA amplified after 30 and 35 cycles were pooled and sequenced in single end by Eurofins MWG (Ebersberg, Germany) using 454 GS-FLX++ Titanium Technologies (454 Life Technologies, USA). Different strategies were compared for data analysis: the FROGS pipeline (Find Rapidly OTUs with Galaxy Solution) (Escudie et al., 2015) or the protocol designed in previous study (Chaillou et al., 2015) were tested. In addition the pipeline using Qiime software currently found in the literature for metabarcoding data sets (Caporaso et al., 2010). Those were combined to different databases. The main features of the strategies tested are summarized Table 12.

FROGS is a pipeline developed to run in a reasonable time in an user-friendly under Galaxy environment. The pipeline includes demultiplexing, and a pre process step to filter and delete sequences with unexpected lengths, with ambiguous bases (N) and which do not contain primer sequence at both 3'- and 5'-ends. The clusterization is performed with Swarm, a robust and fast clustering method for amplicon-based studies without global threshold and independent of sequence order (Mahe et al., 2014). After clustering, detection of chimeras is performed with a specific removal method of FROGS (Vsearch and cross-validation). After filtering multi-affiliation with 2 taxonomy affiliation procedures were performed. FROGS pipeline includes also statistics tools.

Table 13 Comparison of pipeline analysis for the different strategies tested in this study

	FROGS	Qiime (Caporaso et al., 2010)	EBP (Chaillou et al., 2015)
Pre process	Integrated in the pipeline (cutadapt / fastQC software)	Done manually (cutadapt / fastQC software)	Done manually (cutadapt / fastQC software)
Detection of primers	5' and 3'	5'	5'
Detection of chimeras	VQIIME after clustering	DECIPHER before clustering	DECIPHER before clustering
Clustering software	SWARM (Mahe et al., 2014)	Pick de novo included in Qiime software	gsAssembler or CD-Hit (Genomes assembly software)
Reference sequences for each OTUs	-	Most represented sequences	Consensus sequences
16S rDNA Database	Double affiliation default RDP (Cole et al., 2005) SILVA	Possible of double affiliation RDP (Cole et al., 2005) EBP_DB (Chaillou et al., 2015)	RDP EBP_DB (Chaillou et al., 2015)
Normalization / statistics	Integrated in the pipeline	Done manually	Done manually

The protocol design by Chaillou et al. (2015) uses different software, reads were demultiplexed according to barcode sequences with cutadapt and quality of the sequencing is checked using FastQC software (Babraham Bioinformatics). The reads are trimmed and filtered with quality score threshold of 20. Chimeric sequences are detected using Decipher web server (Wright et al., 2012) and are removed from the dataset prior any bioinformatic analysis (Haas et al., 2011). Software used for clustering, initially designed for genome assembly, is used here to cluster 16S rDNA sequences. The clustering is performed with Qiime software (Caporaso et al., 2010) using the longest reads as reference for each operational taxonomic unit (OTU) whereas in the strategy developed by Chaillou et al. (2015) a consensus sequence of each OUT is used as reference. The reference sequences of each OTU are blasted against the Ribosomal Database Project database (RDP II) (Cole et al., 2005) and the EBP/silva database designed by Chaillou et al. (2015) for taxonomic assignation. Relative abundances are estimated by counting the number of reads mapped on OTUs sequences. For both Qiime and EBP methods statistical analysis are performed manually.

Statistical analysis

The rarefaction curves were designed using command citation ("vegan") in R (Oksanen et al., 2016.) and Qiime was used to calculate diversity and richness indices (Caporaso et al., 2010). To establish OTU relative abundance, the numbers of reads were normalized to the median value of total reads as described by Chaillou et al. (2015). For each sample read counts were divided by a normalization factor corresponding to the number of reads in the sample divided by the median value of total reads obtained for the 10 samples.

Bacterial pure cultures for real time quantitative PCR (qPCR)

Strains were cultured on BHI (AES, France) plates with 1.5% agar (Biokar Diagnostics, France) for 36 h at adequate temperature (Table 3). A colony was resuspended into 10 mL of BHI broth and incubated overnight (see Table 13 for incubation conditions). Bacterial cultures were inoculated at 1% on fresh BHI broth and grown for 3-5 h to reach a bacterial suspension of 8 log CFU.mL^{-1}.

A series of 10-fold dilution was performed in BHI broth to obtain bacterial concentrations ranging from 3 to 8 log CFU.mL^{-1}. The exact bacterial concentration was determined after plating on BHI.

DNA extraction for qPCR

A volume of 1 mL of each dilution was centrifuged at 10 000*xg* for 10 min at 4°C. Bacteria pellets were resuspended in a Dulbecco's phosphate buffered saline solution without Ca and Mg (1X) (Eurobio,

France) and DNA was extracted with the High Pure PCR Template Preparation Kit (Roche, France) according to the manufacturer and eluted in 200 µL milliQ water.

Table 14 Bacterial strains used and culture conditions

Bacterial species	Strains	Temperature of incubation	Agitation in BHI broth
Brochothrix thermosphacta	DSM 20171	26°C	140 rpm
Carnobacterium divergens	V41	30°C	-
Pseudomonas fluorescens	CIP 6913.T	30°C	240 rpm
Shewanella putrefaciens	CIP 6929	26°C	140 rpm

Routine PCR procedure

The PCR mixture (50 µL) contained 1X Taq buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3), 0.2 mM dNTP (Euromedex, France), 0.4 µM of each primer, 1.5 U of Taq DNA polymerase (New England Biolabs, USA) and 1 µL of DNA. The PCR protocol encompassed an initial denaturation step (94 °C for 2 min) followed by 35 cycles comprising a denaturation step (94 °C for 30 s), primer annealing steps for 1 min 30 s at 59 °C, and an extension step (72 °C for 1 min). At the end a final extension at 72 °C for 7 min was performed.

qPCR procedure

The qPCR mixture (20 µL) contained 1X Solis BIOdYNE (Estonia) mix (5X Hot firepool evagreen qPCR mix plus (ROX), 0.18 mM each primer and 5 µL of DNA. The quantitative PCR were performed on a Chromo4 system (Biorad, France). The protocol encompassed an initial denaturation step (95 °C for 15 min) followed by 40 cycles comprising a denaturation step (95 °C for 15 s) and a primer annealing step for 1 min at 65 °C for *C. divergens* and *B. thermosphacta*, 60 °C for *Pseudomonas* genus, and 56 °C for *Shewanella* genus. Melting curves were checked from 55 °C to 94 °C. Each sample was quantified in triplicate and the average threshold cycle (C_T) was calculated. Calibration curves were obtained for each strain with DNA obtained from 3 independent extractions performed on pure cultures dilutions ranging from 3 to 8 log CFU.mL^{-1} (section 2.3). Linear regression of the calibration curves were used to convert C_T in estimated bacterial population level in log CFU.mL^{-1}. Quantification of bacteria from chicken leg was performed in triplicate from DNA extracted from microbiota (section 2.1).

Data accession numbers

The fastq formatted and quality filtered read sequences have been deposited at the European Nucleotide Archive (ENA) under the project accession number PRJEB18779 with the accession number ERS1491275.

Results and discussion

In a previous study (Rouger et al., 2017) we determined the bacterial communities from 23 chicken legs by using various selective culture media. We observed that total aerobic mesophilic counts varied from 3 to 8 log CFU.g^{-1} with lactic acid bacteria, *Pseudomonas* sp., and *B. thermosphacta* detected as the dominant bacteria with relative abundance varying between samples. In the present study 10 of those chicken leg bacterial communities (named E, F, G, I, J, M, N, Q, T and U) were chosen as representative the diversity of cuts sold on the French market. From those we investigated the bacterial diversity on a more exhaustive and non-*a priori* way with a NGS approach on the V1-V3 region of 16S rRNA gene from the total metagenomic DNA.

Raw data processing

A total of 220,481 reads were obtained, ranging from 11,883 (sample I) to 33,150 (sample U) per sample. A maximum of 90 reads per sample were removed from the analysis after quality filtering and less than 2,500 reads after chimera removal. On average, 5.6% of reads were removed during the pre-processing steps. From the initial number of reads, the remaining sequences ranged from 86.6% (sample T) to 98.8% (sample M). Finally for the 10 samples a total of 209,122 reads were used for the analysis. To verify that the sequencing coverage was large enough to describe the bacterial diversity, rarefaction curves were established (Figure 21).

It appears from these rarefaction curves that the richness saturation was almost reached for some samples encompassing around 100 species. However for other samples with the same number of reads, a higher richness (up to 200 species) led to curves which did not reach the plateau. A deeper sequencing might have been required for a better coverage of under-represented species. Nevertheless, a large number of species were detected in our samples, which are probably representative of the diversity of the chicken meat bacterial ecosystem.

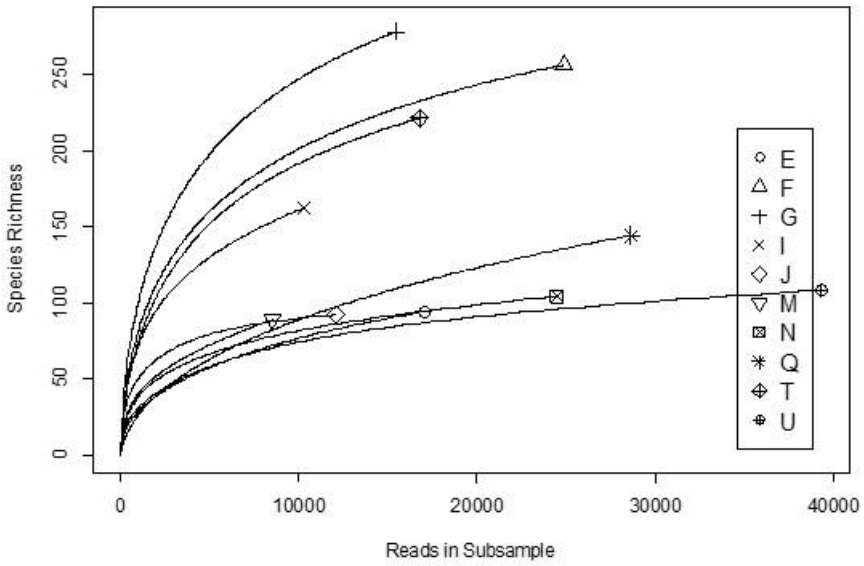

Figure 21 Rarefaction curves from 10 pyrosequencing data set.

Validation of data pipeline analysis

Different pipelines have been developed to analyze datasets of amplicon sequencing. The best known to analyze 454 dataset are, for example, Qiime (Caporaso et al., 2010) and Mothur (Schloss et al., 2009). In others cases, authors use combinations of different software initially developed for others applications (Chaillou et al., 2015). To investigate the robustness of the OTUs identified in our samples, 4 couples (methods/database) were applied, Qiime software (Caporaso et al., 2010) using both RDP database [Qiime/RDP] or the database designed by Chaillou et al. (2015) [Qiime/EBP_DB], method followed by Chaillou et al. (2015) (named [EBP/EBP_DB] in this study) and FROGS using Silva database [FROGS/Silva] (Escudie et al., 2015)

The analysis was performed for each sample but a complete analysis could be obtained for only 3 samples by using FROGS pipeline. Indeed, the 3'-end of the reads was of poor quality and sequence length was too short to match with parameters used during the pre-process. Therefore we used the subset of 3 samples to compare the OTUs and their relative abundances obtained with the 4 different methods. Results are shown Figure 22.

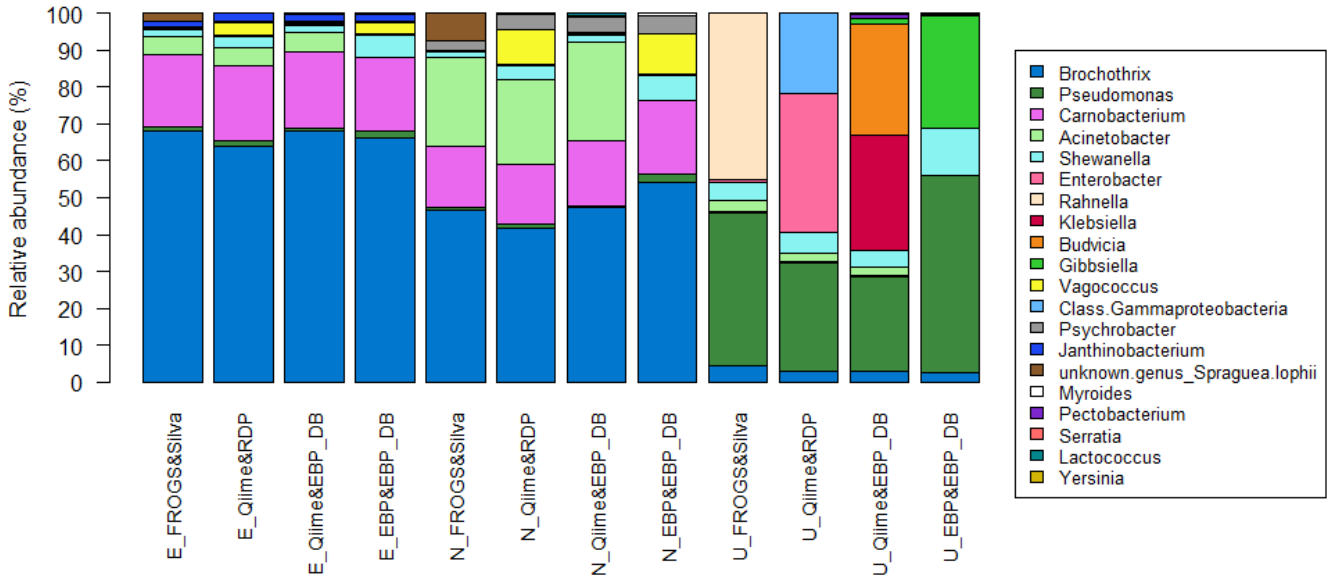

Figure 22 Relative abundance of bacterial genera in 3 different chicken legs samples (E, N and U) with 4 different analysis pipelines

FROGS using Silva database [FROGS/Silva] or with Qiime software using both RDP database [Qiime/RDP] or database design by Chaillou et al., (2015) [Qiime/EBP_DB] or with the pipeline and the database design by Chaillou et al., (2015) [EBP/EBP_DB]

For samples E and N, the [FROGS/Silva], [Qiime/RDP] and [Qiime/EBP_DB] methods produced quite similar results on the identified genera and their relative abundance. The [EBP/EBP_DB] method produced similar results although *Acinetobacter*, a genus belonging to the dominant microbiota detected with other methods, and known to be present on poultry meat (Liu et al., 2006, Lupo et al., 2014) was not detected (Figure 22). For sample U relative abundance and OTUs identification obtained were different depending on the pipeline analysis used. *Pseudomonas* was among the dominant genera according to the 4 strategies. However, *Rahnella, Enterobacter* and gammaproteobacteria, *Klebsiella/Budvicia,* and *Pectobacterium/Gibsiella,* were among the dominant genera identified after treatment with [FROGS/Silva], [Qiime/RDP], [Qiime/EBP_DB], and [EBP/EBP_DB], respectively (Figure 22). Except *Gibsiella* which has been described as an oak phytopathogen (Brady et al., 2010) and the family of undetermined Gammaproteobacteria, the other genera belong to *Enterobacteriacae* family and may therefore be indeed present on poultry cuts. In NCBI database, only partial 16S rDNA sequences are available for *Gibsiella* and *Rahnella* (Stock et al., 2000). In addition, the *Gibsiella* 16S rDNA partial sequence matches with *Pseudomonas fluorescens* and *Serratia* genomes with 99% identity score. The *Gibsiella* identification obtained by [EBP/EBP_DB] method was thus considered as erroneous. As taxonomic assessment was performed only on the 16S rRNA gene V1-V3 region, misidentifications for some OTUs are plausible. [EBP/EBP_DB] method was not further used because of the misidentification of *Gibsiella* in sample U and the absence of detection of *Acinetobacter* in

samples E and N. As well, [Qiime/RDP] was not further used in our study due a lack of identification of Gammaproteobacteria and a putative overestimation of *Enterobacter* in sample U. [FROGS/Silva] method could not be used since analysis could be performed with only 3 out of the 10 datasets. Therefore the [Qiime/EBP_DB] method using the Qiime pipeline (Caporaso et al., 2010) with and assignation of OTU against the EBP database which was developed to identify bacteria of food products at species level (Chaillou et al., 2015) was kept for further analyses.

Bacterial communities present on chicken legs

Abundance Table of 20 dominant species belong to 12 different genera is presented in Table 14.

Only OTUs accounting for more than 0.5% of the total reads were considered. Read counts (total of 197,366 reads) were normalized and relative abundances were calculated for each OTUs. Among the 10 samples, 12 dominant genera encompassing 20 species were identified (Figure 23).

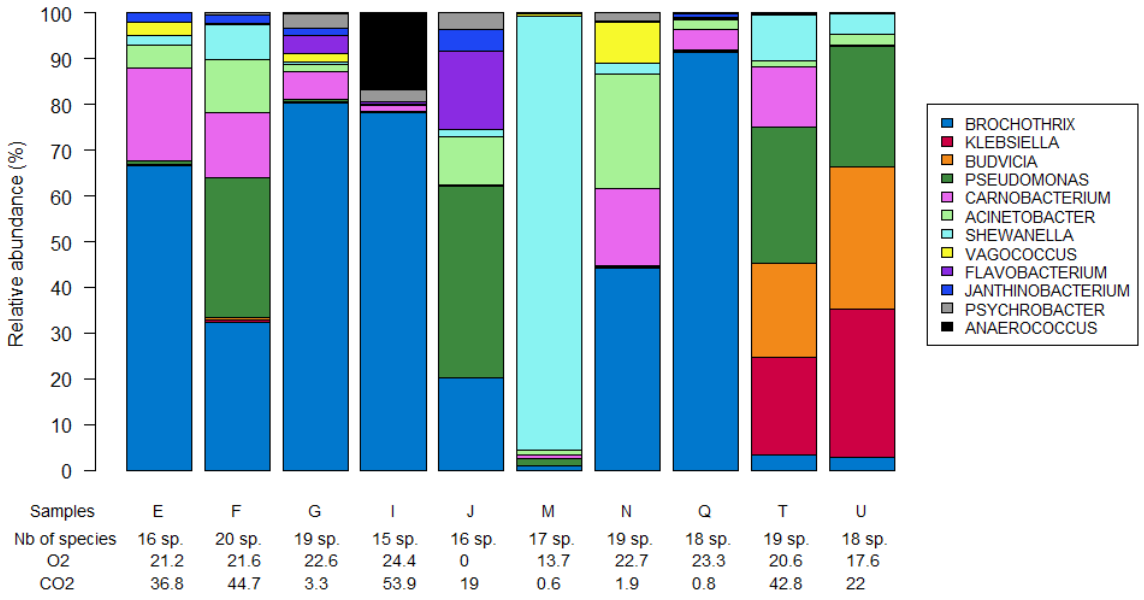

Figure 23 Relative abundance of bacterial genera in 10 chicken legs samples

Qiime pipeline is used with and assignation of OTU against the EBP database. Twelve dominant genera representing more than 0.5% of the total reads are listed. Reads counts (total of 197 366 reads) were normalized and relative abundances were calculated for each OTUs.

Table 16 Number of reads identified at species level

Samples	E	F	G	I	J	M	N	Q	T	U	
Number of OTUs	**108**	**256**	**278**	**162**	**92**	**89**	**104**	**144**	**221**	**108**	**Total number of reads per species**
BROCHOTHRIX THERMOSPHACTA	10993	6929	8920	6207	2037	64	10195	25679	439	1069	**72532**
KLEBSIELLA PNEUMONIAE	26	125	12	3	0	1	43	51	2785	12021	**15067**
BUDVICIA AQUATICA	21	124	11	3	0	1	42	49	2692	11428	**14371**
PSEUDOMONAS CEDRINA	26	763	25	0	129	14	2	43	2492	8520	**12014**
PSEUDOMONAS EXTREMAUSTRALIS	92	5768	23	2	3139	73	44	15	1366	1302	**11824**
CARNOBACTERIUM MALTAROMATICUM	3179	1720	671	61	15	43	3354	1106	1277	89	**11515**
ACINETOBACTER LWOFFII	743	2212	148		886	75	4888	543	138	721	**10354**
SHEWANELLA PROFUNDA	264	1261	23	4	38	5415	280	60	1126	1235	**9706**
VAGOCOCCUS FLUVIALIS	482	72	194	16	14	28	2032	17	1	13	**2869**
FLAVOBACTERIUM ANTARCTICUM	0	19	436	41	1717	0	31	0	5	0	**2249**
ACINETOBACTER GYLLENBERGII	118	319	28		189	8	905	79	27	136	**1809**
JANTHINOBACTERIUM LIVIDUM	321	396	192	5	470	4	40	285	41	16	**1770**
SHEWANELLA XIAMENENSIS	39	147	3		98	869	69	12	108	302	**1647**
PSYCHROBACTER URATIVORANS	4	77	353	200	359	18	420	30	16	1	**1478**
CARNOBACTERIUM PLEISTOCENIUM	0	901		22	2		31	62	452	2	**1472**
ANAEROCOCCUS TETRADIUS	0	1	14	1333	0	1	0	1	0	0	**1350**
SHEWANELLA BALTICA	37	197	23	1	8	486	218	20	68	146	**1204**
CARNOBACTERIUM DIVERGENS	143	409	30	33	3	19	445	94	8	12	**1196**
PSEUDOMONAS FRAGI	1	41	2	1	909	24	12		1	8	**999**
Total of reads per samples	**17019**	**24863**	**15457**	**10252**	**12094**	**8508**	**24475**	**28623**	**16746**	**39329**	**197366**

Dominant species with more than 0.5% of the total reads are listed.

Among those *Brochothrix, Carnobacterium*, and *Pseudomonas* previously described as meat contaminants (Doulgeraki et al., 2012, Chaillou et al., 2015) are the dominant genera of most samples. This is in accordance with the microbiological analysis previously performed by plating methods in which we identified *Pseudomonas*, *B. thermosphacta*, and lactic acid bacteria as the main contaminants (Rouger et al., 2017).

Within the 10 samples of chicken legs *Brochothrix* accounted for 36% of total reads and was present in all samples (Figure 23). At the species level, this genus was represented only by *B. thermosphacta.* This Gram positive species is currently found in different food ecosystems, especially in meat products (Borch et al., 1996, Doulgeraki et al., 2012, Rouger et al., 2017) where it is considered as a major spoilage bacterium (Chaillou et al., 2015, Fougy et al., 2016). *Brochothrix campestris* the other species belonging to the *Brochothrix* genus has been described in soil or other environments and is usually not reported as food spoilage bacterium compared to *B. thermosphacta* (Gribble & Brightwell, 2013).

The genus *Pseudomonas* was the second most abundant genus found in chicken legs with 12% of total reads. The detection of some *Pseudomonas* species in this study, such as *Pseudomonas fragi*, correlates with the previous description of this species as food spoiler and present in chicken microbial ecosystem (Arnaut-Rollier et al., 1999a, 1999b, Mohareb et al., 2015). The presence of two sequence clusters identified as *Pseudomonas extremaustralis* and *Pseudomonas cedrina* in our chicken samples is more doubtful since these species have been rather described as soil bacteria. The genus *Pseudomonas* encompasses many different species which identification based on V1-V3 16S rDNA sequence is difficult (Bergmark et al., 2012). The presence of *P. extremaustralis* and *P. cedrina* in chicken legs would therefore require confirmation.

With 6% of total reads, *Carnobacterium* was the third dominant genus found in chicken meat microbial ecosystem. *Carnobacterium maltaromaticum* was the main species, in agreement with previous studies which reported its isolation from different food products (Leisner et al., 2012, Cailliez-Grimal et al., 2013).

Shewanella was also belonging to the dominant microbiota of chicken legs, accounting for 6% of total reads, although this was mainly due to its dominance in sample M (Figure 23). The species were identified as *Shewanella profunda, Shewanella xiamensis* and *Shewanella baltica.* However identification of *S. profunda* and *S. xiamensis* present mostly in sample M could be also associated to *Shewanella putrefaciens* because of their 16S rDNA sequence similarity (Potron et al., 2011). This last species has been isolated from human microbiota, but also from environment and food products (Holt et al., 2005). *S. baltica*, a species present in oceans has also been reported as a fish and seafood products spoilage organism as *S. putrefaciens* (Vogel et al., 2005, Remenant et al., 2015).

Some other genera representing around 6% of total reads were identified; *Klebsiella* and *Budvicia* were quite abundant in 2 of the 10 chicken leg samples (T and U) and were represented by two species. The first one, *Klebsiella pneumoniae* has been previously described in the human respiratory microbiota and as an opportunistic pathogen and a commensal organism. It is also present in birds and potentially responsible for respiratory tract disease in poultry (Younis et al., 2016). The second one, *Budvicia aquatica,* of the *Enterobacteriaceae* family is usually found in surface water (Bouvet et al., 1985) but may be associated to human diseases (Tomczak and Smuszkiewicz, 2014). The most abundant species from the genus *Acinetobacter* found in our samples was *Acinetobacter lwoffii,* already described on healthy human skin and microbiota (Regalado et al., 2009) but also responsible for bird diseases including chicken ones (Wang et al., 2012). This species was found in particular in samples N and J. Others species like *Acinetobacter soli* and *Acinetobacter venetianus previously* isolated from soil and environment (Al Atrouni et al., 2016) were also observed in this study.

Other genera accounting for only 1% of total reads and present in only some of the 10 samples are listed below. *Psychrobacter urativorans* present in all samples has been reported in frozen meat (Vela et al., 2003, Bowman, 2006). The only species belonging to the genus *Vagococcus* found in the present study was *Vagococcus fluvialis* (samples N and E). The first isolates of this species were recovered from chicken faeces and river water (Hashimoto et al., 1974) 1979) and new isolates were subsequently reported from various animals (pigs, cattle, cats, horse and fishes). Several human clinical isolates and fish probiotics have been described as well (Teixeira et al., 1997, Yi et al., 2005, Sorroza et al., 2012). Among *Flavobacterium* the species *Flavobacterium antarcticum* was identified in sample J. This species was isolated from a terrestrial sample from the Antarctic, issued from a penguin habitat suggesting its adaptation to cold and aquatic environments (Yi et al., 2005). *Anaerococcus tetradius* was detected in sample I. This anaerobe (Murphy & Frick, 2013) is associated with clinical infections like pleural empyema (Ezaki et al., 2001). However, this species renamed in 2001 (former name *Peptostreptococcus tetradius*) was initially considered as close to *Peptostreptococcus barnesae* isolated from chicken feces and renamed *Gallicola barnesae* (Ezaki et al., 2001). Therefore, we cannot exclude a misidentification due to sequence 16S rDNA similarities or to errors in the origin of the *A. tetradius* sequence present in the database. Finally *Janthinobacterium lividum* has been described in samples issued from soils, rivers, lakes and springs but also on skin of amphibians and has been also linked to milk and meat spoilage (Pantanella et al., 2007). This species was found in low abundance in some samples but especially in samples E and J. This psychrotolerant species is aerobic and capnophilic *i.e.* high CO_2 concentrations enhance its growth (Valdes et al., 2015). It has also been reported as potentially important for fighting *Listeria* biofilms in food environments (Fox et al., 2014).

All the genera and species we observed in chicken legs microbiota have already been described in food, animal or water/soil environments. Among those, some have been described as food spoilage bacteria and some as putative human or animal pathogens. This diversity of species could be explained by the presence of non-sterile environment in the farm, especially for free range poultry living outdoors. During the slaughtering process, the water used to rinse carcasses is a potential source of contamination (Goksoy et al., 2004). Finally, manipulators and mechanical evisceration could explain the presence of bacteria associated to human or chicken gut microbiota. The presence of several psychrotrophic and psychrotolerant species in our meat sample microbiota has been already described and reported as the consequence of cold storage shaping of microbial communities balance (Chaillou et al., 2015).

The bacterial communities present in Finnish chicken breasts stored with or without the use of a marinade have been described at the family level by using a metagenomic approach (Nieminen et al., 2012). Our results are in agreement with those obtained with chicken breasts although some bacteria reported in the Finnish poultry products were absent from our results. Indeed among lactic acid bacteria *Carnobacteriaceae, Leuconostocaceae,* and *Lactobacilaceae* have been detected in chicken breasts with *Carnobacteriaceae* abundance much higher than that of the two other families (Nieminen et al., 2012). This may explain that only *Carnobacteria* was observed in our study. As well some species such as the known pathogens *Campylobacter* and *Salmonella*, important for poultry meat safety, were not detected in our datasets. The prevalence of *Campylobacter* is on average 88% of carcasses and 76% of products at the retail level (Saint-Cyr et al., 2016). *Salmonella* was detected in chicken meat at level of 6.5% and the prevalence is 0.34% in the EU countries (EFSA, 2016). However, the contamination level of those two pathogens is usually very low (about 2 log CFU.g^{-1}) (Mead, 2004). Therefore, because of this very low level of contamination the early amplification steps may minimize or exclude under-represented communities. Only very deep NGS sequencing or specific PCR may detect such contaminants. The estimated total viable counts of our samples ranged from 4 (sample G) to 9 (sample J) log CFU.g^{-1}. As the number of reads per sample ranged between ~8,500 and 39,000 (Table 4) and with a cut off of OTU representing at least 0.5% of total reads, such low contamination level would not be detected here. Nevertheless, PCR amplification by using specific primers (Table 1) to detect the presence of *Campylobacter* was negative for the 10 samples (data not shown).

Validation by quantitative PCR and plating methods

Other studies describing the bacterial communities present in meat products, have reported the use of several methods to validate the results as for example in pork sausages (Fougy et al., 2016). In the

present study abundance of the species *C. divergens* and *B. thermosphacta*, and of the genera *Pseudomonas* and *Shewanella* was determined by qPCR on the DNA extracted from the 10 chicken leg microbiota. The qPCR results were then compared to the data obtained by pyrosequencing and also to those obtained by plating method in our previous study for *Brochothrix* and *Pseudomonas* (Rouger et al., 2017) (Figure 24). The regression plots of log CFU.mL^{-1} obtained through the different methods are shown in supplementary Figure 25. For pyrosequencing data, the relative abundance of reads was converted to a percentage of total reads (per sample) with 100% set up as the total aerobic counts measured on plates (expressed in log CFU.mL^{-1}).

Brochothrix

	Cultures	Pyrosequencing	Quantitative PCR
G	3.87±0.00	4.33	4.91±0.22
M*	4.92±0.03	4.57	6.09±0.12
T	4.04±0.02	4.62	5.24±0.23
F	5.05±0.00	4.93	4.72±0.06
U	5.72±0.05	5.40	5.97±0.15
I	5.72±0.08	5.85	5.90±0.18
Q	6.80±0.14	6.92	7.29±0.04
E	7.09±0.00	7.39	6.89±0.10
N	7.36±0.00	7.92	7.77±0.24
J*	7.83±0.00	8.38	6.94±0.14

Shewanella

	Pyrosequencing	Quantitative PCR
G	2.07	1.74±0.06
I	2.75	3.62±0.14
F*	4.29	1.84±0.13
Q	4.47	5.56±0.10
T*	5.09	6.40±0.01
U	5.59	6.19±0.09
E	5.88	6.51±0.00
M*	6.57	4.04±0.12
N	6.66	6.06±0.13
J*	7.22	3.63±0.20

Pseudomonas

	Cultures	Pyrosequencing	Quantitative PCR
G*	3.40±0.03	2.08	2.44±0.05
I	3.26±0.09	2.53	2.14±0.10
Q	4.28±0.25	4.27	3.52±0.06
M	5.70±0.18	4.78	5.40±0.05
F	5.27±0.07	4.91	4.48±0.16
E	6.19±0.17	5.42	5.94±0.06
T	5.76±0.11	5.57	5.20±0.07
N	5.96±0.10	5.67	5.45±0.09
U	7.09±0.00	6.36	6.52±0.04
J	8.92±0.00	8.69	8.54±0.08

Carnobacterium

	Pyrosequencing	Quantitative PCR
G	3.23	3.76±0.07
I	4.12	4.32±0.05
U	4.38	5.19±0.07
M	4.53	5.23±0.07
F	4.57	4.77±0.02
T	5.22	4.66±0.04
Q	5.61	5.75±0.02
J	6.37	5.77±0.05
E	6.87	6.49±0.03
N	7.49	7.04±0.05

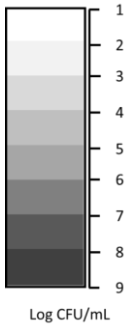

Figure 24 Comparison of bacteria quantification by different methods

Results are expressed in log CFU.mL-1. Counting coined as cultures are issued from plating methods (Rouger et al., 2016). For pyrosequencing data, relative abundance of reads was converted to a percentage of total reads (per sample) with 100% set up as the total viable counts measured on plates. Samples with quantification results differing by more than 1 log CFU.mL-1 depending on the method used are noticed by *.

A relatively good correlation of the ordination of samples according to population level was observed with the different counting estimations (Figure 24). This was particularly true for *Pseudomonas* and confirmed by regression plots (Figure 25) indicating a regression coefficient > 0.95 for comparisons between the three methods. *Brochothrix* quantification data by plating method and by pyrosequencing were also correlated but qPCR quantification results differed (Figure 24 and Figure 25). *Carnobacterium* counting by pyrosequencing and by plating method was more divergent although the

differences were generally below 1 log CFU.mL^{-1}. Only *Shewanella* counting by pyrosequencing and qPCR gave rather large differences.

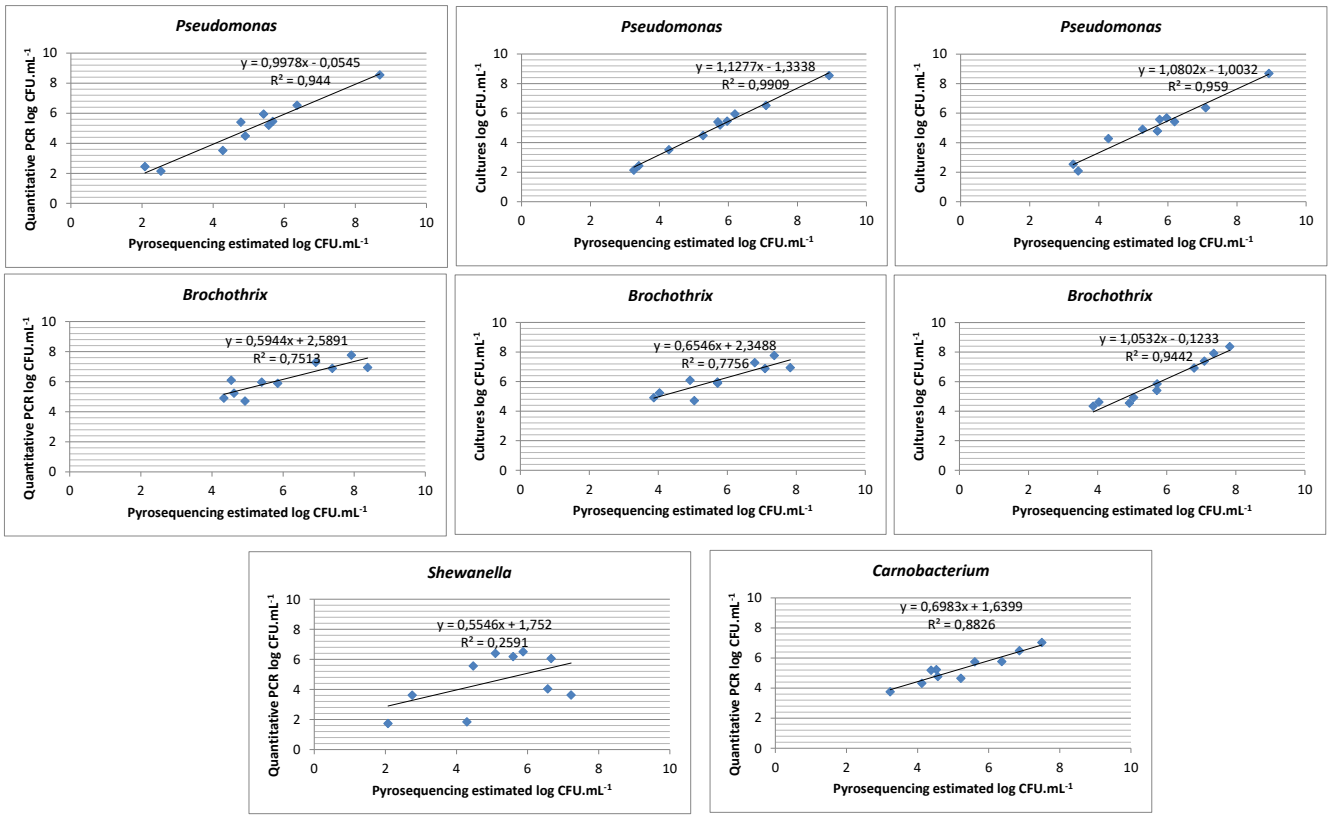

Figure 25 Regression plots of quantification obtained by 3 different method

Plating method data issued from Rouger et al. (2017), pyrosequencing (this study) and quantitative PCR (this study). Each diamond represent one of the 10 chicken leg samples. Results are shown in log CFU.mL-1. For pyrosequencing data, relative abundance of reads was converted to a percentage of total reads (per sample) with 100% set up as the total viable counts measured on plates.

The various divergences we observed may be explained by the limited selectivity of the media used for counting that may lead to an overestimation of a counted population. The qPCR detection limit (between 3 and 8 log CFU.mL^{-1}) may also be responsible for counting uncertainty for low or high population level. The pyrosequencing counts, made from an extrapolation of the number of total reads and total counts for each sample may also be erroneous. As an example, the apparent dominance of *Shewanella* in sample M observed by pyrosequencing (Figure 23) did not correlate with qPCR data (Figure 24). The richness of this sample might have been underestimated because of the high number of reads that were not identified (Figure 23) leading to a low number of OTUs (Table 15). This also is indicated by the rarefaction curve (Figure 21). Nevertheless, these results confirmed the presence and relative abundance for the *B. thermosphacta* and *Pseudomonas*.

Table 17 Richness and diversity indices of the 10 microbial communities issued from chicken legs

	Nb of reads to be analysed after cleaning step	Nb of reads identified by Qiime/EBP analysis	Number of observed OTU by Qiime/EBP analysis	Equitability - Evenness	Shannon - Diversity	Simpson - Diversity	Chao1 - Richness
E	17707	17019	108	0.29	2.58	0.60	1308
F	28083	24863	256	0.45	4.52	0.87	2069
G	15732	15457	278	0.42	3.87	0.68	1195
I	10575	10252	162	0.38	3.39	0.65	1020
J	21736	12094	92	0.36	3.61	0.70	2325
M	14737	8508	89	0.42	4.11	0.75	2073
N	24764	24475	104	0.36	3.38	0.77	2434
Q	29073	28623	144	0.14	1.28	0.25	1275
T	15772	16746	221	0.48	4.51	0.90	1296
U	30943	39329	108	0.34	3.6	0.76	4270

Chicken meat microbial diversity – Influence of slaughtering and storage practices

Once the presence and relative abundance of bacteria validated, we compared the microbial diversity between the 10 chicken leg samples. In Table 15, we listed from 15 (sample I) to 20 (sample F) dominant OTUs. However, the total number of OTUs ranged from 89 (sample M) to 278 (sample G) (Table 15). To complete this diversity analysis different richness and evenness indices were calculated for each samples (Table 15).

An equitability (evenness) index is close to 1 when no species clearly dominates and close to 0 with the presence of dominant species (Heip et al., 1998). In the 10 samples, sample Q showed the lowest (Table 15), as encompassing a single highly dominant species (*Brochothrix*, Fig. 2). At the opposite sample T had the highest equitability index (0.48, Table 15). Accordingly this sample did not exhibit a clear cut dominant species but was rather dominated by 6-8 species, each with a close relative abundance (Table 15). This also correlates with the Shannon index to estimate the diversity. Sample T was the most diversified with the highest Shannon index (4.51) close to that of sample F (4.52) that also harbored a high evenness, and sample Q at the opposite with the smallest Shannon index (1.28). Richness expressed here by the Chao1 index showed sample I as that with the lowest richness and sample U as the richest sample (Table 15).

Thus, we observed that both the nature of the most abundant species present in chicken leg meat but also their relative abundance were different depending on the samples. Interestingly, we noticed that bacterial profiles of samples T and U were similar compared to other samples especially because of the presence of similar ratios of *Klebsiella*, *Budvicia*, and *Pseudomonas* among the dominant microbiota and a higher abundance of *Carnobacterium* and *Vagococcus* in sample T (Figure 23).

According to our previous study, these two samples, sold with two different brand names, were issued from the same slaughterhouse, have been processed the same day and also harbored the same use-by-date (Rouger et al., 2017). However, their contamination was different with a proportion of lactic acid bacteria counts more important in sample T (Rouger et al., 2017) correlating with the present data.

This observation strengthens the link between the meat contamination and the process environment of slaughterhouses (manipulators, surfaces, rinsed water …) as previously proposed (Chaillou et al., 2015).

The chicken leg samples used in this study were packaged under modified atmosphere, and the CO_2 and O_2 percentages in the headspace had been measured just before opening the pack (Rouger et al., 2017). These values issued from our previous article have been reported Figure 23. When O_2 was low in the packs (samples G, M, N and Q) *Pseudomonas* was not present or detected at very low levels. Conversely when packs contained a high percentage of O_2, *Pseudomonas* were identified among the dominant species (samples F, J, T and U), in accordance with the aerophillic phenotype of these bacteria. However, we noticed that in samples E and I, dominated by *Brochothrix*, and, to a lesser extent by *Carnobacterium* in sample E, *Pseudomonas* were absent despite a high concentration of O_2. The highest abundance of the aerobic and capnophilic bacterium *Janthinobacterium* was observed in sample J, as was the case for *Pseudomonas*, a sample stored under air (Rouger et al., 2017). This may suggest a competition between bacteria composing meat microbiota depending on CO_2 and/or O_2 concentration used in the MAP.

Conclusion

In this study we described the microbiota of chicken legs from local supermarkets by the use of V1-V3 16S rRNA gene pyrosequencing. Several strategies were compared to choose the most accurate to determine the dominant species. The data were compared to previous microbiology analysis and qPCR partially confirmed the dominance. Cultural methods are commonly used in food microbiology to detect some specific bacteria but the results may be biased and the specificity of the media is often questioned. Sequencing of 16S rRNA gene is a powerful method currently used to determine the structure of complex ecosystems (Petrosino et al., 2009) by identification of relative abundance of different species composing them. However, 16S rRNA gene sequencing approach has also known biases, especially due to the PCR amplification performed prior the sequencing step (Lee et al., 2012), which can lead to wrong relative abundance and may also generate chimeric sequences. PCR-generated chimeras created during the first step of amplificiation lead to errors during the process of bacterial identification. Special care of this chimeras is required during the pipeline analysis. An other

bias of the 16S rRNA gene sequencing method is the limit of the databases used for bacterial identification. As well, due to the high conservation of the 16S rDNA sequence, it is difficult to discriminate some OTUs at the species or even genus level. The use of housekeeping genes may be very useful for getting more accurate results. Using only 16S rRNA gene requires verifications by additional blast or by the use of other molecular methods like qPCR which itself needs adaptions. Although each of the methods used in this study harbor some biases, we could correlated the data by confirming the presence and relative abundance of some of the dominant species contaminating fresh chicken legs.

In this study we showed the variability of microbial communities present on chicken legs stored at low temperature and collected before the UBD. The samples, collected from supermarkets, did not show any obvious spoilage. Nevertheless we noticed the dominance of different species known as responsible for meat spoilage, such as *B. thermosphacta* or *Pseudomonas.* Although the two main human pathogens associated to poultry consumption, *Campylobacter* and *Salmonella,* were not detected, other putative pathogens were observed.

The microbiota (essentially that of gastrointestinal tract) of broilers and its impact on animal health and on productivity has been described (Stanley et al., 2013, Stanley et al., 2014, Waite & Taylor, 2014, Choi et al., 2015, Mohd Shaufi et al., 2015). However, the contamination of chicken meat by the animal microbiota during the slaughtering process has not yet been deeply investigated. In a way to characterize microorganisms from farm to fork in USA, Oakley et al., (2013) identified the microbiota from broiler chicken production to the carcasses, which are rinsed with a chlorinated solution. In EU, such decontamination of the carcasses is not allowed. Storage at low temperature and gas composition of MAP are used for limiting bacterial growth until UBD. We noticed that contaminants present on chicken legs could originate from animal microbiota, from water, and from slaughterhouse environment. Most of the contaminants were adapted to the storage conditions, being psychrotroph and adapted to the gas composition of the packaging. We also observed a potential competition between the various species composing chicken meat microbiota, depending on the nature of the MAP. Interactions between *Carnobacterium* and *Brochothrix* during food spoilage have been suspected (Laursen et al., 2006). It would therefore be interesting to investigate the microbiota of chicken along the production chain, from living animals to chicken cuts at retrieval for determining the contamination steps and the nature of the contaminants. As well, since microbial contamination of chicken cuts is variable, the influence of storage conditions, in particular that of the MAP gas composition on the dynamics of meat microbiota may help improving chicken meat safety.

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3.3- Ce qu'il faut retenir du chapitre 3

La composition bactérienne de 10 lots de cuisses de poulet conservées sous atmosphère protectrice a été décrite par pyroséquençage et partiellement validée par qPCR. Cette méthode a permis d'obtenir une liste des espèces bactériennes présentes sur la viande de poulet. Les genres majoritairement retrouvés sont *Brochothrix*, *Pseudomonas*, *Carnobacterium* et *Shewanella*. Les différences entre les 10 lots, préalablement observées par méthodes culturales (chapitre 2) ont été confirmées par séquençage à haut débit.

Un possible lien peut être fait entre l'abattoir et les lots de viande. En effet, 2 lots qui proviennent du même abattoir et ont été produits le même jour, présentent des profils bactériens proches en ce qui concerne les espèces retrouvées et leurs proportions. Cependant cette hypothèse devrait être vérifiée en échantillonnant plus de 2 lots. La composition de l'atmosphère protectrice semble également avoir un impact sur les proportions des espèces bactériennes. En effet une corrélation existe entre une forte proportion d'O_2 dans les barquettes et la présence de certaines flores comme *Pseudomonas* en quantité significative. Ces *Pseudomonas* ne sont pas retrouvés quand l'atmosphère est appauvrie en O_2. Cependant dans certains cas, lorsque *Brochothrix* est largement majoritaire et *Carnobacterium* dans une moindre mesure, et malgré une forte concentration en oxygène, les *Pseudomonas* ne sont pas retrouvés, ce qui laisse penser à une compétition entre ces communautés bactériennes dépendant de l'atmosphère gazeuse.

Afin de vérifier cette dernière hypothèse, nous allons étudier l'influence des atmosphères modifiées sur les communautés bactériennes et nous allons également chercher à savoir quelles espèces bactériennes sont actives et qu'expriment-elles au sein du microbiote.

Chapitre 4 Dynamique des écosystèmes microbiens

4.1- Préambule

Nous avons constaté que différentes atmosphères protectrices sont couramment utilisées pour le conditionnement de la viande de poulet et que la charge bactérienne et la nature des contaminants varient suivant les lots. Dans ce chapitre nous avons investigué l'effet de la composition des atmosphères protectrices sur la diversité et l'abondance relative des espèces bactériennes au cours de la conservation. Nous avons également recherché quelles fonctions étaient différentiellement exprimées par les contaminants. Pour ce faire, nous avons utilisé 2 microbiotes (E et U) récoltés en chapitre 2 et dont la composition et les proportions des espèces dominantes sont différentes (chapitre 3). Le microbiote E est très riche en *Brochothrix* et dans une moindre proportion en *Carnobacterium*. Le microbiote U est quant à lui plus pauvre en *Brochothrix* mais il est composé de *Pseudomonas, Budvicia* et *Klebsiella*. Nous avons inoculé ces 2 microbiotes sur de la viande de poulet pauci microbienne selon la méthode développée au chapitre 2. Les viandes ont été stockées à 4°C ou sous les 2 atmosphères couramment utilisées (70% O_2 - 30% CO_2 ou 50% CO_2 - 50% N_2) et un contrôle a été réalisé sous air.

La croissance bactérienne a été suivie au long du stockage par méthodes culturales. Les ADN et ARN bactériens ont été récoltés afin d'être séquencés (métabarcoding, métagénomique et métatranscriptomique) pour voir comment la composition des microbiotes avait évolué suivant les MAP utilisées et pour comprendre quelles espèces bactériennes étaient actives et ce qu'elles exprimaient.

Cette étude est présentée sous forme d'un article scientifique en préparation.

4.2- Optimizing storage parameter to manage chicken meat ecosystem stored under modified atmosphere packaging.

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Abstract

Controlling spoilage microorganisms, especially in raw meat products, is a challenge for the food industry. Microbial communities contaminating meat vary depending on seasonal changes and production processes. In addition, the storage conditions, for example modified atmosphere packaging, have selective effects on the microbiota dynamics. Bacterial interactions during storage of meat are complex and poorly known. Their description should pave the way for improving the quality of fresh meat. Thanks to the recent development of next-generation sequencing methods, widely used for characterizing microbes in different ecosystems, we studied bacterial community dynamics during poultry meat storage under different MAP conditions.

Two microbiotas with very different composition, were used for challenge tests. After their inoculation, the 2 microbiota could overgrow the initial contaminants. Bacterial growth kinetics were monitored at day 2, 4, 7 and 9 after inoculation. DNA for metabarcoding and metagenomics, and RNA for metatranscriptomics could be recovered in sufficient amounts only at day 7 and 9.

We observed that the duplicate challenge tests produced similar results, confirming that microbiota inoculation method diminished the effect of sample variation. Each of the 3 gaseous atmospheres shaped microbial communities differently. Nevertheless, independently from the inoculated microbiotas dominant bacterial communities were converging depending on gas used for packaging. Surprisingly, the active bacteria species, as observed from metatranscriptomics were not systematically the dominant ones. In particular, *Brochothrix thermosphacta*, a spoilage organism, enhanced by storage under 70% O_2 - 30% CO_2 up-regulated only the ribose utilization operon. Conversely, lactobacilli (*Lactobacillus fuchuensis* in microbiota E and *L*actobacillus *sakei* in microbiota U) although subdominant under 50% CO_2 - 50% N_2 atmosphere up-regulated several hundreds of genes among which those dedicated to cell division, transcription and translation showing their growth activity.

Keys word

Metatranscriptomics, metagenomics, metabarcoding, Chicken meat quality, food spoilage

4.2.1.Introduction

World poultry meat production and consumption are steadily increasing and ensuring the bacterial safety of poultry cuts is a challenge.

A large diversity of bacterial species can be hosted by meat products depending on seasonal changes, production processes (Cohen et al., 2007) and storage conditions (Chouliara et al., 2007). Bacterial contamination occurs mostly at the surface and on the skin during slaughtering (Luber, 2009). This contamination originates from animal microbiota (faeces, hide, skin and feathers), from the plant environment (air, equipment, surfaces) and from human handling. The meat microbiota may encompass pathogenic and spoilage bacteria (Doulgeraki et al., 2012) which must be controlled to ensure the safety and the quality of the products (Álvarez-Astorga et al., 2002). The literature reports highly variable total viable counts (from 3 to 9 log CFU/g) depending on storage conditions and poultry cuts (Björkroth, 2005, Balamatsia et al., 2007, Chouliara et al., 2007, Zhang et al., 2012, Al-Nehlawi et al., 2013, Capita et al., 2013, Rouger et al., 2017). In addition large variations exist between samples of the same cuts (Rouger et al., 2017). The use-by-date (UBD) of poultry meat is reached when bacterial contamination reaches around 7 log CFU/g (Okolocha & Ellerbroek, 2005). Storage conditions such as the use of modified atmosphere packaging (MAP) or marinades together with cold temperatures limit the growth of bacteria and shape the bacterial communities initially present on carcasses. It is therefore important to consider meat as complex ecosystems hosting complex microbiota (Fleet, 1999). However, only a few studies dealing with meat microbiota have been reported (del Río et al., 2007b, Cardenas & Tiedje, 2008, Nieminen et al., 2012, Oakley et al., 2013, Benson et al., 2014, Mayo et al., 2014, Chaillou et al., 2015, Fougy et al., 2016).

Understanding the metabolic functions expressed by bacteria during storage, especially the spoiler's bioprotective agents, may also contribute to improve storage conditions for ensuring the microbial control of meat products.

Our strategy was to use standard ecosystems (real, reproducible, storable and known) to limit the natural microbial variability in order to perform reproducible challenge tests. Two different microbiota were inoculated onto model meat matrices, which were then stored under different gaseous atmospheres. Bacterial dynamics and genes expressed by microbiota were monitored.

4.2.2. Materials and Methods

4.2.2.1. Challenge test

Fresh chicken breast fillets were collected from the local supermarket not more 2 days after arrival and transferred to the laboratory at 4°C. They were then rinsed with 70% ethanol. After briefly drying on sterile filter paper, breasts were aseptically cut into 2 cm cubes. One aliquot (1 mL) of two bacterial communities (named E and U in this study) isolated from chicken legs and stored at -80 °C (Rouger et al., 2017) were gently defrosted, and then diluted in tryptone salt solution to obtain an appropriate cell concentration. Meat cubes were inoculated at 5 log CFU/g. Each challenge test was performed in duplicates. After inoculation, 50 g portions were packaged under three different atmospheres: A, (70% O_2 - 30% CO_2) and B, (50% CO_2 - 50% N_2), which are the 2 modified atmospheres routinely used in France for poultry cuts storage; and under air C (20% O_2 - 80% N_2) used as a control. Meat was stored for 9 days at 4 °C. The modified atmosphere composition in the head space was checked at each collection time (2, 4, 7 and 9 days) with triplicate measures of O_2 and CO_2 with an Oxybaby gaz analyzer (WITT Gasetechnik GmbH & Co KG, Germany).

4.2.2.2. Bacterial cultures

4.2.2.2.1. Bacterial enumeration from meat samples

For each collection time, a 40 g-portion of meat was rinsed with tryptone salt solution. CFU were determined after plating serial 10-fold dilutions on various media. The total aerobic viable counts were determined after 2 days incubation at 30 °C on Plate Count Agar (PCA) (Biokar, France). Lactic acid bacteria (LAB) were counted on MRS agar medium pH 5.2 (AES, France) after 4 days incubation at 25 °C under anaerobic conditions (Anaerocult A, Merck, Germany). Enumeration of *Pseudomonas* spp. and *Brochothrix thermosphacta* were performed at 25 °C on Cephalosporine Fucidine Cetrimide CFC (Biokar, France) for 2 days, and Streptomycin Sulfate Thallium Acetate Actidione agar STAA (Oxoid, France) for 3 days, respectively.

4.2.2.2.2. Bacterial pure cultures for real time quantitative PCR (qPCR)

Strains were cultured on BHI plates (AES, France) containing 1.5% agar (Biokar Diagnostics, France) for 36 h at adequate temperature (Table 16). One colony was resuspended into 10 mL of BHI broth and incubated overnight (see Table 16 for incubation conditions). Bacterial cultures were inoculated at 1% on fresh BHI broth and grown for 3-5 h to reach 8 log CFU.mL^{-1}.

A series of 10-fold dilution was performed in BHI broth to obtain bacterial concentrations ranging from 3 to 8 log CFU.mL^{-1}. The exact bacterial concentration was determined after plating on BHI.

Table 18 Bacterial strains used and culture conditions.

Bacterial species	Strains	Temperature of incubation	Agitation in BHI broth
Brochothrix thermosphacta	DSM 20171	26°C	140 rpm
Carnobacterium divergens	V41	30°C	-
Acinetobacter lwoffii	DSM	30°C	240 rpm

4.2.2.3. Nucleic acid extraction

4.2.2.3.1. DNA extraction from pure cultures for qPCR

A volume of 1 mL of bacterial dilutions ranging from 3 to 8 log cells.ml^{-1} was centrifuged at 10 000*xg* for 10 min at 4°C. Bacteria pellets were resuspended in a Dulbecco's phosphate buffered saline solution without Ca and Mg (Eurobio, France) and DNA was extracted with the High Pure PCR Template Preparation Kit (Roche, France) following the manufacturer's instructions and eluted in 200 µL milliQ water.

4.2.2.3.2. Nucleic acid extraction from meat and quality control for sequencing

Immediately after opening, 10 g of meat was added to 10 ml RNA protect cell reagent (Qiagen, Germany) and gently mixed during 15 s. Meat residues and fat were removed from the liquid mixture by centrifugation at 200*xg* for 3 min at 4°C. The supernatant was aliquoted (1 mL) in Eppendorf tubes and centrifuged for 5 min at 10000*xg* at 4°C to collect bacteria. The bacterial pellet was resuspended into 200 µL RNA protect and stored at -80°C until further nucleic acid extraction.

The nucleic acid extraction was performed with the AllPrep RNA/DNA (Qiagen, Germany) according to the manufacturer's procedure. Chemical lysis of bacteria was performed in phenol/chloroform with Qiagen lysis buffer containing β-mercaptoethanol according to the recommendations of the manufacturer. Mechanical lysis was achieved using a FastPrep (MPbiomedicals) for 40 s at a frequency of 5.5 m/s. The cleaning and elution steps for both RNA and DNA were performed on spin membrane columns provided in the kit. Experimental design using DNA for both metabarcoding and metagenomic analyses and RNA for metatranscriptomics is summarized in Figure 26.

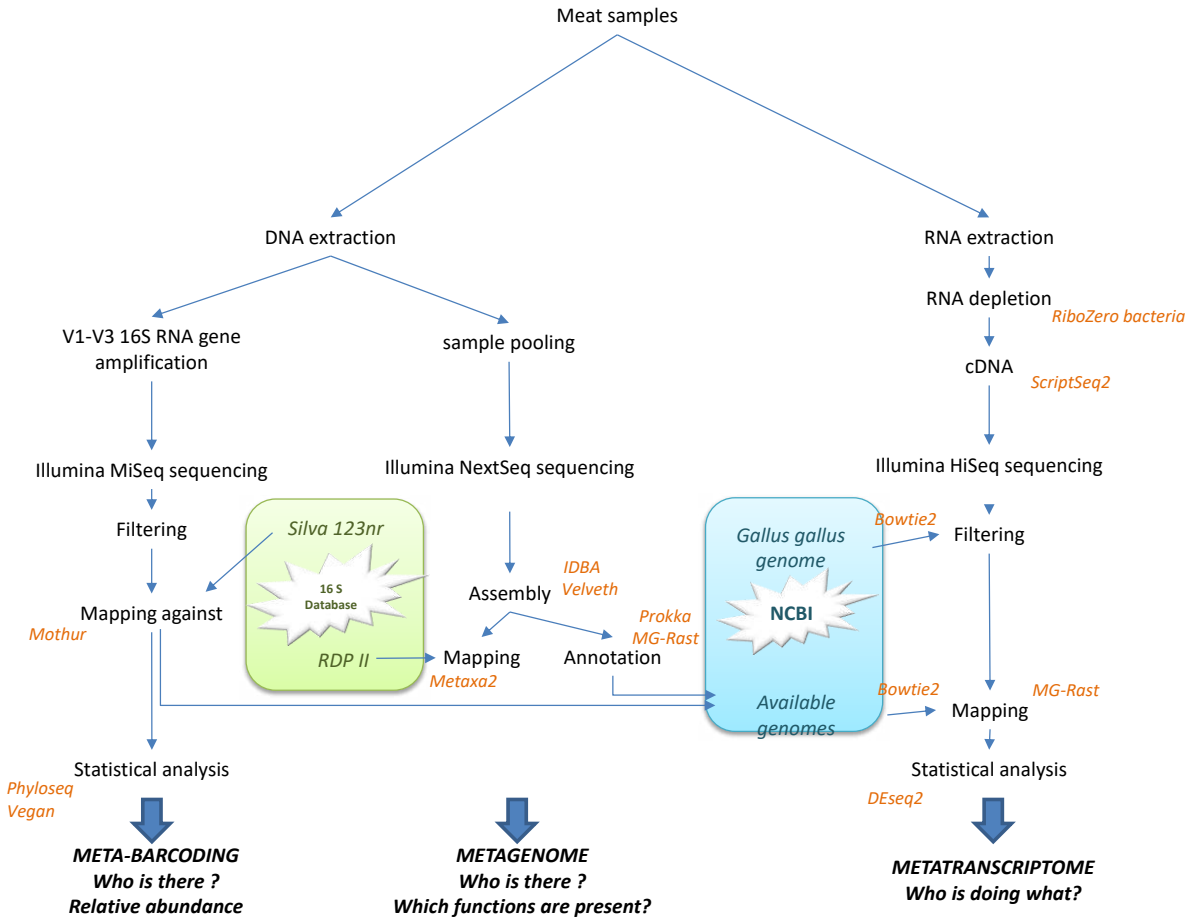

Figure 26 Experimental design of this study and methods used for NGS analysis.

The nucleic acids concentration was measured with a Qubit fluorometer apparatus (Invitrogen, CA, USA) and the quality of RNA was checked on RNA chips on 2100 Bioanalyzer system (Agilent, United States) or automated electrophoresis Experion system (Biorad, France).

4.2.2.4. PCR conditions

4.2.2.4.1. qPCR procedure

The qPCR mixture (20 µL) contained 1X Solis BIOdYNE (Estonia) mix, 5X Hot firepool evagreen qPCR mix plus (ROX), 0.18 mM each primer (Table 17) and 5 µL of DNA. The qPCR were performed on a Chromo4 system (Biorad, France). The protocol encompassed an initial denaturation step (95 °C for 15 min) followed by 40 cycles comprising a denaturation step (95 °C for 15 s) and a primer annealing step for 1 min at 65 °C for *C. divergens*, *B. thermosphacta* and *A. lwoffi*. Melting curves were checked from 55 °C to 94 °C. Each sample was quantified in triplicate and the average threshold cycle (C_T) was calculated. Calibration curves were obtained for the 3 species with DNA obtained from 3 independent extractions performed on pure culture dilutions ranging from 3 to 8 log CFU.mL^{-1}. Linear regression of the calibration curves were used to convert C_T in estimated bacterial population level in log CFU.mL^{-1}.

Table 19 Primers used in this study

Specificity	PRIMER SEQUENCE (5'→3')	PRIMER NAME	FRAGMENT SIZE (BP)	TARGET	REFERENCE
All species	AGAGTTTGATCMTGGCTCAG GTATTACCGCGGCTGCTG	8f 518r	567	16S rRNA gene	Edwards et al, 1989
B. thermosphacta	GGACCAGAGGTTATCGAAACATTAACTG TAATACCAGCAGCAGGAATTGCTT	QSF03-BTH-F QSF03-BTH-R	148	*rpoC*	Fougy et al, 2016
C. divergens	CCGTCAGGGGATGAGCAGTTAC ACATTCGGAAACGGATGCTAAT	CB1 CB2R	340	16S rRNA gene	Scarpellini et al., 2002
A.lwoffii	GAAGCTAGAGTATGGGAGAGGA GTCAGTATTAGGCCAGATGGCT	QSF01-ACI-F QSF01-ACI-R	108	16S rRNA gene	Fougy et al, 2016

4.2.2.4.2. PCR conditions for meta-barcoding sequencing

The V1-V3 region of the 16S rRNA gene (567 bp) was amplified by PCR with primers 8f and 518r (Table 17). Partial Illumina TruSeq adapter sequences were added to the 5' end of the reverse primer. The PCR mixture was composed of 1 x Phusion GC buffer (Thermofisher scientific, France), 200 µM deoxynucleoside triphosphate (dNTP) mix, 0.2 µM each primer, 2.5% dimethyl sulfoxide (DMSO), and 50 to 250 ng of DNA and 1 U of Phusion polymerase (Thermofisher scientific, France). Four replicate reactions were performed for each sample with the following conditions: an initial denaturation step (98 °C for 30 s) followed by 15 cycles comprising a denaturation step (98 °C for 10 s), a primer annealing step (60 °C for 30 s), and an extension step (72 °C for 10 min). At the end a final extension was performed at 72 °C for 10 min. Sequencing adapters and sample specific 8 bp barcodes were added in a second PCR after ExoSAP (Thermofisher scientific, France) purification of the pooled PCR products. The PCR reaction consisted of 1 x Phusion GC buffer, 200 µM dNTP mix, 0.2 µM each adapter (full-length TruSeq P5 and Index containing P7 adapters), 2.5% DMSO, and from 4 to 8 µl of purified PCR product. The cycling conditions consisted of an initial denaturation step (98 °C for 30s) followed by 18 cycles comprising a denaturation step (98 °C for 10 s), a primer annealing step (65 °C for 30 s), and an extension step (72 °C for 10 min). At the end a final extension at 72°C for 10 min was performed. The PCR products were purified and quantified at the Institute of Biotechnology, University of Helsinki, where the MiSeq sequencing was performed.

4.2.2.4.3. Metagenomes preparation for sequencing

Depending on the quantity of DNA required for sequencing, varying numbers of (100 ng) samples with same bacterial profile identified by meta-barcoding were pooled. The DNA solutions were concentrated in a SpeedVac system (Thermofisher scientific, France) to obtain a final volume of 30 µL

of purified DNA. DNA concentration was measured with a NanoDrop Thermo Fischer Scientific, France), prior sequencing.

4.2.2.4.4. Metatranscriptome preparation for sequencing

The RiboZero kit for bacteria (Illumina, United States) was used for rRNA depletion following the manufacturer instructions. Then the RNA solution was fragmented and converted into cDNA with the Illumina ScriptSeq kit (Illumina, United States) before purification on the MiniElute kit (Qiagen, Germany). The sequencing libraries for cDNA (barcodes and adaptors ligations) were performed according to the Illumina procedure prior sequencing.

4.2.2.5. Next Generation Sequencing (NGS) data analysis

All cDNA, PCR-fragment and genomic DNA samples were sequenced with Illumina technologies *i.e.* with HiSeq, MiSeq and NextSeq technologies, respectively (figure 26). After sequencing all the reads are analysed with fastQC software (Babraham Bioinformatics) to check the quality of sequencing.

The Mothur procedure was followed to analyse the metabarcoding sequencing outputs (Schloss et al., 2009). Reads were demultiplexed according to barcode sequences with cutadapt. The reads were trimmed and filtered with a quality score threshold of 20 and a minimum length of 100 bp. Chimeric sequences were detected using Uchime integrated into the Mothur procedure and were removed from the dataset prior any bioinformatic analysis (Haas et al., 2011).

The unique sequences were identified by mapping against the silva_nr_123 database for taxonomic assignation. Those sequences were clustered according to the Mothur procedure and relative abundances were estimated by counting the number of reads mapped on OTUs sequences. After quality trimming, chimera removal, OTU picking and taxonomic assignment of OTUs, the data were transformed to a biom file that contains the OTU table and taxonomic assignment for each OTU. Unwanted sequences as chloroplast sequences, not removed with Mothur procedure were removed with phyloseq package (R) (McMurdie & Holmes, 2013).

Metagenomic reads were assembled with both IDBA (Peng et al., 2012) and Velvet (Zerbino, 2010) software using the following kmer lengths: 57, 61, 74 with Velvet and 80 and 100 with IDBA. Contigs were load on MGrast server (Metagenomic Rapid Annotations using Subsystems Technology - Meyer et al, 2008) for a fast automatic annotation and were also annotated using Prokka (Seemann, 2014).

The use of Metaxa2 (Bengtsson et al., 2011) provided the taxonomic assignation of bacterial species in metagenomes by mapping against the Ribosomal Database Project database (RDP II) (Cole et al., 2005).

For metatranscriptomics, after a quality control check, reads were trimmed according to a quality threshold of 20. The reads were aligned against the *Gallus gallus* genome with Bowtie2 software (Langmead & Salzberg, 2012) to identify chicken reads. The remaining reads were loaded on MG-Rast website (Metagenomic Rapid Annotations using Subsystems Technology) (Meyer et al., 2008) for automatic annotation. After removing reads issued from *Gallus gallus*, the remaining reads were analysed by mapping against a database created with the publicly available genomes from 60 species (corresponding to 16 genera) present in poultry meat microbiota. The reads were aligned against this database with Bowtie2 software to identify the functions expressed and their relative abundances.

4.2.2.6. Statistical analysis

For 16S results output the biom file from mothur was analyzed with the Phyloseq package (R) (McMurdie & Holmes, 2013).

The metabarcoding reads (OTUs) were normalized by dividing the number of reads of each OTU by the sum of all OTU reads per samples. Some α and β diversity measures, Shannon and inverse Simpson indices, were visualized using the raw count data. Then β diversity with Bray-Curtis dissimilarity index and visualization with PCoA ordination on the normalized data were performed. Relative abundance plots were designed by merging data in Phyloseq package.

We used the MG-Rast server, developed to perform statistical analyses, for a fast checking of the metagenomes and metatranscriptomes data. Krona application included in MG-Rast was used to visualize taxonomic diversity of metagenomes.

For metatranscriptomics, differential expression analysis (between 2 conditions) was conducted using the Bioconductor DESeq2 package in R environment R (Love et al., 2014). Library effective size normalization was performed for each metatranscriptomic samples. P-values were adjusted for multiple testing using Bonferroni procedure which assesses the false discovery rate (Reiner et al., 2003). Gene with adjusted p-values < 0.01 and with log2foldchange > 2 or < -2 were considered to be differentially expressed between the two chosen conditions. Venn diagrams were performed with Venny application (Oliveros, 2007-2015).

4.2.2.8. Data accession numbers

The fastq formatted and quality filtered read sequences have been deposited at the European Nucleotide Archive (ENA) under the project accession number xxx with the accession number xxx
The 16S rRNA gene amplicon sequences and the metagenomic sequences were deposited in the Sequence Read Archive (SRA) at EBI (accession number ERP001021). The metagenomic sequences were deposited also in the MG-RAST server (http://metagenomics.anl.gov).

4.2.3. Results

4.2.3.1. Growth dynamics during storage

Challenge tests were performed in duplicate for both microbiota E and U and a non-inoculated control was included. Inoculation level was 2 log higher (~ 5 log CFU/g) than the indigenous microbiota. Meat was then stored at 4 °C under the three different gaseous atmospheres and bacteria were enumerated at day 0 and during storage (T2, T4, T7 and T9). Dynamics of total aerobic mesophilic counts for MAP A are presented in figure 27.

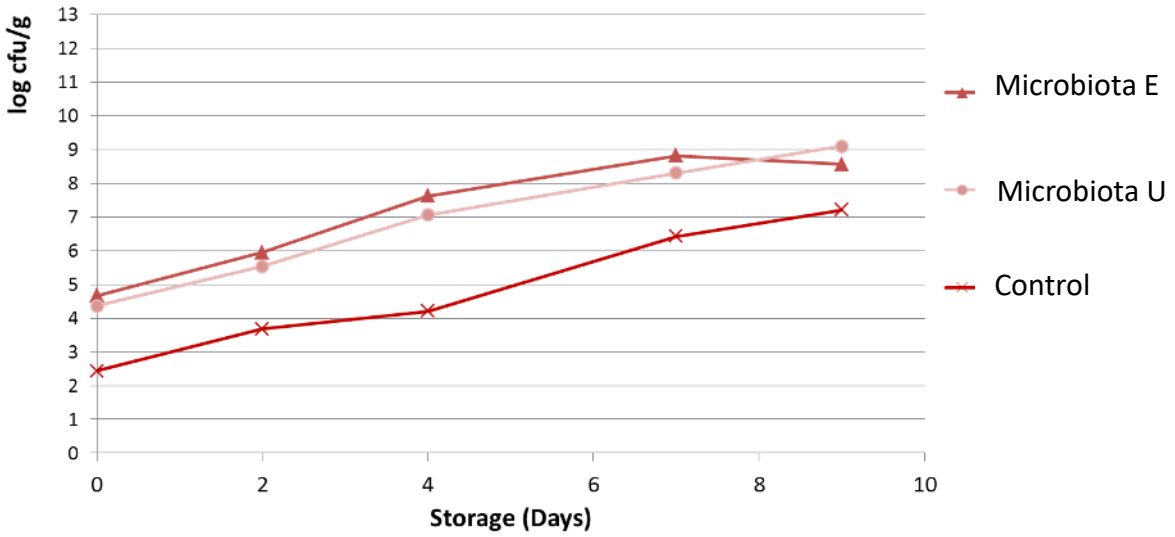

Figure 27 Challenge-tests of microbiotas E and U inoculated on chicken breast dices and incubated under modified atmosphere packaging A (70% O_2 - 30% CO_2) stored at 4°C.
Total aerobic mesophilic bacteria were enumerated at T0, and then at day 2, 4, 7, and 9. A non-inoculated control was also performed.

At day 0, total aerobic mesophilic counts in inoculated samples were around 4.5 log CFU/g. After 9 days the counts reached 8-9 log CFU/g. The indigenous microbiota of the non-inoculated control was 2.43 ± 0.1 log CFU/g at day 0 and reached only 7 log CFU/g at day 9. The 2 log CFU/g difference between inoculated and non-inoculated samples remained during the whole storage period showing that inoculated microbiotas E and U dominated the indigenous contaminants all along the storage. Storage under MAP B and air gave similar results (data not shown) demonstrating that microbiotas E and U overgrow endogenous microbiota whatever the gaseous atmosphere used.

Counts of *B. thermosphacta*, LAB and *Pseudomonas* spp. counts are shown Figure 28.

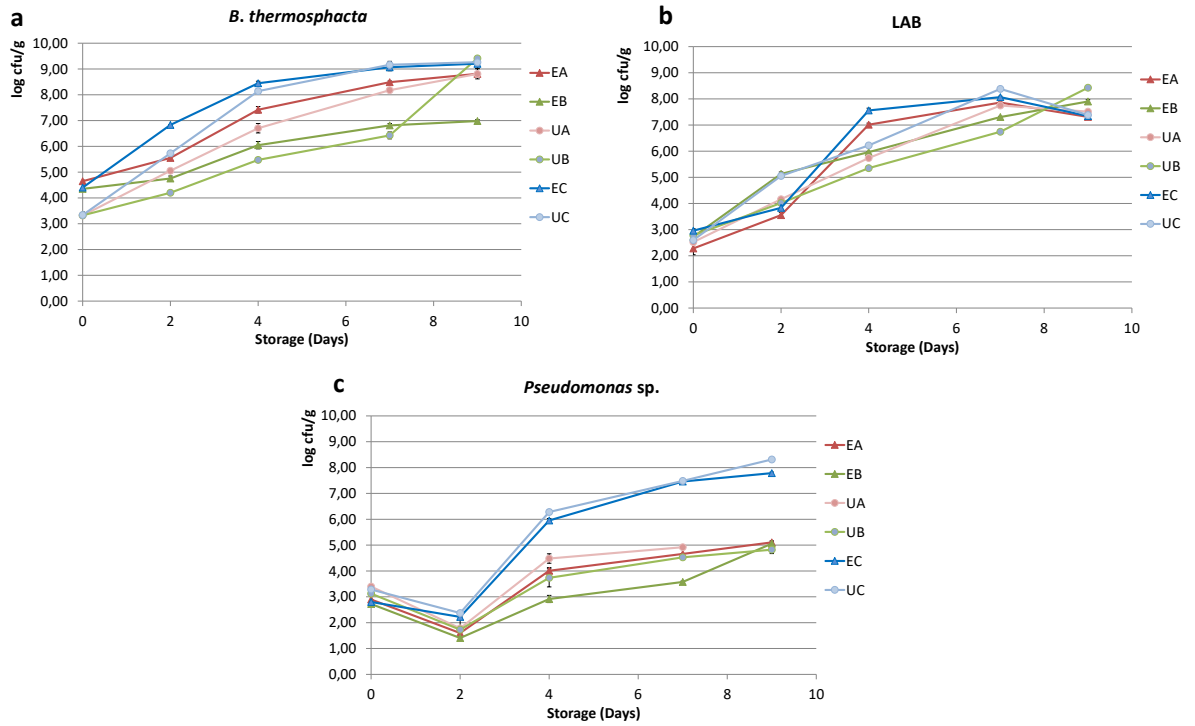

Figure 28 Growth kinetics of *B. thermosphacta* (a), LAB (b) and *Pseudomonas* sp. (c) reimplantation monitored on specific media after inoculation of microbiota E or U and storage under MAP A (70% O_2 - 30% CO_2) or MAP B (50% CO_2 - 50% N_2) or air C (~21% O_2 - 78% N_2).

B. thermosphacta counts were in the same range as the total viable counts. LAB were 1 log lower than the total viable counts while *Pseudomonas* spp. were 3 log lower than the total viable counts for modified atmospheres A and B.

We observed a clear positive effect of air storage on the growth of *Pseudomonas* spp. When compared to MAP A or B (Figure 28 c) and a negative effect of modified atmosphere B on the growth of *B. thermosphacta* (Figure 28 a).

4.2.3.2. Evolution of MAP composition during meat storage

In this study three current poultry meat packaging atmospheres were used: MAP A: 70% O_2 - 30% CO_2, widely used for red meat products, MAP B: 50% CO_2 - 50% N_2 used for various processed meats such as sausages, and air, defined here as C: ~21% O_2 - 78% N_2. The gas composition in each package head space was monitored during 9 days of storage (Figure 29).

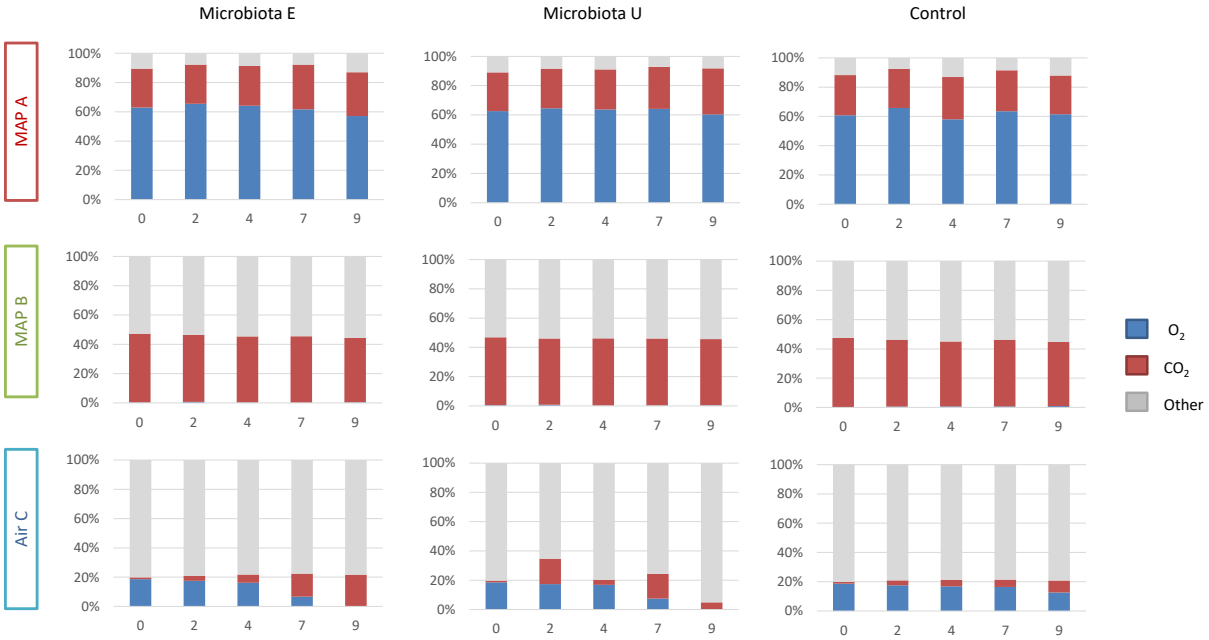

Figure 29 Evolution of gaseous composition in packages during storage of chicken meat at 4°C. O_2 and CO_2 were measured, ratio were completed to 100% for comparison between the 3 conditions for each microbiota inoculated and the control (non-inoculated). Data are the mean of 3 measures.

Under MAP A and B the O_2 and CO_2 concentrations were rather stable during the whole storage. Under air (C) we noticed an O_2 decrease concomitant with CO_2 increase especially in inoculated meat. This may suggest that respiration occurred during storage under air. It could result from the presence of *Pseudomonas* spp. under air but not under MAP B which contained 50% CO2. In MAP A, containing large proportion of O_2, such O_2 consumption and CO_2 production were not observed and *Pseudomonas* spp. growth was weak.

To further investigate the effect of gaseous atmospheres on the microbial communities, nucleic acids were collected from inoculated meat. DNA for metabarcoding and metagenomics and RNA for metatranscriptomics could be recovered in sufficient amounts only at day 7 and 9.

4.2.3.3. Bacterial composition of microbiotas

4.2.3.3.1. Description at genus level (meta-barcoding)

A total of 717 660 reads was obtained, i.e. ~10^5 reads per sample. Identification for taxonomic assignation and estimation of relative abundance of genus and species were performed. The ordination plot drawn Figure 30 shows that sample clusters were clearly depending on the atmosphere composition. Even though the microbiotas inoculated were different at day 0. For each MAP composition, no statistical difference between microbiotas was observed at day 7 and day 9.

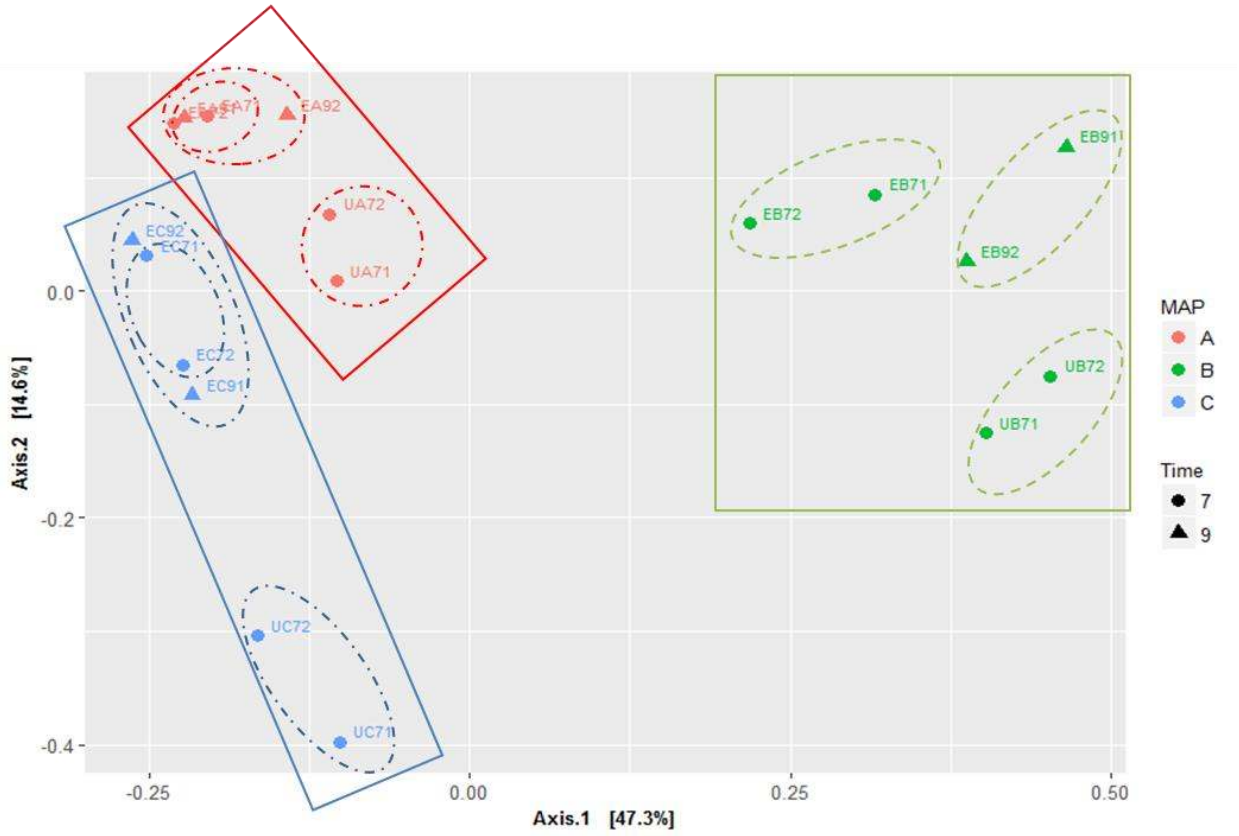

Figure 30 β diversity with Bray-Curtis dissimilarity index and visualization with PCoA ordination on the normalized data

For both microbiota and whatever the day of collection, the duplicates were very homogeneous (see Figure 31). This was confirmed by α and β diversity measures, i.e. Shannon and inverse Simpson indices visualized using the raw count data. Challenge tests performed on this study show in advantages of the use of standard meat microbiota for reproducibility and also repeatability of experiments.

For microbiotas E, observation of microbial profiles (Figure 31) and a statistical ANOVA analysis showed no significant differences between each samples at day 7 or 9.

4.2.3.3.2. Influence of MAP on dominant genera

Under air (C) the most abundant genus was *Brochothrix*. This genus was also dominant under MAP A but under MAP B *Brochothrix* genus was co-dominant with *Carnobacterium*. The 2nd most dominant genus under control conditions (air C) was *Acinetobacter*. This genus was not observed under MAP A or B but only observed under air. Regarding *Carnobacterium* genus, their proportion decreased under air, increased under MAP A and became the dominant genus under MAP B.

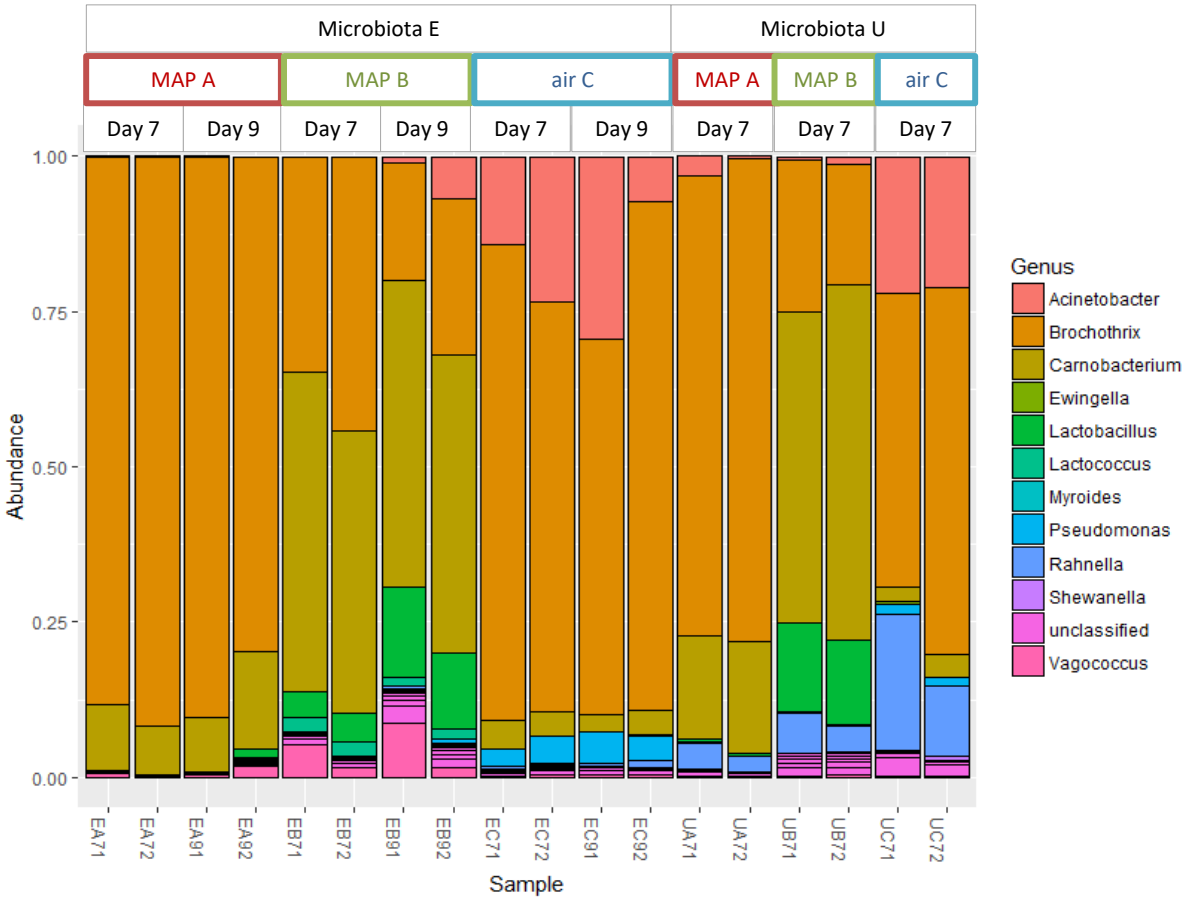

Figure 31 Relative abundance identified by meta-barcoding after inoculation of microbiota E (after 7 and 9 days) or microbiota U (after 7 days) under MAP A (70% O_2 - 30% CO_2) or MAP B (50% CO_2 - 50% N_2) or air C (~21% O_2 - 78% N_2).

Results obtained for the two repeats (1 and 2) are shown.

Therefore, it appeared that whatever the nature of the microbiota inoculated, the gaseous atmosphere shaped the dominant microbial communities during the cold storage. MAP A promoted essentially *Brochothrix* and *Carnobacterium* whereas, to a second extent, MAP B led to *Carnobacterium* dominance followed by *Brochothrix*.

Result of relative abundance were checked and validated by qPCR on 3 dominant species *i.e. B. thermosphacta*, *A. lwoffi* and *C. divergens*. As a selective media exist for *Brochothrix* enumeration, we compared the data obtained by 16S metabarcoding, qPCR and plate counting (Figure 32).

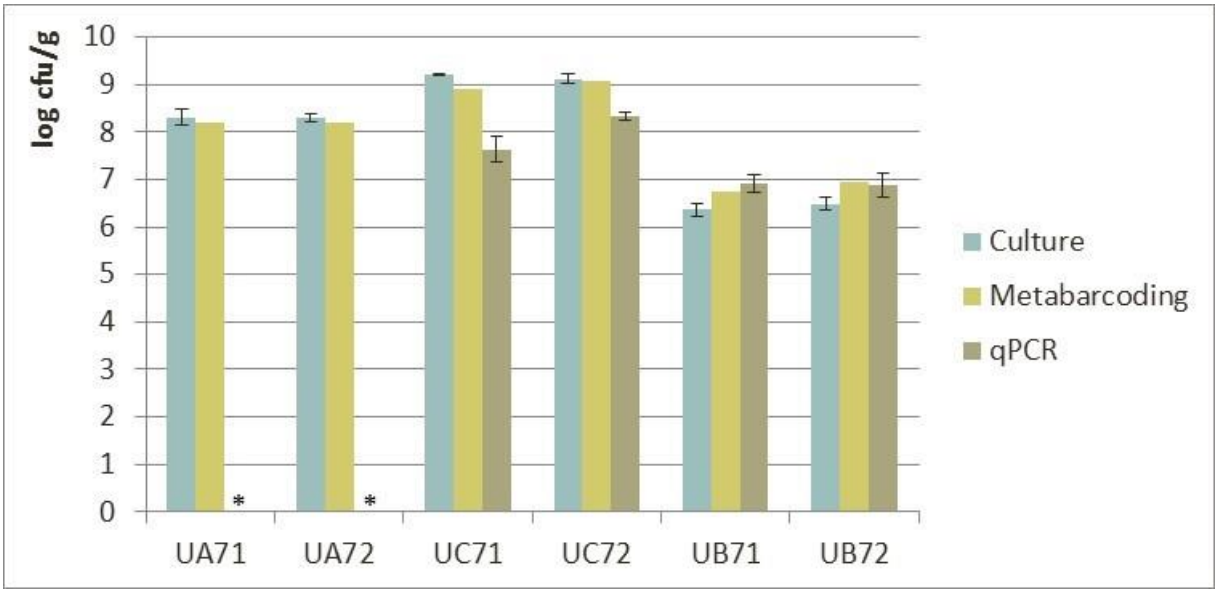

Figure 32 Comparison of *B. thermosphacta* quantification by different methods.
Not determined results are identified by *.

A quite good correlation was observed between results obtained from culture, metabarcoding and qPCR.

4.2.3.3.3. Description of the sub-dominant genera

Regarding the sub-dominant genera, some differences depending on the MAP could be observed. *Pseudomonas* sp. were identified only under air whereas *Lactobacillus* sp. were found only in samples stored under MAP B. In addition, *Rahnella* were identified only in microbiota U.

To summarize *Brochothrix* and *Carnobacterium* were the two dominant genera in the microbiota E and U under MAP A and MAP B. *Brochothrix* was dominant in gaseous atmospheres containing O_2 (MAP A and air C) whereas *Carnobacterium* was dominant under MAP without O_2 and enriched with CO_2 (MAP B). To a lesser extent, *Acinetobacter* was observed only under air. The sub-dominant genera were *Lactobacillus, Pseudomonas* and *Rahnella*. *Pseudomonas* was observed only in meat stored under air correlating with plate count determination.

4.2.3.3.4. Description at species level (metagenomics)

To deeper describe microbiotas at species level and to validate metabarcoding data, metagenomes were also sequenced. Two different metagenomes were constituted by pooling DNA from different samples as follows:

- Metagenome A001 was constructed with the DNA pooled from two replicates of samples E under air C at time 7. This one was use both to validate metabarcoding data and to describe bacterial species.
- Metagenome A002 was constructed with the DNA pooled from two replicates of samples E under MAP B at time 7 and time 9 and with the two replicates of samples U under MAP B at time 7 and under air C at time 9. This one was used to cover the species diversity encountered in our various samples.

The taxonomy identification of bacterial species of metagenomes was performed according to 2 different ways. Each IDBA assembled metagenome was re-annotated with MG-Rast to obtain taxonomy and function assignations. Figure 32 shows the different taxonomy assignations observed for each metagenomes annotated with MG-Rast.

Contamination of prokaryotic DNA by eukaryotic DNA is unavoidable and could be explain by residual chicken DNA or fungal and moulds DNA. Metagenome A002 shows the largest part of chicken DNA contaminations. In addition if abundance is observed, the metagenome is the less diverse despite of the number of samples pooled in this metagenomes samples. MG-Rast taxonomic identification used different databases available on MG-Rast server gives a first idea of the bacterial diversity present in each metagenome.

The richness of the taxon identified in metagenome A001 resulting from DNA extracted from microbiota E after storage for 7 days under air partially correlated with metabarcoding results (Figure 33 A). *Brochothrix* genus accounted for 32% of the reads, but only half was assigned to *B. thermosphacta*, the second half being assigned to the second species belonging to *Brochothrix* genus, namely *B. campestris*. In addition, 14% of the reads in metagenome A001 were assigned to an uncultured bacterium which was not detected by metabarcoding. Several LAB (*L. sakei, L. fuchuensis, L. curvatus, C. maltaromaticum, Carnobacterium galinarum, C. divergens, Enterococcus phoeniculicola Enterococcus faecalis, Enterococcus* sp.) accounted each for 0.8-3% of the reads. *Gammaproteobacteria* including *Acinetobacter* and *Pseudomonas* were also accounted for a few percent of the reads identified. Several *Enterobacteriaceae* associated to insects or plants were also identified.

A large number of species were identified in metagenome A002 including those described above. Several additional minor *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Vagococcus* and *Pediococccus* species were also noticed.

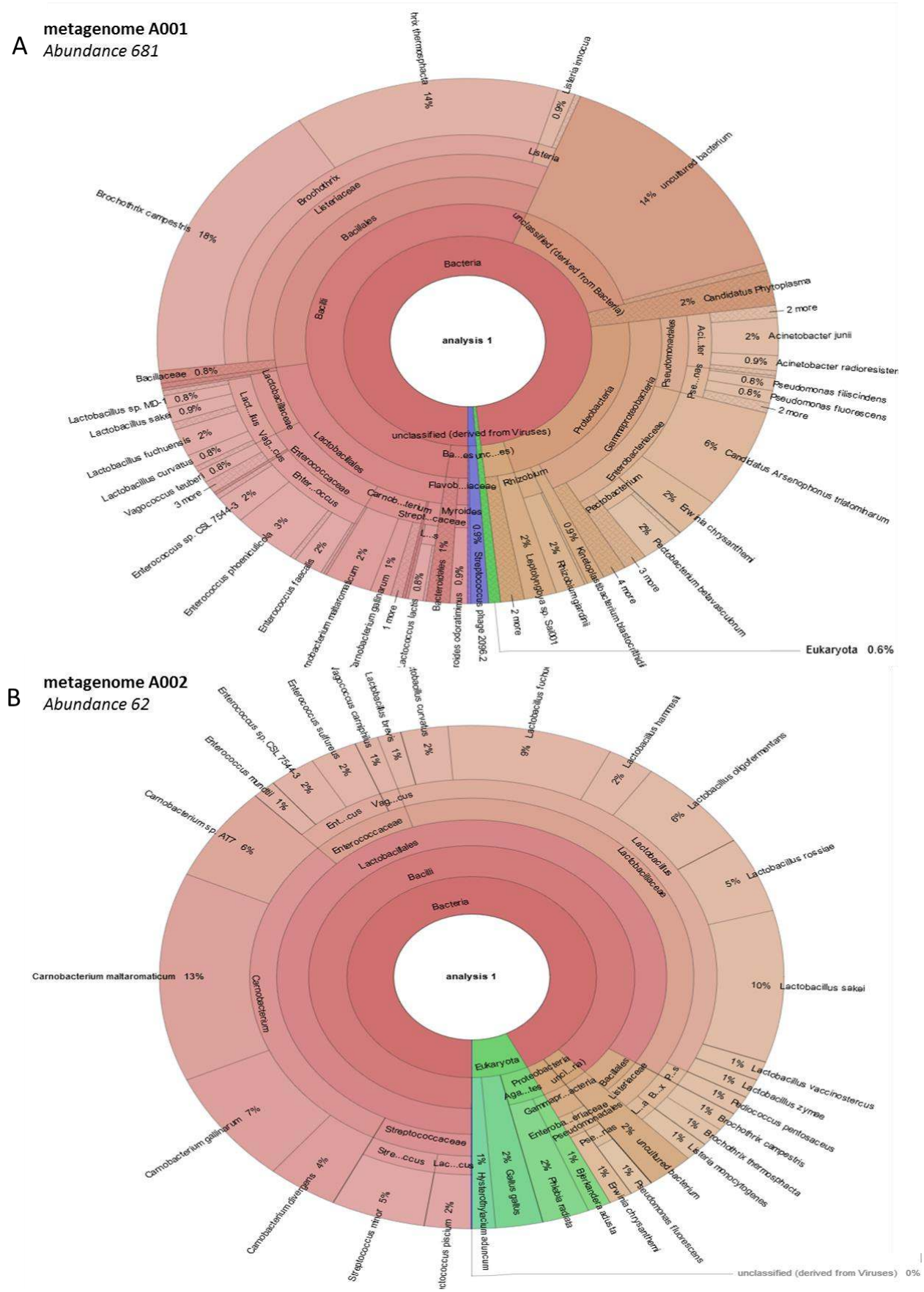

Figure 33 Taxonomy assignation of 3 metagenomes annotated with MG-Rast server.
Eukarotics assignation are in green, viruses in blue and bacteria in red.

The power and relevance of the database used by MG-Rast for taxonomic identification have to be interpreted carefully. To complement the identification details, taxonomic assignation with Metaxa2 and RDP was also performed with metagenomes. Some rRNA of mitochondria and fungi (*Agaricomycetes Saccharomycetales* and *Craniata* families) were found. Regarding bacteria, the families listed below were identified:

- *Flavobacteriaceae* especially *Myroides* genus
- *Listeriaceae* identified as *Brochothrix* or *Listeria* genera
- Carnobacteriaceae represent by *Carnobacterium* genus (C. *divergens* and C. *maltaromaticum* species and another genus *Trichoccus* is found)
- *Enterococcaceae* especially E*nterococcus* and *Vagococcus* genera
- *Lactobacillaceae* represented by *Lactobacillus* genus
- *Leuconostocaceae* identified as *Weissella* genus and additionnal *Leuconostoc* genus found
- *Streptococcaceae* represented by *Lactococcus* genus
- *Shewanellaceae* especially *Shewanella* genus
- *Enterobacteriaceae* represented by *Rahnella*, *Ewingella* and *Yersinia* genera
- *Moraxellaceae* especially *Acinetobacter* and *Psychrobacter* genus
- *Pseudomonadaceae* represent by *Pseudomonas* genus

4.2.3.4. Reference database constitution

Therefore, metabarcoding and metagenomic analyses led to list bacteria present in our microbiota under various condition. The resulting 60 species belonging to 15 genera is summarize table 18. A database with the publicly available genomes from these 60 species was constituted and used as a reference to map RNA reads from metatranscriptomic analysis.

Table 20 List of genome species used in reference database of this study.

Genus	Species	ACCESSION NUMBER
Acinetobacter	*Acinetobacter gyllenbergii*	NIPH_230
	Acinetobacter johnsonii	CIP_64_6
	Acinetobacter junii	CIP_64_5
	Acinetobacter lwoffii	CIP_70_31
	Acinetobacter pittii	MDHS01000001.1
	Acinetobacter soli	CIP_110264
	Acinetobacter venetianus	CIP_110063
Anaerococcus	*Anaerococcus lactolyticus*	NZ_JRMW00000000.1
	Anaerococcus prevotii	NC_013171.1
	Anaerococcus tetradius	NZ_ACGC00000000.1
Brochothrix	*Brochothrix thermosphacta*	NZ_KK211200.1
Budvicia	*Budvicia aquatica*	NZ_ATYS00000000.1
Carnobacterium	*Carnobacterium divergens*	NZ_JQLO00000000.1
	Carnobacterium maltaromaticum	NC_019425.2
	Carnobacterium pleistocenium	NZ_JQLQ00000000.1
Flavobacterium	*Flavobacterium antarcticum*	NZ_ATTM00000000.1
	Flavobacterium cauense	NZ_AVBI00000000.1
	Flavobacterium hydatis	NZ_JPRM00000000.1
	Flavobacterium sasangense	NZ_JMLU00000000.1
	Flavobacterium suncheonense	NZ_AUCZ00000000.1
Janthinobacterium	*Janthinobacterium agaricidamnosum*	NZ_HG322949.1
	Janthinobacterium lividum	NZ_JFYR00000000.1
Klebsiella	*Klebsiella. pneumoniae*	NC_016845.1
Lactobacillus	*Lactobacillus aviarius*	NZ_AYZA00000000.1
	Lactobacillus crispatus	NC_014106.1
	Lactobacillus curvatus	NZ_AGBU00000000.1
	Lactobacillus fuchuensis	NZ_BAMJ00000000.1
	Lactobacillus gasseri	NZ_CP006809.1
	Lactobacillus helveticus	NC_021744.1
	Lactobacillus ingluviei	NZ_CAKF00000000.1
	Lactobacillus johnsonii	NC_005362.1
	Lactobacillus oris	NZ_CP014787.1
	Lactobacillus panis	AZGM01000001.1
	Lactobacillus pontis	NZ_AZGO00000000.1
	Lactobacillus sakei	NC_007576.1
	Lactobacillus salivarius	NC_007929.1
	Lactobacillus sanfranciscensis	NC_015978.1
	Lactobacillus vaginalis	NZ_ACGV00000000.1
Lactococcus	*Lactococcus lactis*	AE005176.1
Myroides	*Myroides. odoratimimus*	CIP_101113
Pseudomonas	*Pseudomonas agarici*	NZ_CP014135.1
	Pseudomonas caeni	NZ_ATXQ01000001.1
	Pseudomonas chlororaphis	CP014867.1
	Pseudomonas extremaustralis	NZ_AHIP01000001.1
	Pseudomonas japonica	NZ_BBIR00000000.1
	Pseudomonas kilonensis	JZXC01000001.1
	Pseudomonas moraviensis	NZ_CM002330.1
	Pseudomonas protegens	NC_004129.6
	Pseudomonas putida	NC_002947.4
	Pseudomonas taetrolens	NZ_JYLA01000001.1
	Pseudomonas viridiflava	NZ_JXQO00000000.1
Psychrobacter	*Psychrobacter alimentarius*	CP014945.1
	Psychrobacter glacincola	NZ_LIQB00000000.1
	Psychrobacter maritimus	NC_021158.1
	Psychrobacter urativorans	NZ_CP012678.1
Rahnella	*Rahnella. aquatilis*	CP003244.1
Shewanella	*Shewanella baltica*	NC_016901.1
	Shewanella frigidimarina	NC_008345.1
	Shewanella putrefaciens	NC_009438.1
	Shewanella xiamenensis	NZ_JGVI00000000.1

4.2.3.5. Functions are expressed by microbiotas according to MAP condition

After cDNA sequencing, 291 540 352 reads (length average 159 pb) were analysed. Seven to 32 million reads per samples were obtained from the sequencing platform (Table 19).

Table 21 Summary of cDNA reads obtained per samples.

Samples	Number of raw reads	% of reminded [1]	Analyzed reads
EA71	16 772 877	46.1%	7 734 906
EA72	13 575 758	32.1%	4 360 036
UA71	14 770 219	43.2%	6 384 846
UA72	19 226 158	38.3%	7 372 131
EB71	12 282 944	33.9%	4 166 373
EB72	8 638 601	33.8%	2 922 399
UB71	18 245 442	27.8%	5 076 623
UB72	16 310 892	16.2%	2 649 516
EC71	32 455 902	68.3%	22 151 916
EC72	12 705 057	44.8%	5 692 480
UC71	10 594 822	37.4%	3 961 199
UC72	22 921 146	46.0%	10 545 175
EA91	10 471 159	20.3%	2 129 701
EA92	7 452 867	27.2%	2 029 028
EB91	26 519 103	16.0%	4 254 134
EB92	18 314 308	21.9%	4 011 535
EC91	15 707 723	39.7%	6 234 736
EC92	14 575 374	41.3%	6 025 038
Total	**291 540 352**		**107 701 772**

[1] % of total raw reads remaining after removal of *G. gallus* reads and filtering

More than half of the reads (63%) were identified as *G. gallus* reads and removed from the analysis. The remaining 107 701 722 reads were further analysed. To identify functions expressed in metatranscriptome samples, functions assignation was performed both on MG-Rast server with M5NR database (Wilke et al 2012) and using the 60 genome database. The MG-Rast analysis identified protein sequences classified by their main functions. After checking that duplicates were homogeneous the differences between the main functions expressed, depending on atmosphere and/or time of storage, were evaluated (Figure 34).

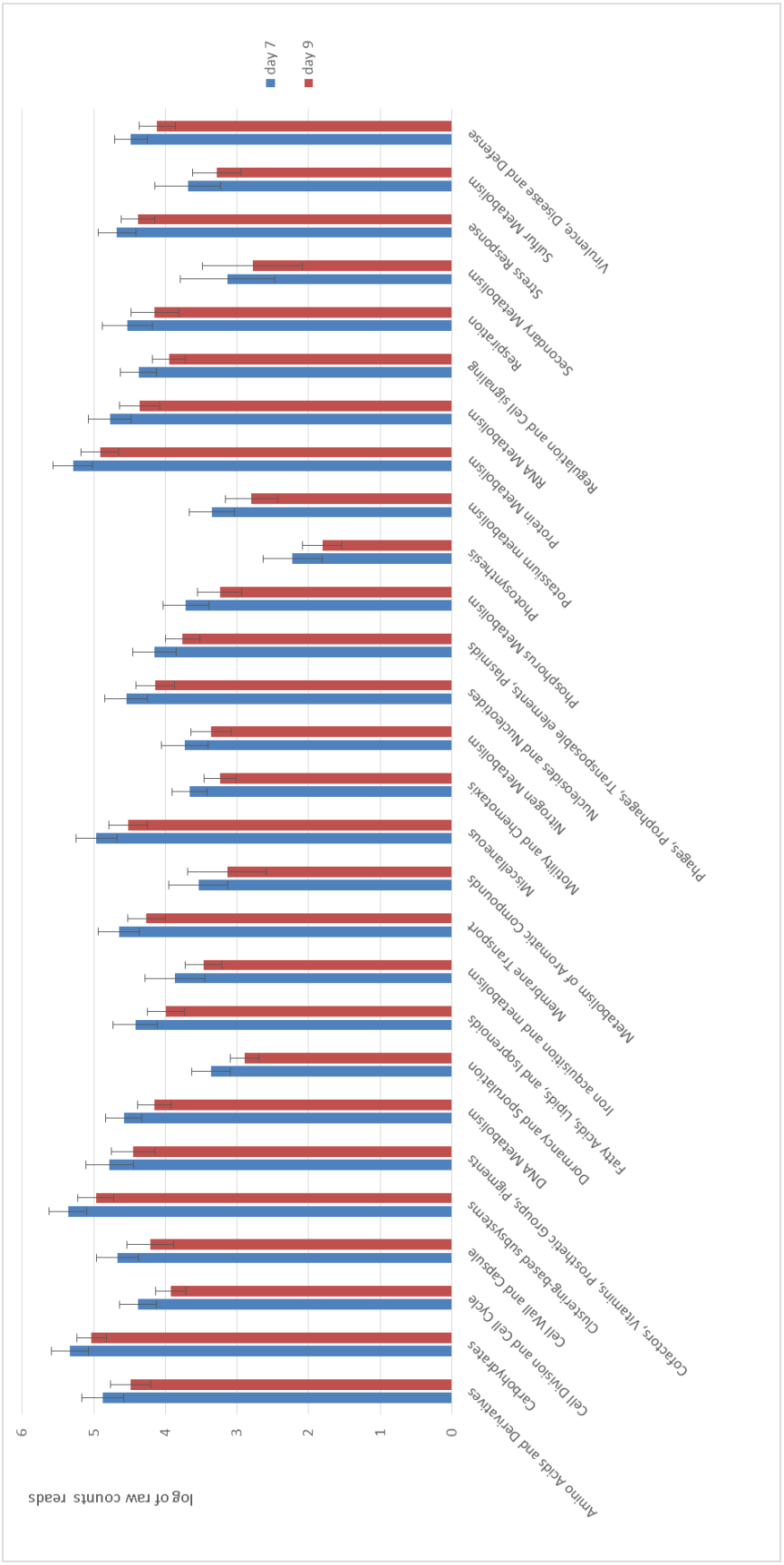

Figure 34 Classification of genes expressed by microbiota E after 7 (blue) and 9 (red) days of storage under MAP A (70% O_2 - 30% CO_2) or MAP B (50% CO_2 - 50% N_2) or air C (~21% O_2 - 78% N_2).

No significant time effect was observed according to the main function categories. A 0.5-1 log difference between day 7 and 9 was observed in MAP A and air C samples, whereas no time effect was observed for MAP B (data not shown). As already observed from metabarcoding, the metatranscriptomic data obtained after MG-Rast annotation, showed that samples from day 7 and day 9 were similar.

A comparison of functional categories expressed by microbiota E (day 7 + day 9) under different MAP is shown Figure 35.

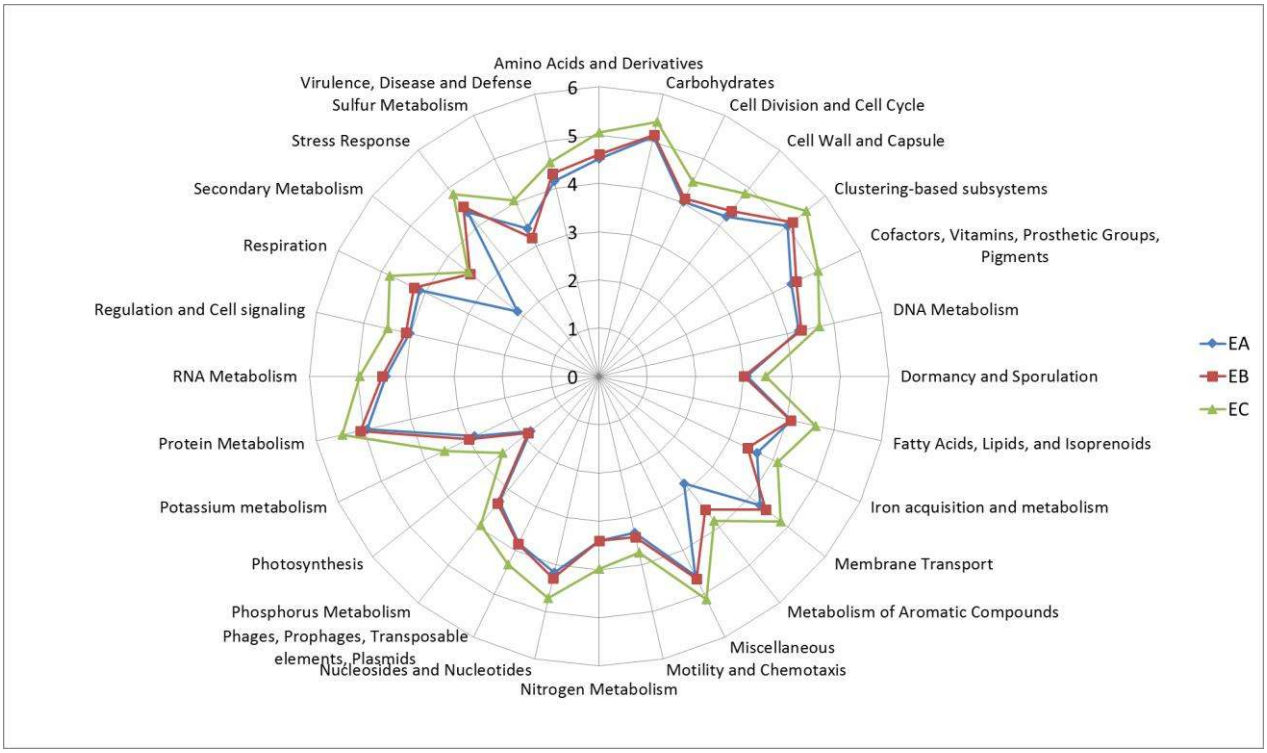

Figure 35 Log of count reads per functional categories observed for each MAP A (70% O_2 - 30% CO_2) or MAP B (50% CO_2 - 50% N_2) or air C (~21% O_2 - 78% N_2).

Globally, it appears that more functions were expressed under air (C). That could suggest that both MAP A and B indeed limit bacterial activity. The most abundant functional categories observed for each MAP condition were carbohydrate metabolism, clustering based subsystems and protein metabolism. In a smaller proportion, some functions identified as Photosynthesis or Dormancy and sporulation classes could be artefactual and required further assignation. Interestingly, metabolism of aromatic compounds and secondary metabolism functions, which could be involved in spoilage, were lower in samples stored under MAP A.

The annotation performed with MG-Rast was useful to have a first overview of different functions expressed in meat samples but to assign expressed genes to species, we mapped metatranscriptome reads against database constituted with the genomes of 60 species available from the NCBI database.

4.2.3.6. Functions are expressed by species according to MAP condition

On average only, 34% of metatranscriptomic reads could be mapped against bacterial genomes from the database. Number of reads mapped per samples were variable from 26% to 48% of the total filtered reads whilst database was created with identified species from metabarcoding and metagenomic result. Actually, mapping against metagenome annotated with Prokka provided identification level of metatranscriptome up to 47% on average. The remaining reads could be identified as mitochondrial or yeast or fungi which were not removed during the procedure of RNA extraction or depletion but were not checked.

After mapping, identified genes according to each conditions were sorted and normalized to see genes differentially expressed. This analysis was performed a first time with all samples using air C sample as reference. Since no statistical difference was observed between samples from day 7 and day 9 (Figure 36), data for samples of time 7 and 9 were pooled. Then agglomerative hierarchical clustering (AHC) of metatranscriptome samples were drawn (figure 36).

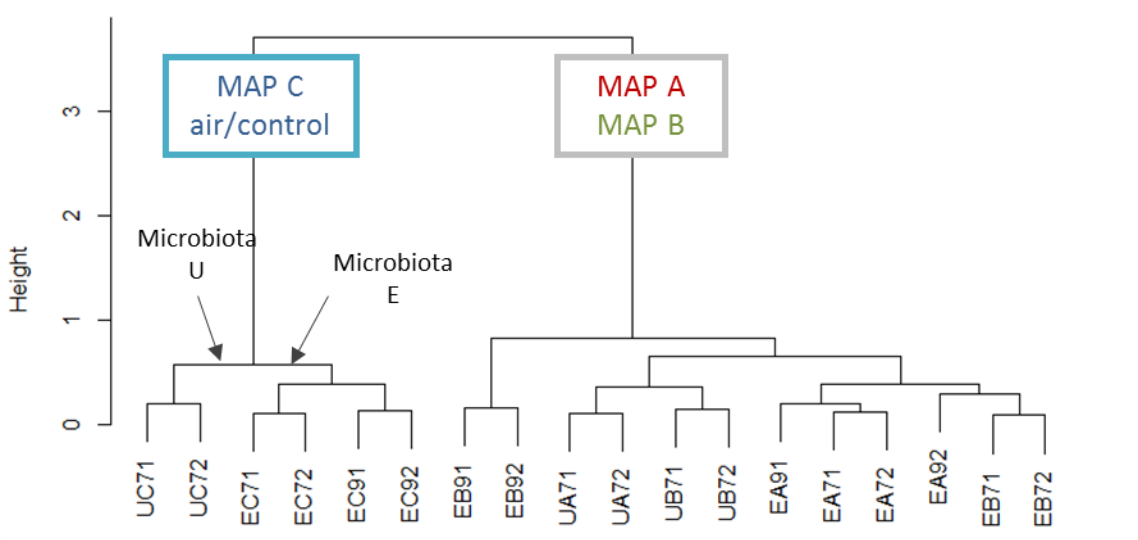

Figure 36 Agglomerative hierarchical clustering (AHC) of metatranscriptome samples from total read counts of 24 032 genes

Agglomerative hierarchical clustering of metatranscriptome data clearly cluster samples with air storage statistically different from to the other MAP. Interestingly we noticed that in the MAP clusters, samples were also cluster according to the microbiota except for samples EB91 and EB92. In addition we found that duplicates were almost all cluster by two, except for samples EA91 and EA92 (Figure 36).

For both microbiota, samples issued from MAP A, B or C were compared in order to determine genus that were differentially expressed depending on the storage atmosphere (Figure 37). As modified atmospheres (A or B) are proposed to improve the shelf-life of poultry meat, we focused on the genes that were up-regulated under those MAPs, by comparison to storage under air (Figure 38).

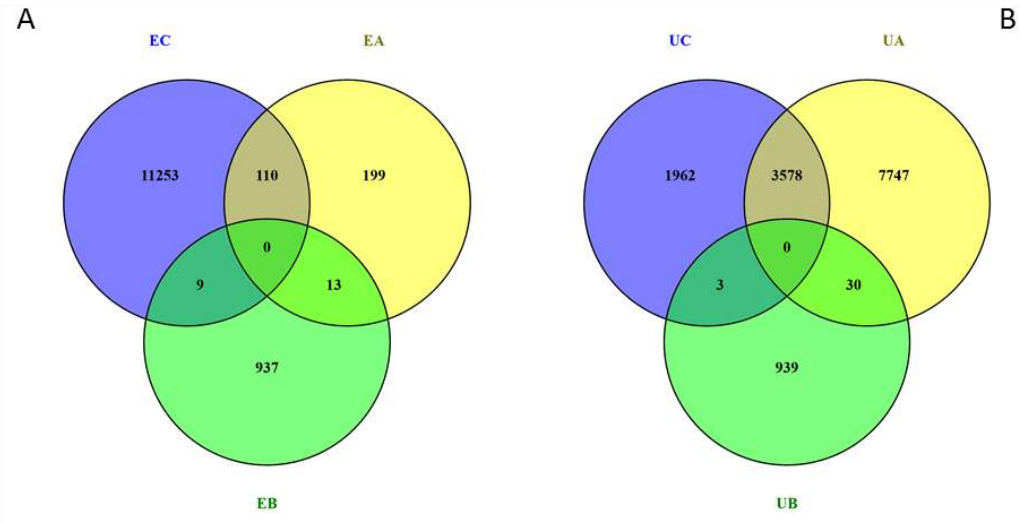

Figure 37 Venn diagram with the number of genes differentially expressed according to the MAP condition MAP A (70% O_2 - 30% CO_2) or MAP B (50% CO_2 - 50% N_2) or air C (~21% O_2 - 78% N_2) for microbial communities E and U.

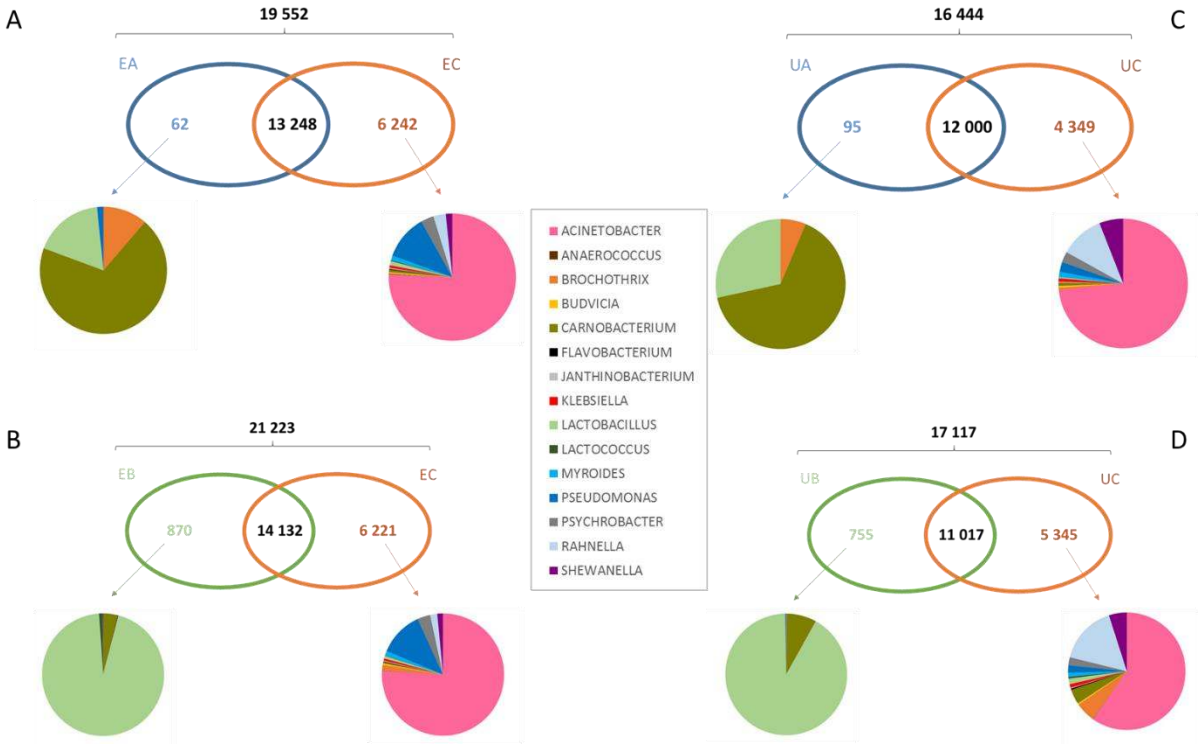

Figure 38 Differentially expressed genes and their taxonomic assignation depending on the conditions.

The number of genes differentially expressed by microbiota E (left panel) and U (right panel) are indicated. For each microbiota, the metatranscriptomes from MAP A (70% O_2 - 30% CO_2) or MAP B (50% CO_2 - 50% N_2) were compared to those obtained after storage under air (C) (~21% O_2 - 78% N_2) giving the 4 Venn diagrams. Black numbers at the top of each diagram indicate the total number of genes. Grey numbers indicated those whose expression did not vary. Colored numbers indicated differentially expressed genes. Each pie chart details the taxonomic assignation of each pool of differentially expressed genes.

The first observation was that more genes were up-regulated under air (from 4349 to 6262 genes) than under condition A (62 to 95 genes) or condition B (755 to 870 genes). This could be explain by a high metabolic activity of bacterial species under air/control compared to MAP A or B that are proposed to improve meat shelf life. We identified that most part of up-regulated genes could be attributed to *Acinetobacter* genus and from *Pseudomonas* in microbiota E or *Rahnella* and *Shewanella* in microbiota U.

As we decided to focus on the effect of storage under modified atmosphere, we examined which functions were especially up-regulated by microbiota E and U, after storage under MAP A or B, by comparison to air storage. Indeed, as modified atmosphere packages are supposed to improve microbial safety of poultry meat, it is meaningful to identify if undesirable functions are still expressed under MAP, or on the contrary if beneficial functions are over-expressed. The detailed list of such up-

regulated genes is shown in Annex 1. The assignation of these genes to the various species is shown (Figure 39).

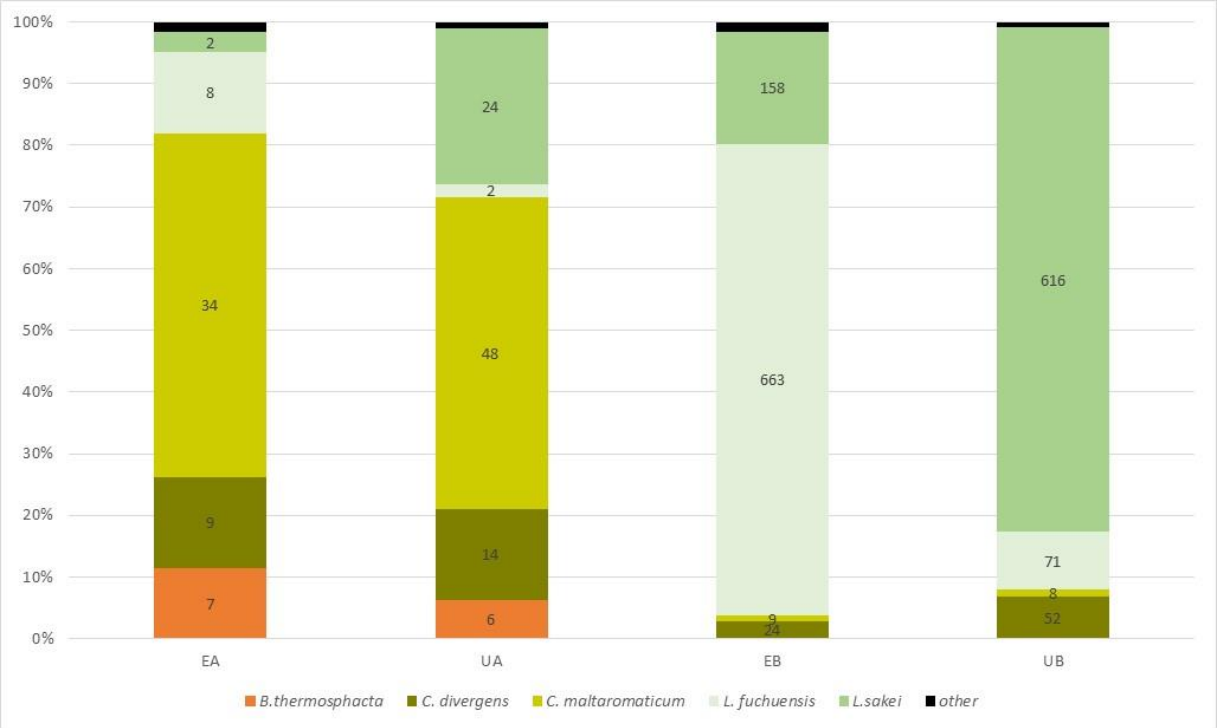

Figure 39 Species assignation of up-regulated genes
The number of genes up-regulated by microbiota E and U under condition A (70% O_2 - 30% CO_2) or B (50% CO_2 - 50% N_2) compared to air.

Under MAP A storage, a large part of up-regulated genes was attributed to *C. maltaromaticum* and then to *B. thermosphacta, C. divergens*, *L. sakei* and *L. fuchuensis* were observed. In MAP B the active species were mostly *L. sakei* followed by *C. divergens* in microbiota U and *L. fuchuensis* followed by *C. divergens* in microbiota E.

In order to describe which functions were up-regulated, each gene position and identification was verified by comparing to genome annotations available on Mage Microscope annotation platform. Genomes of *B. thermosphacta* DSM 20171, *L. sakei* 23K, *Carnobacterium maltaromaticum* LMA28 or *C. divergens* V41 were used. When EC number was available and relevant those were used to reconstruct metabolic pathways. Because curated annotation was not available on the platform for *L. fuchuensis,* we considered the annotations provided by bowtie2, and then compared the annotations to the genome of *L. sakei*, as both species are closely related.

4.2.3.6.1. Microbiota E and U under O_2 and CO_2 enriched atmosphere

The up-regulated genes in MAP A vs air were similar in microbiota E and U. Although *B. thermosphacta* was the dominant species (Figure 31) only 7 (in microbiota E) and 6 (in microbiotas U) genes were up-regulated by this species. The over-expression of the ribose operon *rbsBCADK,* encoding the ribose ABC-trasnporter (RbsA, RbsB, RbsC) the pyranase (RbsD) and the ribokinase (RbsK) (Figure 40) suggests that ribose is used as a preferred carbon source under MAP A. The ribose operon RbsR gene was no up-regulated, but surprisingly a gene encoding a putative transcriptional regulator, located upstream for the ribose operon but on the opposite orientation was also up-regulated.

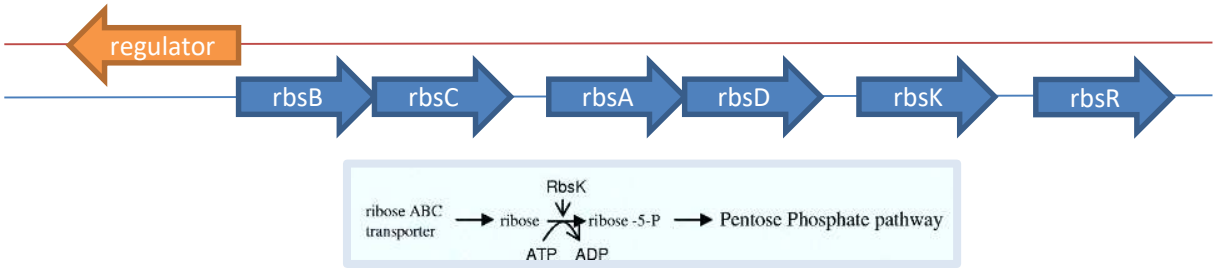

Figure 40 The ribose operon in *B.thermosphacta* adapted from Autieri et al. (2007)

Regarding lactic acid bacteria, *C. maltaromaticum* up-regulated more genes (34 in microbiota E and 48 genes in microbiota U) than *C. divergens* (9 genes in microbiota E and 14 genes in microbiota U) or *L. fuchuensis* (8 genes in microbiota E and 2 genes in microbiota U) and *L. sakei* (2 genes in microbiota E and 24 genes in microbiota U) (Figure 39). *B. thermosphacta, C. divergens*, *C. maltaromaticum* and *L. sakei* up-regulated also the ribose operon. A regulator of unknown functions described was also identified downstream the ribose operon of *C. maltaromaticum* but was not overexpressed. Intriguingly, the rbsR repressor was also up-regulated.

Besides ribose, other genes involved in sugar utilization were also up-regulated. *C. maltaromaticum* up-regulated the gluconate kinase gene showing the utilization of pentulose and hexulose and also an operon involved in maltodextrin degradation (*malDEL* and *mdxE*). In addition, in microbiota U genes encoding several PTS dependent enzymes II putatively involved in maltose or cellobiose transport and utilization were up-regulated suggesting the utilization of complex sugar as carbon sources. At the same time, several genes encoding enzymes involved in the glycolysis pathway were over-expressed by each LAB. Gene encoding enzymes as GlpF (glycerol permease), or GlpO (α-glycerophosphate) were expressed by *L. sakei* in microbiota U suggesting glycerol utilization as carbon source. GlpF internalizes glycerol and GlpO catalyzes the dihydroxyactetone (DHA-P) production from glycerol-P with O_2 as cofactor. Lactobacilli expressed also gene encoding enzymes involved in the last steps of glycolysis to drive compounds to acetate pathway as lactate oxidase or pyruvate oxidase. In addition, transport of

thiamine was also up-regulated by *C. maltaromaticum*. Thiamine is a co-factor for several reactions of glycolysis and amino acid metabolism. Chicken meat is rich in thiamine which could therefore be indeed used as a co-factor (Kim and Bowers 1988).

In both microbiota E and U, we also observed the up-regulation of several genes involved in iron or heme transport. *C. maltaromaticum* up-regulated 10 such in microbiota E and 12 in microbiota U while *C. divergens* up-regulated 4 genes involved in iron/heme transport in microbiota E and 7 in microbiota U. Those proteins are identified as permeases belonging to the fecCD family protein transport or are ABC transporters. *C. maltaromaticum* also expressed a gene encoding a heme degrading monooxygenase (IsdG and IsdC) which releases iron from heme after internalization in the cell. Bacteria could also defend themselves from ROS by over-expressing genes encoding the manganese-dependent super oxide dismutase (Mn-SOD), heme-dependent catalase or NADH oxidase. Mn-SOD gene was over-expressed by *C. maltaromaticum* in microbiota E and catalase gene by *L. sakei* in microbiota U. In addition, several genes encoding proteins identified as involved in oxidative stress response were listed as reductases and oxidase: ferredoxin reductase or nitroreductase in microbiota E (4 genes) by *C. maltaromaticum* and *L. fuchuensis* and by *C. maltaromaticum* and *L. sakei* in microbiota U (7 genes).

Therefore, MAP A which was enriched in O_2 which could considered as an oxidative stress condition for microbiotas. Oxidative stress in bacteria is a contentious subject (Imlay 2015). ROS created by oxidative stress such as O_2^- and H_2O_2 can damage enzymes and may also cause mutations. Activated by heme, the catalase has a major role in oxidative stress decrease by degradation of H_2O_2. Iron released in the cell by fecCD or fecE transporters could be further reduced by different ferredoxins which was found as differentially expressed in this study. Iron may also be used as a cofactor for the NADH dehydrogenase (co-factor Iron-S) or ribonucleotide reductase reactions (co-factor Fe cation 1.17.4.1) explaining why *Carnobacteria* over-expressed genes involved in transport of iron and heme.

We also observed that function involved in the use of amino acids as nitrogen source, as for example allantoin, derived from purine. Lactic acid bacteria in E and U microbiotas up-regulated genes encoding this pathway which convert allantoin to ammonia and CO_2. Several genes were expressed by *Carnobacterium* as the allantoinase gene or allD, allC and allE genes (Figure 41). This correlates with the presence of allantoin in poultry plasma after feeding the animals with inosine-supplemented diets (Simoyi et al., 2003).

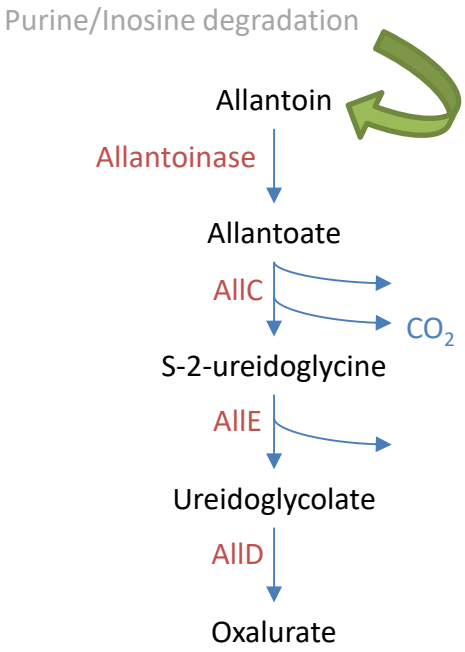

Figure 41 Schema of allantoin pathway adapted from Lee et al. (2013)

Genes encoding transport and/or degradation of other amino acids were also up-regulated as for example methionine in *C. maltaromaticum* or threonine in *L. sakei*. Genes coding for the enzymes of the arginine deiminase pathway were also up-regulated by *L. sakei.* Arginine can be used as a source of energy, carbon and nitrogen for this bacterium (Figure 42) (Champomier Vergès et al., 1999). This pathway has been shown to be expressed when limited amounts of glucose were available and also under aerobiosis (Champomier Vergès et al., 1999).

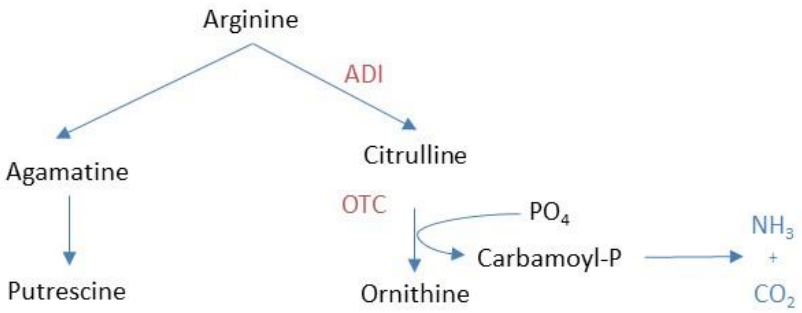

Figure 42 Arginine degradation pathway adapted from Champomier Vergès et al. (1999)

4.2.3.5.2. Genes up-regulated in MAP B (enriched in CO_2 and N_2) vs air in microbiota E and U

Compared to what we observed under MAP A, more genes were up-regulated in MAP B vs air storage. No gene was over-expressed by *B. thermosphacta* (Figure 42). Genes up-regulated by *Carnobacterium* were quite similar with those found in MAP A involved mainly in the transport of complex sugars and the use of the PTS system to import carbon sources. Ribose operon and enzymes involved in the sugar degradation and fermentation were up-regulating attesting for an active sugar metabolism. Expressed by *C. divergens* only, metabolism of glycerol using FMN as co-factor is also up-regulated. As describe in enriched O_2 atmosphere (see previous section) ADI pathway were also up-regulated. Some anaerobic regulator quite poorly known where also reported suggesting a response to anaerobic condition. The major difference with the observation made in MAP A up-regulated genes was the activity of 2 *Lactobacillus* species: *L. fuchuensis* up-regulated 663 genes in microbiota E and 71 in microbiota U while *L. sakei* up-regulated 158 genes in microbiota E and 616 in microbiota U.

Nevertheless those two closed species regulated similar functions. From the ~750 genes up-regulated by both *L. sakei* and L. *fuchuensis* in each microbiotas, 12% in microbiota E and 25% in microbiota U was coding for unknown or putative functions and were not be further analyzed. Functions involved in cell wall synthesis or cell division, translation, replication, transcription, energy production were identified suggesting an active bacterial growth and multiplication. In microbiota E where *L. fuchuensis* was the most active species, among the 663 up-regulated genes, 47 were involved in cell wall synthesis and cell division, 40 were encoding functions associated to replication regulation, and 105 were in translation and transcription functions. In microbiota U, the 616 up-regulated genes expressed by *L. sakei* were reported as follows: 63 genes were involved in cell wall synthesis and cell division, 31 in replication regulation, and 132 in translation and transcription functions. This suggest that in each microbiota *L. sakei* and *L. fuchuensis* harbored an active cellular machinery in microbiota U and E, respectively.

Other functions linked to meat environment were also up-regulated by *L. sakei* in microbiota U. IN particular the genes enabling the utilization of different carbon sources available in meat were up-regulated by *L. sakei* in microbiota U (Figure 43).

The PTS mannose complex involved in mannose uptake was identified through over-expression of 6 genes encoding EII^{man} ABCD ensuing transport and phosphorylation of mannose and the 2 PTS general enzymes EI and HPr. In addition the PTS enzyme II cellobiose was also reported suggesting the utilization of complex sugars. Genes encoding ribose operon as previously described were also up-regulated. Utilization of other sugars as fructose, galactose, glucose, and mannose were also induced

as indicated by genes encoding several permeases and transporters (GlcU), regulator (FurR) and enzymes involved in degradation (GapA, GalE). Additionally, several genes such as those encoding glycolytic enzymes (Pyruvate kinase, 6-phopho-fructo-kinase, fructose 1,6-biphopahse aldolase, gpmA for example) were up-regulated attesting the utilization of different sugars by the bacteria (Figure 43). Regeneration of NADP(H) was also up-regulated because it was used as cofactor of different stage of glycolysis.

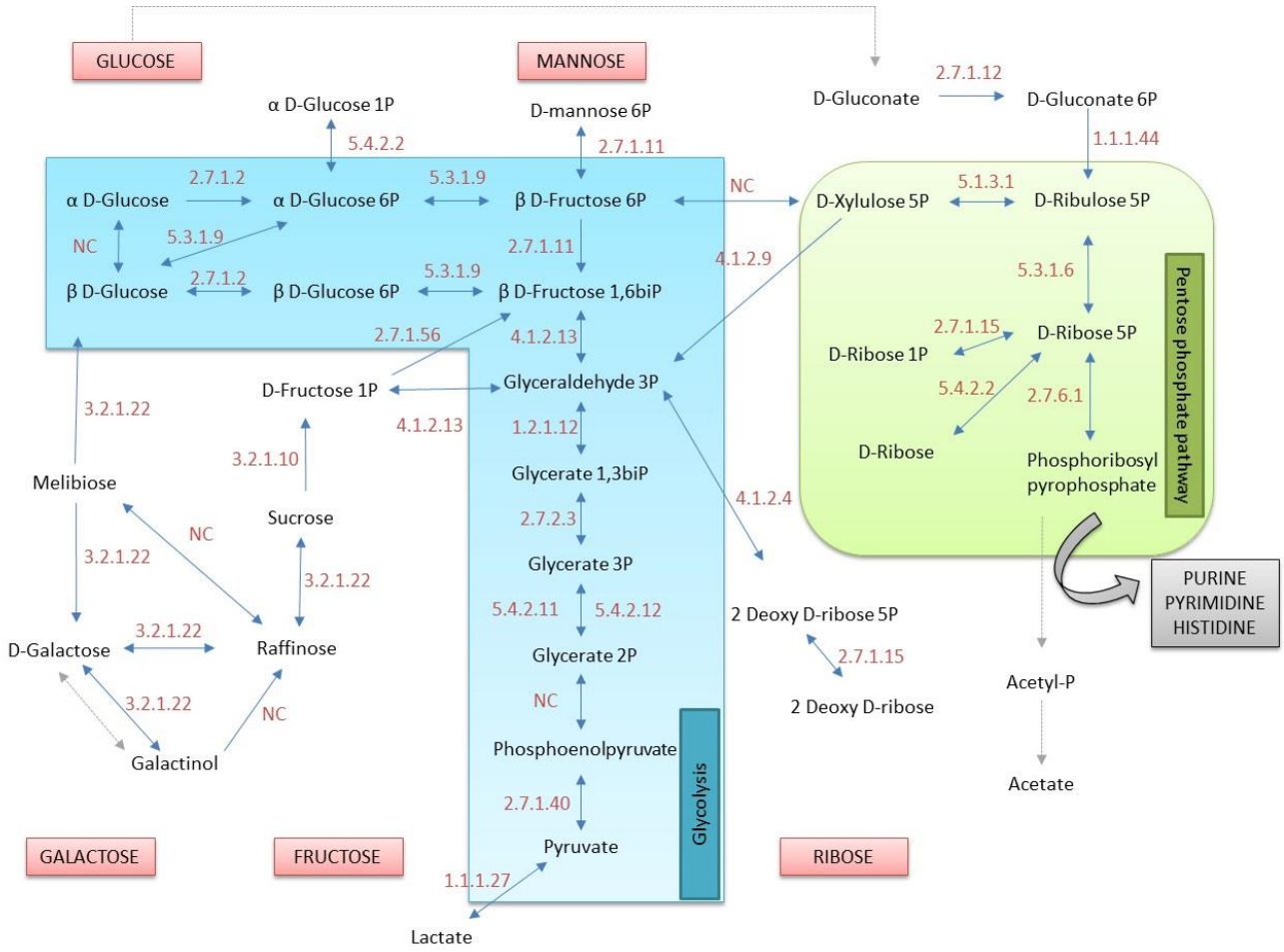

Figure 43 Sugars related functions up-regulated by *L. sakei* in microbiota U.
The genes encoding the various functions are shown. NC indicates that the corresponding genes were not detected as over expressed. EC number of enzymes are indicated.

Utilization of sugar was ensured through heterolactic fermentation and pentose phosphate pathways by acetate kinase and pyruvate degradation metabolism. Production of compound as acetoin may also be induced through the up-regulation of acetoacetate decarboxylase, or synthase enzymes expressed by *L. sakei.*

Pyruvate generated by glycolysis could lead acetoin production because 2 genes encoding acetolactate synthase (EC 2.2.1.6) and α-acetolactate decarboxylase (EC 4.1.1.5) were up-regulated suggesting the production of (R)-2-acetoin, a precursor of butanoate which can be responsible for off-odors.

Acetoacetyl-coA C-acetyltransferase (EC 2.3.1.9) and mevalonate kinase (EC 2.7.1.36) genes were up-regulated suggesting the production of mevalonate from acetyl-coA produced after glycolysis. Pyruvate is also used for Coenzyme A synthesis a co-factor important for several metabolic pathways. This co-factor could also be synthesized from amino acid (alanine, methionine) and fatty acid metabolisms as suggested by the up-regulation of different enzyme involved in those metabolisms.

Genes encoding several functions involved in amino acid metabolism (MetK) or purine metabolism were also up-regulated by *L. sakei.* Amino acid as alanine, arginine, asparagine, and nucleobases as purines (guanine and xanthine) and pyrimidines could be sources of nitrogen or biosynthesized by cells. Metabolism of purine and pyrimidine degradation was as well up-regulated by *L. sakei* with 11 genes involved in this metabolism (for example those encoding PurA, PurE and different reductases and kinases). This observation was correlated to the high number of functions identified as involved in cell machinery and growth of *L. sakei* among the up-regulated genes. In the same way, 6 genes of the F0F1 ATPase involved in energy production were up-regulated. Chaillou et al. (2005) described energy production pathways used by *L. sakei* from the meat. In microbiota U, all those pathways of energy producing were up-regulated in this study suggesting that *L. sakei* was well active in sample U.

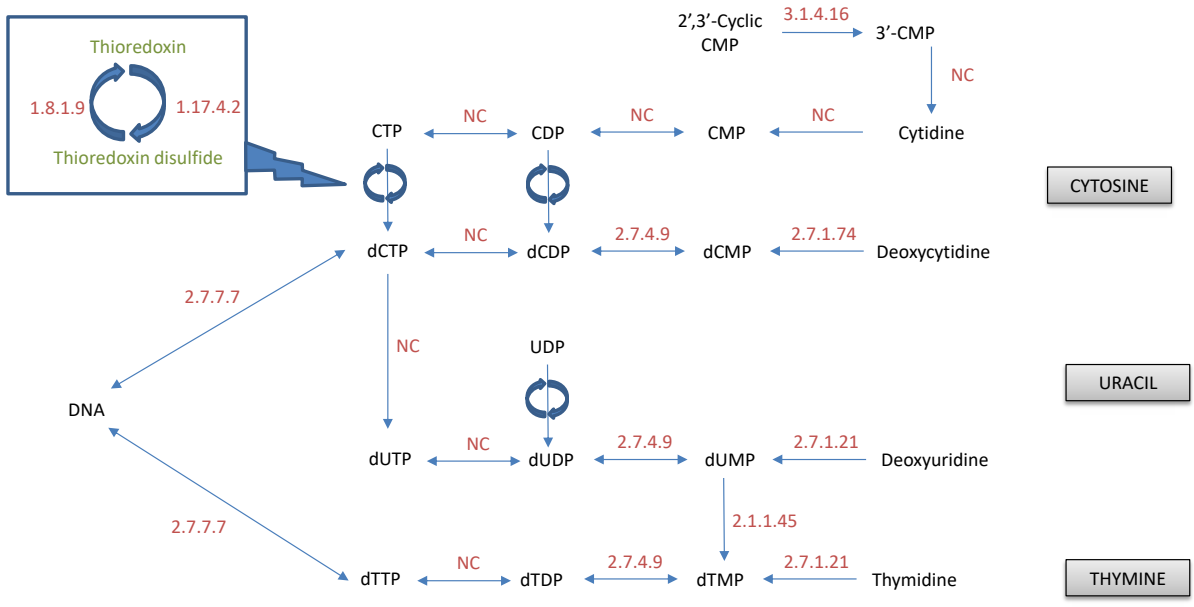

Figure 44 Pyrimidine biosynthesis related functions up-regulated by *L. sakei* in microbiota U. The genes encoding the various functions are shown. NC indicates that the corresponding genes were not detected as over expressed. EC number of enzymes are indicated.

In addition, genes encoding enzymes involved in the metabolism of pyrimidines were also noticed as up-regulated. Those encode a thioredoxin reductase (EC 1.8.1.9) a putative 2',3'-cyclic nucleotide 2'-phosphodiesterase (EC 3.1.4.16), a deoxyadenosine kinase (EC 2.7.1.74), a thymidine kinase (EC

2.7.1.21) a thymidylate kinase (EC 2.7.4.9) and 2 sub-units of the DNA polymerase. This suggest pyrimidine biosynthesis toward DNA replication (Figure 44).

L. sakei requires also vitamins. From up-regulated genes, we identified the induction of several enzymes involved in thiamine (Vitamin B6) metabolism (EC 2.7.4.7, EC 2.8.1.7 or ThiD EC 2.7.6.2). Thiamine biosynthesis or salvage from meat environment might ensure the presence of this co-factor necessary for different reactions.

Conversely as what was observed under aerobic conditions, no gene for iron or heme transport was induced. However we noticed the up-regulation of 13 genes involved in stress response as a cold shock protein which may lead to resistance to cold storage (chicken meat stored at 4°C) or other stress response proteins with uncharacterized functions. In microbiota U, *L. sakei* expressed ClpP protein in suggesting an atypical adaptation response favoring by a zinc anaerobic reductase.

Because of a lack for curated annotation of the *L. fuchuensis* genome, the predicted functions expressed by *L. fuchuensis* in microbiota E were confronted to *L. sakei* genome. Functions over expressed by *L. sakei* were quite similar to that up-regulated functions of *L. fuchuensis* in microbiota E suggesting that *L. fuchuensis* and *L. sakei* had the same behavior. The cobalamin biosynthesis protein CbiM and ATP-cobalamin adenosyltransferase (EC 2.5.1.17) converting cobalamin to its co-enzyme from were up-regulated only by *L. fuchuensis*. However the *cbiM* gene annotation is uncertain as it is also identical to genes identified a cobalt transporters.

Nevertheless, it was interesting to notice that the predominant activity of *L. sakei* in microbiota U compared to the predominant activity of *L. fuchuensis* in microbiota E was also identified in atmosphere enriched in O_2 validating that initial microbiota U and E were different.

4.2.4.Discussion

Two different microbiotas were used to inoculate fresh chicken meat and were able to overgrow the indigenous contaminants. Microbiotas dynamics were followed during 9 days to investigate the gaseous atmosphere influence and metatranscriptomic analysis allowed to describe the behavior of various species composing the microbiotas during storage. The duplicates performed in this study showed similar results validating our meat model system.

4.2.4.1. Gaseous atmosphere shape the bacterial populations and metabolic functions expressed

We compared the relative abundance of bacterium present in meat samples shared under various atmospheres and the most actives ones. Relative abundance was deduces from metabarcoding and partially confirm either by plating methods, qPCR and metagenomics. Metabolic activities were

deduced from the amount of genes that were up-regulated in one of the 3 atmosphers. The results are shown Table 20.

Table 22 Comparison of bacteria present and active depending on storage condition

	Present genus[a]	Active genus[b]
MAP A	*Brochothrix* > *Carnobacterium* EA UA	*Carnobacterium* > *Lactobacillus* > *Brochothrix* E and U
MAP B	*Carnobacterium* > *Brochothrix* > *Lactobacillus* EB UB	*Lactobacillus* >> *Carnobacterium* E and U
air C	*Brochothrix* > *Acinetobacter* > *Rahnella* EC UC	*Acinetobacter* > *Pseudomonas* E *Acinetobacter* > *Rahnella* U

[a] Taxonomic assignation by metabarcoding (figure 30)
[b] Taxonomic assignation of up-regulated genes differentially expressed (figure 38)

Even though *B. thermosphacta* was dominant in both microbiota E and U, this species was not more active in any of the 3 storage conditions. Only the ribose operon was over-expressed under MAP A or MAP B by comparison to storage under air. Carnobacterium (*C. maltaromaticum* and *C. divergens*) were dominant in MAP A and B and also up-regulated gens for carbohydrate metabolism, iron transport or oxidative stress response, depending on the MAP. *Acinetobacter* were the most active bacteria under air storage. Surprisingly, lactobacilli (*L. fuchuensis* and *L. sakei*) although sub-dominant were the most active under MAP A and MAP B.

B. thermosphacta is well-known as dominant spoiled bacterium, occurring on different MAP (Pin et al., 2002) and differently affected by storage condition when compared to other spoilage bacteria such as *Pseudomonas* for example (Stanborough et al., 2017). *B. thermosphacta* preferentially uses glucose as carbon source on meat (Gill & Newton, 1977) but we showed that it could use ribose after 7 or 9 days of cold storage. The glucose concentration of meat decreases during storage, as previously shown by Lilyblade and Peterson (1962) imposing bacteria to use alternative carbon sources available from meat. *B. thermosphacta* may also use glycerol as carbon source (Stanborough et al., 2017) although we did not detect any up-regulation of such genes.

The influence of MAP is not only managed by O_2 but also by CO_2. Oxygen seemed to favor *B. thermosphacta* while CO_2 was more favorable to *Carnobacterium*. The absence of impact of CO_2 on Gram positive bacteria such as *B. thermosphacta* was previously reported (Johansson et al., 2011). Nevertheless, *B. thermosphacta* can use sugars to produce lactate under anaerobic conditions and acetoin in the presence of O_2 (Gill and Newton 1977). However our results did not reveal such influence. We may hypothesis that a fine tuning of gene expression by *B. thermosphacta* is responsible for its adaptation but below the threshold limit we used. Indeed the genome of *B. thermosphacta*

seems particularly rich in transcriptional regulators and stress response genes (Stanborough et al., 2017).

Regarding other active bacteria species in meat microbiotas, *Carnobacterium* identified as dominant in anaerobic MAP condition up-regulated various functions in both anaerobic and aerobic conditions. As *B. thermosphacta*, *C. divergens* and *C. maltaromaticum* has been shown to be able to use different carbon sources like lactose and galactose (Iskandar et al., 2016). Many species in meat microbiotas up-regulated genes to use various sugars by using PTS systems, suggesting that other carbon sources than glucose and ribose are available in meat. O_2 proportions in the MAP also influenced the expression of functions by *Carnobacterium* as for example the heme and iron transport up-regulated in aerobic conditions while switched off in anaerobic conditions. This observation require further investigations to understand microbial ecology of meat and to manage the spoilage caused by various species during meat storage.

4.2.4.2. Importance of subdominant species in microbiotas.

The most relevant information from metatranscriptomic study is the high number of up-regulated functions issued from *L. sakei* and *L. fuchuensis,* which were subdominant according to metabarcoding and metagenomic results. The study reveals thus, the importance of subdominant species. The presence of dominant LAB as *Leuconostoc gasicomitatum* in marinated chicken meat (Nieminen et al., 2012) was not observed in our study. *L. gasicomitatum* was described as unable to use amino acid on meat unlike *L. sakei* (Johansson et al., 2011). After 9 days of storage, the nutriments available on meat may become limiting for *L. gasicomitatum* growth. Conversely, *L. sakei* is well adapted to meat ecosystem because this species harbors both PTS systems and the ribose operon (Chaillou et al., 2005). Pyruvate metabolism of *L. sakei* can also be modified when glucose is depleted in the environment by using arginine pathway as described Figure 43.This condition occurred in stationary phase of *L. sakei* growth. Also, the environmental pH influenced the expression of arginine pathway that could be and additional argue of *L. sakei* adaptation in spoiled meat (Rimaux et al., 2012). Arginine deiminase pathway already reported to be involved in smoked salmon spoilage (Jørgensen et al., 2000, Fernandez & Zuniga, 2006). In addition, regulation of ribose transport and catabolic machinery of *L. sakei* was well known (McLeod et al., 2011).

4.2.4.3. Different sources of carbon and nitrogen could be used in chicken meat

To summarize, major metabolic pathways used by bacteria present in chicken meat microbiotas in this study were shown Figure 45.

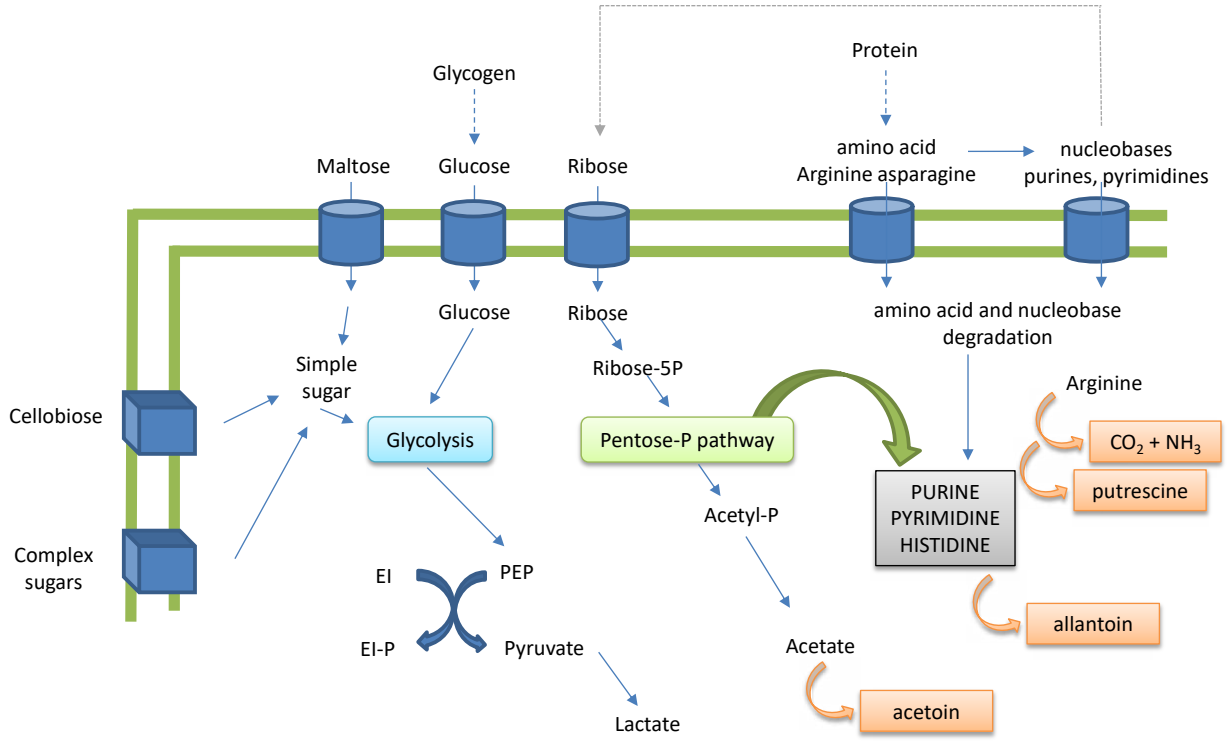

Figure 45 Major metabolic pathways used by bacteria in chicken meat microbiota.
Scare boxes represent PTS transport system while cylinder boxes represent permeases.

As described by Stentz et al., (2001) meat is rich in different substrates but poor in sugars (glucose and ribose mainly). Muscles contain glycogen (Stentz et al., 2001) which can be used as carbon source by some bacteria. Just after slaughtering, the main sugars present in meat are glucose and fructose but ribose is also present in smaller amounts (Aliani et al., 2013). After 6 days of storage, glucose concentration decreases while ribose and inositol increase during the storage (Lilyblade and Peterson, 1962). Glucose is limited for bacteria growth but alternative carbon sources as amino acids or lactate can be used (Gill & Newton, 1977). Hypothesis about possible carbon source utilization have been proposed for *L. sakei* (Stentz et al., 2001) and could be extrapolated to other bacteria present in meat (Figure 43).

4.2.4.4. Key role of species in chicken meat spoilage

LAB such as *L. fuchuensis*, *L. sakei*, *C. maltaromaticum* and *C. divergens* have been previously associated to fresh meat spoilage and isolated from vacuum beef package (Sakala et al., 2002). Production of off-odors, altering the sensory quality of raw meat, could incriminate these species in meat spoilage. The role of LAB in meat spoilage was reported but this role is also still discussed (Pothakos et al., 2015). All LAB are not involved in sensory spoilage. *L. sakei* for example may have a

bioprotective function in meat and may exert antagonistic activity against undesired microorganisms (Champomier-Vergès et al., 2002, Chaillou et al., 2014, Jones et al., 2009). The relevant observation in this study was the predominance of *L sakei* activity in microbiota U and that of *L fuchuensis* predominance in microbiota E in particular anaerobic MAP B condition. Sensory tests would be requires to state on the putative protective or spoiling role of this LAB in poultry meat. This study shows the potential of very powerful NGS technologies applied in food microbiology. Metagenomic and metatranscriptomic data enabled a detailed analysis of poultry meat microbial ecology and highlighted the bacterial behaviour during poultry meat storage.

Acknowledgements

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4.3- Ce qu'il faut retenir du chapitre 4

Le but de ce chapitre était de comprendre le comportement des espèces bactériennes au sein de microbiotes de viande de poulet conservée sous atmosphère protectrice. Pour cela, 2 microbiotes préalablement isolés (chapitre 2) et décrits par pyroséquençage (chapitre 3) ont été inoculés sur des dés de viande de poulet et stockés durant 9 jours à 4°C sous 2 MAP différentes (A et B) et sous air (C).

La croissance bactérienne a été suivie de l'inoculation jusqu'à 9 jours de stockage à 4°C par méthodes culturales montrant ainsi que les communautés bactériennes inoculées ont été capables de se développer. Nous avons donc réussi à mimer les conditions dans lesquelles se trouvent les communautés bactériennes au cours d'un stockage jusqu'à la DLC.

A 7 et 9 jours de stockage, les ADN et ARN bactériens ont été récoltés afin d'être séquencés (métabarcoding, métatranscriptomique, métagénomique). Les résultats de métabarcoding et de métagénomique nous ont permis de mettre en évidence la prédominance de *B. thermosphacta* dans les microbiotes conservés sous MAP enrichie en O_2 alors que dans les viandes conservées en anaérobiose, *B. thermosphacta* mais aussi *C. maltaromaticum* and *C. divergens* étaient majoritaires. Il est important de noter que ces résultats semblent valider le fait que les compositions gazeuses des

barquettes de viande conditionnent les espèces bactériennes des microbiotes en favorisant/ sélectionnant certaines espèces.

Les résultats de métatranscriptomique ont généré une liste conséquente de fonctions différentiellement exprimées suivant les atmosphères de stockage. Dans un souci de simplification, nous avons spécifiquement recherché les fonctions surexprimées dans les conditions de MAP A ou B par rapport à celles exprimées sous air. Cela nous a permis de mettre en évidence que les espèces dont l'activité est induite par une atmosphère protectrice, ne sont pas toujours les espèces dominantes. En effet, des lactobacilles, non détectés par pyroséquençage au chapitre 2 et détectés comme sous dominants dans nos challenges tests semblent être plus actifs après stockage sous MAP que sous air. Nous avons également mis en évidence la dominance de *B. thermosphacta* dans les différentes conditions de stockage, avec peu de différences des fonctions exprimées. Cette bactérie semble donc insensible aux conditions gazeuses de stockage.

Ces résultats permettent de mieux connaitre les moyens de maitrise des communautés bactériennes, utilisés pour prolonger la DLC et assurer la sécurité et la qualité des produits. Il serait également intéressant d'analyser les fonctions surexprimées dans les microbiotes stockés sous air.

Il faut cependant noter que des difficultés ont été rencontrées lors des extractions d'acides nucléiques à partir de viande de poulet (Figure 46). Comme évoqué lors du chapitre 2, elles se sont révélées parfois limitantes au cours de cette dernière étude. En effet, lors d'un 1^{e} challenge test avec une inoculation de la flore à 3 log UFC/g, une forte contamination d'ARN eucaryotes a été identifiée (figure 46 A). Nous avons émis les hypothèses suivantes :

- Présence de nucléase dans la viande
- Quantité limitante de bactéries
- Inaccessibilité des bactéries lors de la lyse

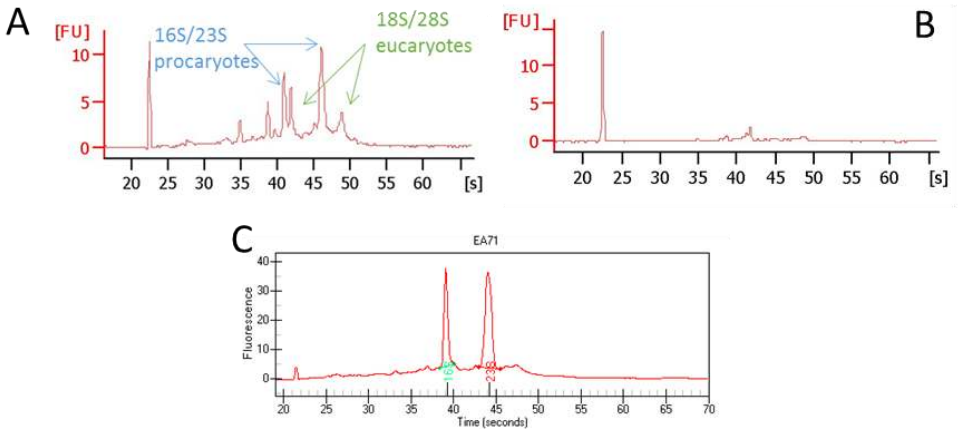


Figure 46 Résultats de puces ARN Agilent ou Experion montrant la qualité des ARN extrait lors de 3 challenges tests (A, B et C).

Un 2nd challenge test avec une inoculation initiale de 5 log CFU/g a été réalisé lors duquel différents tests ont été effectués pour optimiser le protocole d'extraction d'acides nucléiques bactériens à partir de viande de poulet (Annexe 2). Après optimisation, un 3e challenge test a été réalisé et nous avons pris le soin de vérifier dès J_0 l'absence de nucléase dans la viande en effectuant une extraction d'ARN et d'ADN à J_0. L'ARN a pu être extrait à partir de 4 jours de stockage. Cependant une concentration suffisante d'ARN pour le séquençage n'a pu être obtenue qu'après 7 et 9 jours de stockage (Figure 46 C).

Discussion et perspectives

Les bactéries présentes sur la viande de volaille ont été décrites le plus souvent par des méthodes culturales mais l'état de l'art nous montre le peu d'informations qui ont été rapportées. Cela résulte d'une part des biais inhérents à ces méthodes mais aussi à l'extrême variabilité des découpes, transformations et préparations à base de viande de poulet. La variabilité des lots de viande de poulet aussi bien dans la nature des contaminants que dans la charge de contamination rend difficile la comparaison des résultats de la littérature portant sur la viande de volaille. Nous avons donc mis au point au cours de ce projet une procédure permettant de reconstituer un microbiote standard utilisable pour des expérimentations répétables et reproductibles, s'affranchissant ainsi de la variabilité entre les lots de viande.

Les méthodes de séquençage à haut débit, un nouveau regard sur les communautés microbiennes de la viande à destination des industriels.

L'essor des technologies de séquençage à haut débit a rendu possible l'investigation des communautés bactériennes dans leur globalité favorisant ainsi des études d'écologie microbienne. L'utilisation de ces approches a permis une analyse approfondie des contaminants bactériens de la viande de volaille. Elle nous a permis de montrer la richesse et la diversité des communautés bactériennes de la viande de poulet. Outre le fait de générer des connaissances dans la description des espèces bactériennes, les NGS permettent également d'étudier le comportement d'un microbiote dans sa globalité. Dans notre étude nous avons généré des connaissances sur les microbiotes de la viande de poulet conservée sous atmosphère protectrice fournissant des résultats utiles pour la communauté scientifique mais aussi pour les industriels de la filière. Ces méthodes permettent d'avoir un regard différent sur les procédures mises en place en abattoir par exemple par méthode culturale pour déterminer les critères de sécurité et la DLC des produits. Bien que ces récentes technologies soient utilisées en recherche, nous pouvons apporter des informations et des résultats utiles et applicables aux industriels de la viande de poulet. De nouveaux échanges sont donc possibles avec les industriels de la filière se basant sur des données d'écologie microbienne, démystifiant ainsi l'utilisation de ces méthodes en milieu industriel. Ces méthodes nécessitent en revanche une capacité de traitement de donnée difficilement accessible au niveau industriel. Rien que dans le cadre de cette thèse, une quantité importante de données a été produite. Nous avons cherché à extraire les informations les plus pertinentes pour notre

projet. Cependant, une analyse complémentaire devrait être réalisée pour explorer les données de façon plus exhaustive.

Influence de l'atmosphère protectrice sur les microbiotes

Grace aux microbiotes standards développés au cours de la première partie du projet, nous avons pu évaluer l'impact d'un facteur abiotique (MAP) sur la dynamique des communautés bactériennes de la viande de poulet. En effet, l'utilisation d'une atmosphère gazeuse pour augmenter la DLC des produits de volaille, est une pratique très courante en France mais dont l'utilisation par les industriels semble assez empirique. Pour cela 2 microbiotes différents ont été reconstitués et 2 atmosphères protectrices ont été comparées à un conditionnement sous air. La puissance des outils de NGS a permis de mettre en avant la diversité des microbiotes de la viande de poulet. Aucune espèce bactérienne non décrite à ce jour n'a été retrouvé parmi les dominants contrairement à ce qui a pu être observé dans d'autre cas (Chaillou et al. 2015). *Brochothrix*, *Carnobacterium, Pseudomonas* et *Shewanella* sont les principaux genres bactériens retrouvés sur des cuisses de poulet conservées sous atmosphère gazeuse modifiée.

Bien qu'il soit aisé de comprendre que les atmosphères gazeuses vont avoir un rôle de protection en inhibant certaines espèces bactériennes, nous avons montré que la composition gazeuse de l'atmosphère conditionne les microbiotes. Lorsque 2 microbiotes différents sont inoculés sur la viande, après 9 jours de stockage il semble que les profils bactériens obtenus soient similaires pour une même atmosphère modifiée utilisée est la même. L'atmosphère enrichie en O_2 et CO_2 semble favoriser la dominance de *Brochothrix* au dépend de *Carnobacterium* alors que lorsque l'atmosphère est enrichie en CO_2 et N_2 *Carnobacterium* et *Brochothrix* sont identifié comme les 2 communautés microbiennes dominantes. Des espèces sous dominantes ont aussi été identifiées. Les lactobacilles par exemple ne sont détectés que sous atmosphère protectrice anaérobie alors que les *Pseudomonas* le sont en aérobiose.

L'analyse des fonctions exprimées par ces microbiotes a montré que la composition de l'atmosphère protectrice influence également l'activité des espèces sous dominantes. Nous avons vu en effet que le fait d'appliquer une atmosphère modifiée n'entraine que très peu la surexpression de gènes, mais entraine la sous expression de beaucoup plus de gènes. Ce résultat semble montrer un ralentissement général du métabolisme au sein des communautés microbiennes, menant sans doute une altération plus lente du produit, en accord avec les observations empiriques des industriels sur la durée de vie de leurs produits. Nous avons également montré que les espèces sous dominantes telles que les

lactobacilles en anaérobiose pouvaient être très actives. Dans un même temps, les espèces dominantes telles que *B. thermosphacta* semblent être bien adaptées à l'écosystème de la viande puisque peu ou pas de fonctions sont exprimées de manière différentielle suivant les conditions de stockage. Il est donc important de noter l'intérêt des communautés microbiennes minoritaires et de ne pas focaliser les recherches uniquement sur les espèces dominantes. Cela nécessite donc d'investiguer un peu plus les flores minoritaires qui dans notre exemple n'avait pas été détectées par pyroséquençage par exemple.

La viande de poulet, un verrou scientifique lors des extractions d'acides nucléiques bactériens ?

L'utilisation de ces méthodes de séquençage à haut débit a nécessité l'extraction d'acides nucléiques bactériens à partir de viande de volaille. Malgré une mise au point du protocole de collecte des bactéries et de l'optimisation des étapes d'extraction, les ADN bactériens de seulement 10 des 23 lots de viande de poulet ont pu être isolés et amplifiés (chapitre2). Lors des extractions d'ARN et ADN dans le cadre du challenge test utilisé dans le chapitre 4, des difficultés ont également été soulevées. Ces difficultés ont permis de répondre à un appel à projet visant à lever des verrous scientifiques proposé par le RFI (Food for tomorrow- Région Pays de la Loire) en avril 2016. Ce projet de 6 mois, nommé Extraction of Nucléique Acid BottlEneck in poultry meat a été financé (Annexe 3). Les principaux objectifs de ce projet ont été d'identifier des échantillons dits « négatifs » dont les acides nucléiques ne peuvent être extraits en qualité ou en quantité suffisantes pour une utilisation NGS, afin d'identifier la cause de ce biais d'extraction (accessibilité des bactéries, présence d'inhibiteurs de PCR ou de nucléases). Enfin, un protocole a été mis au point pour favoriser l'extraction d'acides nucléiques et leur utilisation à partir d'une matrice viande de poulet.

Elucider le rôle de chacune des espèces du microbiote dans le phénomène l'altération

Bien que des espèces identifiées comme sous dominantes et cependant actives au sein du microbiote, comme *L. sakei* ou *L. fuchuensis* aient déjà été décrites dans la littérature dans des produits carnés altérés, il est difficile de préciser quels rôles ces espèces pourraient avoir sur la viande de volaille. Nous avons vu que certaines voies métaboliques sont exprimées témoignant de l'utilisation de certains sucres ou d'acides aminés. Il faudrait alors approfondir les expérimentations en ciblant ces espèces afin de comprendre les interactions qui peuvent résider au sein des microbiotes. L'intérêt de notre microbiote standard permet de reproduire des expériences pour valider la présence de ces sous

dominants et de mieux comprendre l'importance de leurs métabolisme au sein de l'écosystème. Dans un écosystème aussi riche en nutriments que les produits carnés, les capacités à utiliser un substrat joue un rôle très important pour la compétition inter espèces et donc, dans la dynamique des communautés microbiennes. Les différentes voies métaboliques mises en œuvre successivement suite à la production ou l'utilisation des substrats par les micro-organismes, conditionnent ainsi le devenir des communautés bactériennes au cours de véritables successions écologiques. Ces expérimentations pourraient également être combinées à des analyses sensorielles afin d'identifier si une ou plusieurs espèces peuvent conduire à la production de composés colorés ou odorants par exemple. Cela nécessiterait également d'améliorer la définition des critères d'altération. En effet, aujourd'hui, pour les découpes de volailles, aucun critère sensoriel ne sert de marqueur du phénomène. En industrie, la DLC est fixée après évaluation de la charge bactérienne totale et des bactéries lactiques déterminées par méthodes culturales. Une formule applicable à ces résultats permet de fixer un seuil et de fixer un délai de consommation assurant une qualité optimal et la sécurité du produit. Suite à nos travaux et en approfondissant les hypothèses relevées, l'expression ou la présence d'une espèce bactérienne en particulier, détectable par un gène cible (biomarqueur) par exemple pourrait permettre d'identifier l'altération d'un produit. En combinant des approches de séquençage à haut débit et des tests sensoriels, on pourrait savoir si la présence et le développement d'espèces, telles que des lactobacilles au dépend de *Carnobacterium* ou de *B. thermosphacta,* favorisent ou inhibent l'altération du produit. A terme des outils simples de détection de certaines espèces cibles pourraient être utilisés en industrie afin de déterminer la possibilité d'altération de certains lots de viande par exemple.

Pour conclure,

Nous avons généré au cours de ce projet des informations descriptives des microbiotes de viande de poulet et nous avons commencé à comprendre les mécanismes d'adaptation des bactéries à certaines conditions de stockage, permettant de mieux caractériser et donc à long terme de mieux comprendre l'altération de la viande. Des approches de bio préservation pour lutter contre altération en maitrisant les communautés bactériennes pourraient alors être envisagées en modifiant des facteurs biotiques ou abiotiques, favorables au développement de « bonnes » bactéries naturellement présentes sur la viande. Un modèle comme celui développé dans ce travail de thèse serait pour cela très utile, mais les contaminations de la viande de volaille devraient également être maitrisées très en amont de la chaine de production, depuis l'animal jusqu'à l'aliment.

Valorisation des travaux de thèse

Articles dans des journaux scientifiques internationaux à comité de lecture

- **Rouger A**., Remenant B., Prévost H., Zagorec M., (2017)*, A method to isolate bacterial communities and characterize ecosystems from food products:Validation and utilization in a reproducible chicken meat model*. International Journal of Food Microbiology 247 (2017) 38–47
- **Rouger A**., Moriceau N., Prévost H., Remenant B., Zagorec M., *Diversity of bacterial communities in French chicken cuts stored under modified atmosphere packaging.* Soumis dans Food Microbiology (FM_2017_102).
- **Rouger A**., Tresse O., Zagorec M., *Bacterial contamination occurring on poultry meat: A review*, Soumis dans Food Microbiology (FM_2017_316)**.**
- **Rouger A**., Hultman J., Moriceau N., Björkroth J., Zagorec M., *Optimizing storage parameter to manage chicken meat ecosystem stored under modified atmosphere packaging,* en preparation

Article de vulgarisation scientifique

- Macé S., **Rouger A**., Haddad N., Zagorec M., Tresse O., 2014. Viabilité de *Campylobacter* en fonction du stress oxydant et de la flore endogène des aliments, Rapport bibliographique à destination du pôle de compétitivité Valorial.
- Présentation du sujet de thèse dans le cadre de la formation « vulgarisation scientifique » de l'école doctorale, 2 articles « Plus redoutable que la salmonellose » et « Comprendre pour mieux combattre » parus dans le e-journal de l'ED Biologie santé N°2, octobre 2014.
- Participation à la rédaction d'un encart présentant les travaux de l'unité de recherche dans le dossier de presse de INRA "Volailles: les chercheurs veillent au grain" à destination du grand public.

Communications en congrès internationnaux

Présentation orales

- **Rouger A.**, Remenant B, Prévost H, Zagorec M, *Deciphering bacterial diversity and ecological interactions on poultry meat to improve food quality and safety.* XXII European Symposium on the Quality of Poultry Meat, 2015, Nantes (France)
- **Rouger A.**, Hultman J, Remenant B, Prévost H, Björkroth J, Zagorec M, *Bacterial communities' dynamics and interactions during poultry meat storage to improve food quality and safety*. 3rd International Conference on Microbial Diversity: The challenge of Complexity, 2015, Perugia (Italie)

→ Award: FEMS Young Scientists Meeting Grant

- **Rouger A.**, Hultman J., Remenant B., Prévost H., Björkroth J., Zagorec M., *Understanding bacterial community dynamics to improve the quality of poultry meat during refrigerated storage.* 25th International ICFMH Conference – FoodMicro, 2016, Dublin (Irlande)

→ Award: Best oral communication of Young investigator to the profession of food microbiology and hygiene by ICFMH

- Zagorec M., **Rouger A.**, Hultman J., Remenant B., Prévost H., Björkroth J., *Microbial communities of poultry meat and their behavior during storage.* SIBAL 2016 Internationnal symposium on Lactic Acid Bacteria, 2016, San Miguel de Tucuman (Argentina)

Posters

- **Rouger A.**, Remenant B, Prévost H, Zagorec M, *Deciphering bacterial diversity and ecological interactions on poultry meat to improve food quality and safety*. 24th International ICFMH Conference – FoodMicro, 2014, *Nantes (France)*
- **Rouger A.**, Remenant B, Prévost H, Zagorec M, *Constitution of a microbial model ecosystem of poultry meat*. XXII European Symposium on the Quality of Poultry Meat, *2015, Nantes (France)*
- **Rouger A.**, Remenant B, Prévost H, Zagorec M, *Understanding diversity of bacterial communities from poultry meat to improve food quality and safety*. 6th Congress of European Microbiologists (FEMS), 2015, Maastricht (The Netherlands)

Communications en congrès nationaux

Présentation orales

- **Rouger A.**, Remenant B, Prévost H, Zagorec M, *Description de la diversité des communautés bactériennes de la viande de volaille pour améliorer la qualité et la sécurité.* 20ème colloque du Club des Bactéries Lactiques, 2015, Lille (France)

- **Rouger A.**, Remenant B, Prévost H, Zagorec M, *Description de la diversité bactérienne présente sur la viande de volaille afin d'augmenter la qualité et la sécurité des aliments.* Journées scientifiques de l'école doctorale VENAM, 2015, Angers (France)

Posters

- **Rouger A.**, Remenant B, Prévost H, Zagorec M, *Etude des interactions bactériennes dans l'écosystème des découpes de volaille.* Journées scientifiques de l'école doctorale VENAM, 2013, Le Mans (France)
- **Rouger A.**, Remenant B, Prévost H, Zagorec M, *Caractérisation de l'écosystème microbien de viande de volaille.* Congrès National de la Société Française de Microbiologie, 2014, Paris, (France)
- **Rouger A.**, Remenant B, Prévost H, Zagorec M, *Description de la diversité bactérienne présente sur la viande de volaille afin d'augmenter la qualité et la sécurité des aliments*. Journées Recherche Industrie Microbiologie : Management des Ressources Microbiennes, 2014, Narbonne (France)

Annexe 1 Differentially expressed genes

Differentially expressed genes up-regulated in EA condition.

	EA_descriptions	ec number	Descriptions
1	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS00940_|_allantoinase_|_219647:221014_Reverse	3.5.2.5	Allantoine/purine degradation
2	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS08115_|_Zn-dependent_hydrolase_|_1772010:1773248_Reverse	3.5.3.9	Allantoine/purine degradation allC
3	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS08090_|_ureidoglycolate_dehydrogenase_|_1767281:1768330_Reverse	1.1.1.154	Allantoine/purine degradation AllD
4	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS08095_|_ureidoglycolate_dehydrogenase_|_1768441:1769499_Reverse	1.1.1.154	Allantoine/purine degradation AllD
5	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS04470_|_cysteine_desulfurase_SufS_subfamily_protein_|_1014935:1016173_Forward	2.8.1.7	biosynthesis of iron-sulfur clusters, thio-nucleosides in tRNA
6	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS05880_|_fecCD_transport_family_protein_|_1309965:1310930_Forward		Iron/heme Transport
7	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14375_|_fecCD_transport_family_protein_|_3056718:3057725_Reverse Transport fer?		Iron/heme Transport
8	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14380_|_heme_ABC_transporter_substrate-binding_protein_IsdE_|_3057715:3058617_Reverse		Iron/heme Transport
9	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14385_|_sortase_B_cell_surface_sorting_signal_domain-containing_protein_|_3058686:3060827_Reverse		Iron/heme Transport
10	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14390_|_heme_uptake_protein_IsdC_|_3061082:3061732_Reverse		Iron/heme Transport
11	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14560_|_periplasmic-binding_family_protein_|_3110149:3111105_Reverse		Iron/heme Transport
12	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14565_|_fecCD_transport_family_protein_|_3111133:3112137_Reverse		Iron/heme Transport
13	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14575_|_fecE_|_ABC_transporter_family_protein_|_3113253:3114035_Reverse		Iron/heme Transport
14	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS00240_|_glpK_|_glycerol_kinase_|_57269:58777_Forward Glycerol	2.7.1.30	glycolysis
15	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS00245_|_alpha-glycerophosphate_oxidase_|_58810:60639_Forward	1.1.3.21	glycolysis
16	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS09680_|_superoxide_dismutase_|_2076272:2076880_Reverse	1.15.1.1	oxidative stress
17	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13460_|_Organic_hydroperoxide_resistance_protein_2_|_2855479:2855916_Forward	1.11.1.15	oxidative stress
18	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS04735_|_ferredoxin--NADP(+)_reductase_|_1073913:1074905_Reverse	1.18.1.2	Redox
19	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS07240_|_nitroreductase_|_1589851:1590450_Forward	1.6.6.-	Reductase Redox
20	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS16575_|_heme-degrading_monooxygenase_IsdG_|_3498725:3499075_Forward	1.14.99.3	release Fe from heme after internalization
21	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13615_|_D-ribose_ABC_transporter_substrate-binding_protein_|_2887581:2888510_Reverse		Ribose operon
22	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13620_|_rbsC_|_2888533:2889501_Reverse		Ribose operon
23	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13625_|_ABC_transporter_family_protein_|_2889504:2890985_Reverse		Ribose operon
24	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13630_|_D-ribose_pyranase_|_2891093:2891488_Reverse		Ribose operon
25	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13635_|_ribokinase_|_2891463:2892368_Reverse	2.7.1.15	Ribose operon
26	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13640_|_transcriptional_regulator_|_2892365:2893345_Reverse		Ribose operon
27	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS02550_|_gluconate_kinase_|_607876:609414_Forward	2.7.1.12	Sugar pentulose and hexulose kinases
28	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS05490_|_hypothetical_protein_|_1235086:1235646_Forward		Unknown
29	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS05840_|_hypothetical_protein_|_1301641:1302255_Forward		Unknown
30	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS06275_|_hypothetical_protein_|_1393538:1393723_Forward		Unknown
31	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13360_|_hypothetical_protein_|_2836248:2836730_Forward		Unknown
32	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS15115_|_hypothetical_protein_|_3244191:3244448_Reverse		Unknown
33	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS04180_|_GTPase_HflX_|_958413:959675_Reverse		Unknown function
34	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS04730_|_hypothetical_protein_|_1073512:1073748_Forward		Unknown, adjacent Ironredoxin
35	BR52_CARNOBACTERIUM_DIVERGENS_RS03260_|_iron_ABC_transporter_ATP-binding_protein_|_653738:654496_Forward		Iron/heme Transport
36	BR52_CARNOBACTERIUM_DIVERGENS_RS05415_|_heme_ABC_transporter_substrate-binding_protein_IsdE_|_1110213:1111091_Reverse		Iron/heme Transport

37	BR52_CARNOBACTERIUM_DIVERGENS_RS05420_|_hypothetical_protein_|_1111150:1113309_Reverse		Iron/heme Transport
38	BR52_CARNOBACTERIUM_DIVERGENS_RS05425_|_heme_uptake_protein_IsdC_|_1113320:1113985_Reverse		Iron/heme Transport
39	BR52_CARNOBACTERIUM_DIVERGENS_RS04885_|_D-ribose_ABC_transporter_substrate-binding_protein_|_994672:995604_Reverse		Ribose operon
40	BR52_CARNOBACTERIUM_DIVERGENS_RS04895_|_D-ribose_transporter_ATP-binding_protein_|_996571:998052_Reverse		Ribose operon
41	BR52_CARNOBACTERIUM_DIVERGENS_RS03635_|_hypothetical_protein_|_730032:730211_Forward		Unknown
42	BR52_CARNOBACTERIUM_DIVERGENS_RS05405_|_hypothetical_protein_|_1108501:1109262_Reverse		Unknown
43	BR52_CARNOBACTERIUM_DIVERGENS_RS10480_|_hypothetical_protein_|_2194131:2194457_Reverse		Unknown
44	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02915_|_carbamate_kinase_|_16548:17477_Reverse		ADI pathway
45	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02920_|_ornithine_carbamoyltransferase_|_17493:18494_Reverse		ADI pathway
46	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03380_|_oxaloacetate_decarboxylase_|_39825:41225_Forward		glycolysis
47	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03490_|_pyruvate_oxidase_|_60204:62039_Reverse		glycolysis
48	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09495_|_lactate_oxidase_|_207:1313_Forward		glycolysis
49	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02560_|_glutathione_peroxidase_|_19411:19887_Forward		oxidative stress
50	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11810_|_catalase_|_8329:9770_Forward		oxidative stress
51	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02830_|_tRNA_modification_GTPase_|_69621:71009_Forward		Translation
52	LCA_LACTOBACILLUS_SAKEI_RS08770_|_50S_ribosomal_protein_L23_|_1735697:1735981_Reverse		Translation
53	LCA_LACTOBACILLUS_SAKEI_RS09420_|_tRNA_uridine(34)_5-carboxymethylaminomethyl_synthesis_enzyme_MnmG_|_1876244:1878136_Reverse		Translation
54	PFL_PSEUDOMONAS_PROTEGENSRS13070_|_3D-(3,5/4)-trihydroxycyclohexane-1,2-dione_acylhydrolase_(decyclizing)_|_2866680:2868617_Forward		Unknown
55	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110350_|_transcriptional_regulator_|_334158:334670_Forward		Iron dependent, NADH regeneration
56	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110355_|_D-ribose_ABC_transporter_substrate-binding_protein_|_334939:335856_Reverse		Ribose operon
57	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110360_|_ribose_ABC_transporter_permease_|_335869:336804_Reverse		Ribose operon
58	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110365_|_D-ribose_transporter_ATP-binding_protein_|_336806:338284_Reverse		Ribose operon
59	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110375_|_ribokinase_|_338704:339585_Reverse	2.7.1.15	Ribose operon
60	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0109085_|_aldehyde_reductase_|_87376:88548_Forward		oxidative stress
61	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110325_|_sodium:dicarboxylate_symporter_|_329644:331026_Reverse		proton/sodium-glutamate symport protein

Differentially expressed genes up-regulated in UA condition.

	UA_descriptions	ec number	remarques
1	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS04895_|_methionine_adenosyltransferase_|_1090842:1092032_Forward	2.5.1.6	AA degradation
2	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS08065_|_carbamate_kinase_|_1762191:1763132_Reverse	2.7.2.2	allantoine/arginine degradation/carbamate kinase
3	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS08115_|_Zn-dependent_hydrolase_|_1772010:1773248_Reverse	3.5.3.9	Allantoine/purine degradation allC
4	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS08100_|_hypothetical_protein_|_1769545:1770330_Reverse	3.5.3.-	Allantoine/purine degradation allE
5	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS02735_|_642717:643205_Forward		Amino acid permease family protein
6	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS11940_|_alpha-glycosidase_|_2542681:2544459_Reverse	3.2.1.-	bbmA
7	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS05845_|_cobalt_transport_protein_|_1302514:1302975_Forward		cobalt Transport
8	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS09305_|_DeoR_family_transcriptional_regulator_|_2003950:2004702_Forward		COG1349 Transcriptional regulators of sugar metabolism
9	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS00240_|_glpK_|_glycerol_kinase_|_57269:58777_Forward	2.7.1.30	glycolysis
10	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS00245_|_alpha-glycerophosphate_oxidase_|_58810:60639_Forward	1.1.3.21	glycolysis
11	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS00250_|_glycerol_transporter_|_60891:61607_Forward		glycolysis
12	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS11910_|_beta-phosphoglucomutase_|_2534635:2535295_Reverse	5.4.2.6	glycolysis (maltose)
13	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13360_|_hypothetical_protein_|_2836248:2836730_Forward		hypothetical
14	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS05840_|_hypothetical_protein_|_1301641:1302255_Forward		Hypothetical membrane protein
15	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS05850_|_ABC_transporter_family_protein_|_1303121:1304602_Forward		Iron/heme Transport
16	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS05880_|_fecCD_transport_family_protein_|_1309965:1310930_Forward		Iron/heme Transport
17	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS05885_|_ABC_transporter_family_protein_|_1310934:1311692_Forward		Iron/heme Transport
18	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS05890_|_ABC_transporter_substrate-binding_protein_|_1311724:1312686_Forward		Iron/heme Transport
19	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS07950_|_fecCD_transport_family_protein_|_1736394:1737389_Reverse		Iron/heme Transport
20	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14375_|_fecCD_transport_family_protein_|_3056718:3057725_Reverse		Iron/heme Transport
21	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14380_|_heme_ABC_transporter_substrate-binding_protein_IsdE_|_3057715:3058617_Reverse		Iron/heme Transport
22	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14385_|_sortase_B_cell_surface_sorting_signal_domain-containing_protein_|_3058686:3060827_Reverse		Iron/heme Transport
23	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14390_|_heme_uptake_protein_IsdC_|_3061082:3061732_Reverse		Iron/heme Transport
24	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14560_|_periplasmic-binding_family_protein_|_3110149:3111105_Reverse		Iron/heme Transport
25	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14565_|_fecCD_transport_family_protein_|_3111133:3112137_Reverse		Iron/heme Transport
26	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS11580_|_DNA_helicase_UvrD_|_2471406:2473698_Reverse		isdC heme?
27	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS08105_|_sugar_(and_other)_transporter_family_protein_|_1770427:1771683_Reverse		Major facilitator family transporter
28	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS11925_|_sugar_ABC_transporter_permease_|_2538522:2539373_Reverse		malD
29	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS11930_|_binding--dependent_transport_system_inner_membrane_component_family_protein_|_2539375:2540679_Reverse		malE
30	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS11945_|_oligo-1,6-glucosidase_|_2544472:2546169_Reverse	3.2.1.10	malL
31	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS11935_|_maltodextrin-binding_protein_mdxE_|_2540973:2542241_Reverse		mdxE
32	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS08095_|_ureidoglycolate_dehydrogenase_|_1768441:1769499_Reverse	1.1.1.154	OxydoReductase/Allantoine/purine degradation allD
33	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS06695_|_energy-coupled_thiamine_transporter_ThiT_|_1485986:1486540_Forward		probable thiamine trnasporter
34	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS12170_|_PTS_lactose_transporter_subunit_IIC_|_2589974:2591224_Reverse	2.7.1.69	PTS lactose
35	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS15270_|_transcriptional_regulator_|_3279819:3280250_Reverse		putative regulator upstream from an oxidoreductase
36	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS04735_|_ferredoxin--NADP(+)_reductase_|_1073913:1074905_Reverse	1.18.1.2	Redox
37	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS07960_|_pyridine_nucleotide-disulfide_oxidoreductase_|_1738420:1739475_Reverse	1.8.1.9	redox
38	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS16550_|_nitroreductase_|_3493135:3493719_Forward		Redox
39	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS04215_|_NADH_dehydrogenase_|_965870:967075_Forward	1.6.99.3	Redox Fe-S
40	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS16575_|_heme-degrading_monooxygenase_IsdG_|_3498725:3499075_Forward	1.14.99.3	release Fe from heme after internalization
41	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13615_|_D-ribose_ABC_transporter_substrate-binding_protein_|_2887581:2888510_Reverse		Ribose operon
42	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13620_|_rbsC_|_2888533:2889501_Reverse		Ribose operon

43	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13625_|_ABC_transporter_family_protein_|_2889504:2890985_Reverse		Ribose operon
44	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13630_|_D-ribose_pyranase_|_2891093:2891488_Reverse		Ribose operon
45	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13635_|_ribokinase_|_2891463:2892368_Reverse		Ribose operon
46	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13640_|_transcriptional_regulator_|_2892365:2893345_Reverse		Ribose operon
47	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS05490_|_hypothetical_protein_|_1235086:1235646_Forward		unknown
48	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14370_|_SrtB_family_sortase_|_3056000:3056743_Reverse		unknown but conserved
49	BR52_CARNOBACTERIUM_DIVERGENS_RS10480_|_hypothetical_protein_|_2194131:2194457_Reverse		hypothetical
50	BR52_CARNOBACTERIUM_DIVERGENS_RS03205_|_ABC_transporter_permease_|_643498:644574_Forward		Iron/heme Transport
51	BR52_CARNOBACTERIUM_DIVERGENS_RS03210_|_hemin_ABC_transporter_ATP-binding_protein_|_644578:645249_Forward		Iron/heme Transport
52	BR52_CARNOBACTERIUM_DIVERGENS_RS05405_|_hypothetical_protein_|_1108501:1109262_Reverse		Iron/heme Transport
53	BR52_CARNOBACTERIUM_DIVERGENS_RS05410_|_ABC_transporter_permease_|_1109249:1110223_Reverse		Iron/heme Transport
54	BR52_CARNOBACTERIUM_DIVERGENS_RS05415_|_heme_ABC_transporter_substrate-binding_protein_IsdE_|_1110213:1111091_Reverse		Iron/heme Transport
55	BR52_CARNOBACTERIUM_DIVERGENS_RS05420_|_hypothetical_protein_|_1111150:1113309_Reverse		Iron/heme Transport
56	BR52_CARNOBACTERIUM_DIVERGENS_RS05425_|_heme_uptake_protein_IsdC_|_1113320:1113985_Reverse		Iron/heme Transport
57	BR52_CARNOBACTERIUM_DIVERGENS_RS06500_|_MFS_transporter_|_1328162:1329595_Reverse		Major Facilitator Superfamily
58	BR52_CARNOBACTERIUM_DIVERGENS_RS09060_|_PTS_beta-glucoside_transporter_subunit_EIIBCA_|_1873685:1875574_Reverse	2.7.1.191	PTS beta glucoside EIIABC
59	BR52_CARNOBACTERIUM_DIVERGENS_RS07165_|_PTS_lactose_transporter_subunit_IIC_|_1465838:1467091_Reverse		PTS cellobiose family EIIC
60	BR52_CARNOBACTERIUM_DIVERGENS_RS04895_|_D-ribose_transporter_ATP-binding_protein_|_996571:998052_Reverse		Ribose operon
61	BR52_CARNOBACTERIUM_DIVERGENS_RS04910_|_999342:1000316_Reverse		Ribose operon regulator interaction with HPr?
62	BR52_CARNOBACTERIUM_DIVERGENS_RS00995_|_sodium:dicarboxylate_symporter_|_209259:210653_Forward		transport allantoine?
63	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01880_|_phosphoketolase_|_39208:41571_Forward	4.1.2.9	degradation vers glycolyse
64	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04280_|_NAD(FAD)-dependent_dehydrogenase_|_27141:28493_Forward		Redox
65	LCA_LACTOBACILLUS_SAKEI_RS03300_|_alpha-glycerophosphate_oxidase_|_653687:655513_Forward		~glpO maltaromaticum degradation vers glycolyse
66	LCA_LACTOBACILLUS_SAKEI_RS07890_|_N-acetylglucosamine-6-phosphate_deacetylase_|_1564739:1565878_Reverse	3.5.1.25	C NAG utilisation
67	LCA_LACTOBACILLUS_SAKEI_RS02940_|_NADH_peroxidase_|_585192:586544_Forward	1.11.1.1	catalase replace
68	LCA_LACTOBACILLUS_SAKEI_RS02630_|_2-amino-3-ketobutyrate_CoA_ligase_|_527761:528948_Forward	2.3.1.29	degradation threonine
69	LCA_LACTOBACILLUS_SAKEI_RS09415_|_ABC_transporter_ATP-binding_protein_|_1874495:1876119_Reverse		drug resistance ABC transporter ATPase
70	LCA_LACTOBACILLUS_SAKEI_RS03305_|_glycerol_transporter_|_655538:656257_Forward	1.1.3.21	glpF degradation vers glycolyse
71	LCA_LACTOBACILLUS_SAKEI_RS00135_|_hypothetical_protein_|_26675:27040_Forward		hypothetical
72	LCA_LACTOBACILLUS_SAKEI_RS01915_|_ferrichrome_ABC_transporter_substrate-binding_protein_|_408363:409286_Reverse		Iron transport
73	LCA_LACTOBACILLUS_SAKEI_RS02635_|_UDP-glucose_4-epimerase_|_528968:529915_Forward	1.1.1.103	L-threonine dehydrogenase
74	LCA_LACTOBACILLUS_SAKEI_RS00150_|_2,5-diketo-D-gluconic_acid_reductase_|_29633:30484_Reverse	1.1.1.274	oxydo reductase
75	LCA_LACTOBACILLUS_SAKEI_RS00760_|_peptidase_|_155691:157391_Reverse		Putative Bifunctional glycolsyltransferase
76	LCA_LACTOBACILLUS_SAKEI_RS05395_|_dihydrolipoyl_dehydrogenase_|_1075805:1077211_Reverse	1.8.1.4	redox
77	LCA_LACTOBACILLUS_SAKEI_RS05605_|_F0F1_ATP_synthase_subunit_epsilon_|_1113647:1114081_Reverse	3.6.3.14	redox
78	LCA_LACTOBACILLUS_SAKEI_RS05925_|_pyruvate_oxidase_|_1170993:1172828_Forward	1.2.3.3	redox
79	LCA_LACTOBACILLUS_SAKEI_RS06940_|_L-lactate_oxidase_|_1363470:1364576_Reverse	1.13.12.4	redox
80	LCA_LACTOBACILLUS_SAKEI_RS04710_|_ribonucleoside-diphosphate_reductase_subunit_alpha_|_927727:929898_Reverse	1.17.4.1	reductase fe
81	LCA_LACTOBACILLUS_SAKEI_RS00930_|_ribose_transporter_RbsU_|_193092:193976_Forward		Ribose operon
82	LCA_LACTOBACILLUS_SAKEI_RS00935_|_D-ribose_pyranase_|_193997:194392_Forward		Ribose operon
83	LCA_LACTOBACILLUS_SAKEI_RS00940_|_ribokinase_|_194412:195320_Forward		Ribose operon
84	LCA_LACTOBACILLUS_SAKEI_RS05220_|_obgE_|_GTPase_ObgE_|_1037400:1038692_Reverse		ribosome-associating GTP binding protein putatively involved in stress
85	LCA_LACTOBACILLUS_SAKEI_RS01885_|_thiol_reductase_thioredoxin_|_403233:403544_Forward		stress oxydant
86	LCA_LACTOBACILLUS_SAKEI_RS09425_|_tRNA_uridine(34)_5-carboxymethylaminomethyl_synthesis_GTPase_MnmE_|_1878167:1879555_Reverse		translation
87	LCA_LACTOBACILLUS_SAKEI_RS09450_|_rnpA_|_ribonuclease_P_protein_component_|_1883731:1884093_Reverse	3.1.26.5	translation

88	LCA_LACTOBACILLUS_SAKEI_RS01370_|_phosphoketolase_|_286496:288859_Forward	4.1.2.9	utilisation sucre
89	LCRIS_LACTOBACILLUS_CRISPATUS_RS02860_|_membrane_protein_|_544276:544524_Reverse		membran protein
90	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110355_|_D-ribose_ABC_transporter_substrate-binding_protein_|_334939:335856_Reverse		Ribose operon
91	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110360_|_rbsC_|_ribose_ABC_transporter_permease_|_335869:336804_Reverse		Ribose operon
92	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110365_|_D-ribose_transporter_ATP-binding_protein_|_336806:338284_Reverse		Ribose operon
93	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110370_|_D-ribose_pyranase_|_338309:338707_Reverse		Ribose operon
94	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110375_|_ribokinase_|_338704:339585_Reverse		Ribose operon
95	Q329_BROCHOTHRIX_THERMOSPHACTA_RS0110350_|_transcriptional_regulator_|_334158:334670_Forward		transcriptional regulator

Differentially expressed genes up-regulated in EB condition.

	EB_descriptions	ec number	remarques
1	ANAEROCOCCUS,TETRADIUS|GG666300,1_cds_EEI82594,1_549_[protein=BMC_domain_protein]_[protein_id=EEI82594,1]_[location=76391,,76801]		unknown
2	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS11940_|_alpha-glycosidase_|_2542681:2544459_Reverse	3.2.1.-	bbmA clivage sucres complexes
3	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS12155_|_PTS_sugar_transporter_subunit_IIB_|_2588476:2588793_Reverse	2.7.1.69	celA3
4	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS12170_|_PTS_lactose_transporter_subunit_IIC_|_2589974:2591224_Reverse	2.7.1.69	celB
5	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS12150_|_PTS_lactose/cellobiose_specific_transporter_subunit_IIA_|_2588114:2588443_Reverse	2.7.1.69	celC
6	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13615_|_D-ribose_ABC_transporter_substrate-binding_protein_|_2887581:2888510_Reverse		Ribose operon
7	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13620_|_rbsC_|_2888533:2889501_Reverse		Ribose operon
8	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13625_|_ABC_transporter_family_protein_|_2889504:2890985_Reverse		Ribose operon
9	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13635_|_ribokinase_|_2891463:2892368_Reverse	2.7.1.15	Ribose operon
10	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS13640_|_transcriptional_regulator_|_2892365:2893345_Reverse		Ribose operon
11	BR52_CARNOBACTERIUM_DIVERGENS_RS06620_|_carbamate_kinase_|_1358057:1359001_Reverse	2.7.2.2	ADI pathway
12	BR52_CARNOBACTERIUM_DIVERGENS_RS06630_|_ornithine_carbamoyltransferase_|_1360590:1361606_Reverse	2.1.3.3	ADI pathway
13	BR52_CARNOBACTERIUM_DIVERGENS_RS06615_|_cyclic_nucleotide-binding_protein_|_1357075:1357776_Reverse		ADI pathwayTranscriptional regulator ArcR essential for anaerobic expression of the ADI pathway, Crp/Fnr family
14	BR52_CARNOBACTERIUM_DIVERGENS_RS08860_|_rpmE2_|_50S_ribosomal_protein_L31_|_1825395:1825658_Reverse		50S_ribosomal_protein
15	BR52_CARNOBACTERIUM_DIVERGENS_RS06635_|_arginine_deiminase_|_1361637:1362875_Reverse	3.5.3.6	ADI pathway
16	BR52_CARNOBACTERIUM_DIVERGENS_RS08350_|_cadmium_transporter_|_1718139:1720262_Reverse	3.6.3.3	Cation-transporting ATPase (P-type ATPase)
17	BR52_CARNOBACTERIUM_DIVERGENS_RS07155_|_PTS_mannose_transporter_subunit_IIA_|_1464875:1465204_Reverse	2.7.1.69	EIIA PTS (lichenan?)
18	BR52_CARNOBACTERIUM_DIVERGENS_RS10005_|_FMN-binding_protein_|_2082543:2083478_Reverse		FMN-binding_protein
19	BR52_CARNOBACTERIUM_DIVERGENS_RS10425_|_hypothetical_protein_|_2183123:2184301_Reverse		Major Facilitator Superfamily
20	BR52_CARNOBACTERIUM_DIVERGENS_RS03030_|_nucleic_acid-binding_protein_|_610663:611223_Forward		nucleic_acid-binding_protein
21	BR52_CARNOBACTERIUM_DIVERGENS_RS06640_|_guanine_permease_|_1363383:1364684_Forward		pbuO hypoxanthine/guanine permease regulated by PurR
22	BR52_CARNOBACTERIUM_DIVERGENS_RS09060_|_PTS_beta-glucoside_transporter_subunit_EIIBCA_|_1873685:1875574_Reverse		PTS beta glucoside EIIABC
23	BR52_CARNOBACTERIUM_DIVERGENS_RS02840_|_bifunctional_acetaldehyde-CoA/alcohol_dehydrogenase_|_571790:574393_Forward		reductase
24	BR52_CARNOBACTERIUM_DIVERGENS_RS04885_|_D-ribose_ABC_transporter_substrate-binding_protein_|_994672:995604_Reverse		Ribose operon
25	BR52_CARNOBACTERIUM_DIVERGENS_RS04890_|_rbsC_|_ribose_ABC_transporter_permease_|_995619:996569_Reverse		Ribose operon
26	BR52_CARNOBACTERIUM_DIVERGENS_RS04895_|_D-ribose_transporter_ATP-binding_protein_|_996571:998052_Reverse		Ribose operon
27	BR52_CARNOBACTERIUM_DIVERGENS_RS04900_|_D-ribose_pyranase_|_998064:998459_Reverse		Ribose operon
28	BR52_CARNOBACTERIUM_DIVERGENS_RS04905_|_ribokinase_|_998434:999339_Reverse	2.7.1.15	Ribose operon
29	BR52_CARNOBACTERIUM_DIVERGENS_RS04910_|_LacI_family_transcriptional_regulator_|_999342:1000316_Reverse		Ribose operon
30	BR52_CARNOBACTERIUM_DIVERGENS_RS11980_|_PTS_beta-glucoside_transporter_subunit_EIIBCA_|_2517893:2519845_Forward		Sucrose PTS EIIABC
31	BR52_CARNOBACTERIUM_DIVERGENS_RS03205_|_ABC_transporter_permease_|_643498:644574_Forward		Transport Fer/heme
32	BR52_CARNOBACTERIUM_DIVERGENS_RS06490_|_tRNA_(guanosine(46)-N7)-methyltransferase_TrmB_|_1326423:1327067_Reverse	EC:2.1.1.33	tRNA modification
33	BR52_CARNOBACTERIUM_DIVERGENS_RS07135_|_hypothetical_protein_|_1461890:1462111_Reverse		unknown
34	BR52_CARNOBACTERIUM_DIVERGENS_RS11135_|_gldA_|_glycerol_dehydrogenase_|_2330976:2332106_Forward		glycerol metabolism
35	GJA_Janthinobacterium agaricidamnosum_RS20030_|_gapA_|_type_I_glyceraldehyde-3-phosphate_dehydrogenase_|_4671307:4672317_Reverse		glycolysis
36	~~IW20_FLAVOBACTERIUM_HYDATIS_RS06095_|_IW20_FLAVOBACTERIUM_HYDATIS_RS06095_|_GLPGLI_family_protein_|_363194:364102_Reverse~~		
37	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03725_|_chorismate_mutase_|_36615:36902_Forward		shikimate metabolsim dans la biosynthèse d'acides aminés aromatiques tels que la phénylalanine et la tyrosine
38	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01210_|_GTP_pyrophosphokinase_|_85690:87921_Forward	2.7.6.5	(p)ppGpp synthetase; (p)ppGpp) are involved in regulating growth and several different stress responses
39	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03600_|_L-serine_dehydratase,_iron-sulfur-dependent_subunit_beta_|_13553:14203_Forward		4Fe-4S dependent protein
40	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00605_|_5-bromo-4-chloroindolyl_phosphate_hydrolase_|_123576:124262_Forward		5-bromo-4-chloroindolyl_phosphate_hydrolase
41	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00515_|_cysteine_desulfurase_|_110214:110555_Reverse	2.8.1.7	AA (alanine) biosynthesis
42	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09330_|_peptidase_M23_|_17019:17657_Forward		AA catabolism
43	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09020_|_D-alanine/D-serine/glycine_permease_|_17300:18676_Forward		aa permease
44	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12090_|_aspartate_aminotransferase_|_6124:7727_Forward		aa synthesis
45	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05240_|_glyA_|_serine_hydroxymethyltransferase_|_32529:33776_Forward		aa synthesisvitamin B6 dependent
46	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08495_|_glutamate:protein_symporter_|_11643:12917_Forward		aa transport
47	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04270_|_serine/threonine_dehydratase_|_24568:25608_Forward		aa utilization

48	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04405_|_multidrug_ABC_transporter_ATP-binding_protein_|_52536:53264_Forward		ABC transporter
49	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06700_|_multidrug_ABC_transporter_ATP-binding_protein_|_41573:43513_Forward		ABC transporter
50	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06965_|_multidrug_ABC_transporter_ATP-binding_protein_|_6885:8669_Reverse		ABC transporter
51	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06970_|_multidrug_ABC_transporter_ATP-binding_protein_|_8670:10415_Reverse		ABC transporter
52	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07230_|_ABC_transporter_|_7709:10591_Reverse		ABC transporter
53	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07270_|_multidrug_ABC_transporter_ATP-binding_protein_|_17943:18812_Reverse		ABC transporter
54	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02885_|_sulfate_ABC_transporter_ATP-binding_protein_|_8615:10951_Reverse		ABC Transporter
55	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04445_|_bacteriocin_cleavage/export_ABC_transporter_|_5:1993_Forward		ABC transporter
56	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04950_|_ABC_transporter_ATP-binding_protein_|_27138:29075_Forward		ABC Transporter
57	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06385_|_phosphate_ABC_transporter_ATP-binding_protein_|_13455:14213_Forward		ABC Transporter
58	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07665_|_spermidine/putrescine_ABC_transporter_substrate-binding_protein_|_20913:21980_Reverse		ABC transporter
59	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04250_|_peptide_ABC_transporter_ATP-binding_protein_|_21412:22113_Forward		ABC transporter
60	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08050_|_manganese_transporter_|_29567:31135_Forward		ABC transporter (Mn?) Manque 1 ss u qui n'est pas surexprimée
61	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02640_|_amino_acid_ABC_transporter_permease_|_30826:31485_Forward		ABC Transporter permease
62	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06130_|_ABC_transporter_permease_|_3415:5514_Reverse		ABC Transporter permease
63	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00050_|_multidrug_ABC_transporter_ATP-binding_protein_|_7201:9084_Reverse		ABC transporter, operon with dfrA and thyA
64	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04670_|_acetate_kinase_|_38463:39644_Forward	2.7.2.1	acetate kinase 2, purines nucleosides degradation, pyruvate degradation
65	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07045_|_acetate_kinase_|_22116:23309_Reverse	2.7.2.1	acetate kinase 2, purines nucleosides degradation, pyruvate degradation
66	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05685_|_acetolactate_synthase_|_19981:21663_Forward	2.2.1.6	acetolactate synthase, acetoin biosynthesis, potential spoilage, pyruvate degradation
67	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00240_|_acyltransferase_|_48101:50014_Reverse		Acetyl transferase of unknown function
68	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11290_|_acyltransferase_|_3306:4186_Reverse		Acetyl transferase of unknown function
69	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01030_|_acyl_carrier_protein_|_56247:56489_Forward		Acyl carrier, lipid metabolism
70	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02140_|_glutamate/gamma-aminobutyrate_family_transporter_YjeM_|_18669:20159_Forward		amino acid/polyamine transporter
71	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04275_|_amino_acid_permease_|_25705:27021_Forward		Aminoacid permease
72	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06670_|_amino_acid_permease_|_34150:35979_Reverse		Aminoacid permease
73	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02680_|_ribonucleotide_reductase_assembly_protein_NrdI_|_38518:38982_Forward		Anaerobic ribonucleoside-triphosphate reductase-activating protein
74	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11400_|_ribonucleoside-triphosphate_reductase_activating_protein_|_9755:10332_Reverse		Anaerobic ribonucleoside-triphosphate reductase-activating protein
75	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09705_|_pyrroline-5-carboxylate_reductase_|_8785:9594_Reverse	1.5.1.2	Arginine and proline metabolism
76	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00930_|_arginine_repressor_|_35304:35756_Forward		Arginine repressor
77	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04350_|_arginine_repressor_|_40930:41403_Forward		ArgR family transcriptional regulator
78	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11760_|_arsenate_reductase_|_4521:4939_Forward		arsenate reductase
79	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11320_|_ATPase_|_37681:40346_Reverse		ATPase
80	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11305_|_aromatic_ring-opening_dioxygenase_LigA_|_18541:20570_Reverse		biosynthèse de certains acides aminés aromatiques
81	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11520_|_carboxypeptidase_|_19723:22193_Reverse		catabolisme AA?
82	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11530_|_dipeptidase_PepV_|_30317:31719_Reverse		catabolisme AA?
83	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00150_|_SMC-Scp_complex_subunit_ScpB_|_28437:29060_Reverse		Cell division
84	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00155_|_segregation_and_condensation_protein_A_|_29044:29802_Reverse		Cell division
85	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00165_|_site-specific_tyrosine_recombinase_XerD_|_30252:31136_Reverse		Cell division
86	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01235_|_division/cell_wall_cluster_transcriptional_repressor_MraZ_|_89843:90274_Forward		Cell division
87	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01245_|_cell_division_protein_FtsL_|_91261:91638_Forward		Cell division
88	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01270_|_cell_division_protein_FtsQ_|_97284:98138_Forward		Cell division
89	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01285_|_cell_division_protein_SepF_|_100863:101294_Forward		Cell division
90	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01290_|_cell_division_protein_|_101295:101579_Forward		Cell division
91	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02715_|_chromosome_partitioning_protein_ParB_|_43967:44842_Reverse		Cell division
92	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05300_|_rod_shape-determining_protein_|_43165:44154_Forward		Cell division
93	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05320_|_cell_division_protein_FtsW_|_44985:46184_Forward		Cell division
94	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06340_|_cell_division_ATP-binding_protein_FtsE_|_4587:5273_Forward		Cell division
95	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06345_|_cell_division_protein_FtsX_|_5263:6150_Forward		Cell division
96	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07820_|_septation_inhibitor_protein_|_12855:13526_Reverse		Cell division
97	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07825_|_rod_shape-determining_protein_MreD_|_13543:14073_Reverse		Cell division
98	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09055_|_cell_division_protein_FtsK_|_5102:7453_Reverse		cell division

99	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09370_|_septum_formation_initiator_family_protein_|_8596:8994_Forward		cell division
100	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09390_|_cell_division_protein_FtsH_|_11704:13815_Forward		cell division
101	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11655_|_cell_division_inhibitor_MinD_|_12059:12852_Reverse		cell division
102	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11660_|_rod_shape-determining_protein_MreC_|_14075:14934_Reverse		cell division
103	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12050_|_cell_division_protein_FtsI_|_2944:5054_Forward		cell division
104	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09740_|_chloride_channel_protein_|_768:1979_Reverse		cell division
105	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07365_|_flippase_|_4203:5612_Reverse		Cell division?
106	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10710_|_fibronectin-binding_protein_|_60864:62578_Reverse		cell surface, adhesion
107	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11975_|_UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-_alanine_ligase_|_5863:7229_Forward		cell wall
108	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01795_|_aspartate_racemase_|_19860:20570_Forward	5.1.1.13	Cell wall biogenesis/degradation
109	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01255_|_phospho-N-acetylmuramoyl-pentapeptide-_transferase_|_93801:94763_Forward		Cell Wall biosynthesis
110	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09210_|_UDP-N-acetylglucosamine_1-carboxyvinyltransferase_|_15709:16968_Forward		Cell wall biosynthesis
111	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01835_|_UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,_6-diaminopimelate_ligase_|_28593:30137_Forward		Cell Wall biosynthesis?
112	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00735_|_UDP-N-acetylmuramate--L-alanine_ligase_|_174:1508_Forward		Cell wall synthesis
113	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03685_|_pilus_assembly_protein_HicB_|_29808:30134_Reverse		Cell Wall synthesis
114	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03820_|_54303:55691_Forward		Cell Wall synthesis
115	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05210_|_UDP-N-acetylmuramyl_peptide_synthase_|_26911:28260_Reverse		Cell Wall synthesis
116	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07345_|_UTP--glucose-1-phosphate_uridylyltransferase_|_694:1578_Reverse		Cell Wall synthesis
117	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07385_|_dTDP-glucose_4,6-dehydratase_|_7782:8810_Reverse		Cell Wall synthesis
118	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07390_|_dTDP-4-dehydrorhamnose_3,5-epimerase_|_8829:9410_Reverse		Cell Wall synthesis
119	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07435_|_exopolysaccharide_biosynthesis_protein_|_17138:17881_Reverse		Cell Wall synthesis
120	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07790_|_N-acetylmuramoyl-L-alanine_amidase_|_6707:8035_Forward		Cell wall synthesis
121	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08345_|_D-alanine--D-alanine_ligase_|_4993:6045_Reverse		Cell wall synthesis
122	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08765_|_N-acetylmuramoyl-L-alanine_amidase_|_12122:14080_Reverse		cell wall synthesis
123	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10655_|_murD_|_UDP-N-acetylmuramoyl-L-alanyl-D-glutamate_synthetase_|_94779:96148_Forward		cell wall synthesis
124	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09935_|_D-alanyl-lipoteichoic_acid_biosynthesis_protein_DltD_|_5780:7048_Reverse		cell wall synthesis?
125	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09940_|_D-alanine--poly(phosphoribitol)_ligase_subunit_2_|_7050:7286_Reverse		cell wall synthesis?
126	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10140_|_undecaprenyl-phosphate_alpha-N-acetylglucosaminyl_1-phosphate_transferase_|_3294:4388_Forward		cell wall synthesis?
127	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10825_|_N-acetylglucosamine-6-phosphate_deacetylase_|_40051:41195_Forward		cell wall?
128	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11560_|_UDP-glucose_4-epimerase_|_15288:16279_Forward		cell wall?
129	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09460_|_alanine_racemase_|_9906:11057_Forward		cell well synthesis
130	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04305_|_SorC_family_transcriptional_regulator_|_30627:31664_Forward		CggR transcriptional regulator of gapA
131	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06905_|_peptidyl-prolyl_cis-trans_isomerase_|_41991:42575_Forward	5.2.1.8	Chaperoning folding
132	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00440_|_def_|_peptide_deformylase_|_91398:91952_Forward	3.5.1.88	cleaves off formyl group from N-terminal methionine residues of newly synthesized proteins; binds Fe2+
133	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06495_|_ATP-dependent_Clp_protease_proteolytic_subunit_|_36203:36787_Reverse		ClpP protease, adaptation to atypical conditions
134	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04590_|_carboxysome_structural_protein_EutM_|_26397:26690_Forward		CoA-dependent aldehyde dehydrogenase synthesis coenzyme B12-dependent pathway of ethanolamine degradation
135	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04630_|_carboxysome_structural_protein_EutM_|_33049:33324_Forward		CoA-dependent aldehyde dehydrogenase synthesis coenzyme B12-dependent pathway of ethanolamine degradation
136	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04645_|_ethanolamine_utilization_protein_EutN_|_34528:34803_Forward		CoA-dependent aldehyde dehydrogenase synthesis coenzyme B12-dependent pathway of ethanolamine degradation
137	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04660_|_aldehyde_dehydrogenase_EutE_|_35868:37289_Forward	1.2.1.10	CoA-dependent aldehyde dehydrogenase synthesis coenzyme B12-dependent pathway of ethanolamine degradation
138	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04665_|_alcohol_dehydrogenase_|_37311:38429_Forward		CoA-dependent aldehyde dehydrogenase synthesis coenzyme B12-dependent pathway of ethanolamine degradation
139	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11170_|_ethanolamine_utilization_protein_EutP_|_40226:40654_Forward		CoA-dependent aldehyde dehydrogenase synthesis coenzyme B12-dependent pathway of ethanolamine degradation
140	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04650_|_ATP--cobalamin_adenosyltransferase_|_34815:35393_Forward		cobalamin metabolism
141	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00565_|_cobalamin_biosynthesis_protein_CbiM_|_118270:119277_Forward		cobalamin_biosynthesis_protein_CbiM
142	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00365_|_coaD_|_pantetheine-phosphate_adenylyltransferase_|_77045:77539_Reverse	2.7.7.3	coenzyme A biosynthesis
143	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04045_|_type_I_pantothenate_kinase_|_33167:34096_Reverse	2.7.1.33	coenzyme A synthesis
144	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00055_|_hypothetical_protein_|_9197:9658_Reverse		Conserved protein of unknown function in operon with dfrA and thyA
145	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08320_|_cyclopropane-fatty-acyl-phospholipid_synthase_|_1379:2566_Reverse	2.1.1.79	cyclopropane-fatty-acyl-phospholipid synthase
146	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00790_|_glpK_|_glycerol_kinase_|_9289:10806_Forward	2.7.1.30	degradation vers glycolyse
147	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10765_|_adhP_|_zinc-dependent_alcohol_dehydrogenase_|_54662:55588_Forward		dehydrogenases catalyze the opposite reaction as part of fermentation to ensure a constant supply of NAD^+

148	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02180_|_peptidase_M20_|_27179:28507_Forward	3.4.-.-	Dipeptidase
149	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03620_|_peptidase_C69_|_17475:18896_Forward	3.4.-.-	Dipeptidase
150	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08365_|_peptidase_M13_|_9194:11089_Forward	3.4.-.-	Dipeptidase
151	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08855_|_peptidase_U34_|_6752:8161_Reverse	3.4.-.-	Dipeptidase
152	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10360_|_peptidase_U34_|_10:1446_Reverse	3.4.-.-	Dipeptidase
153	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09720_|_peptide_ABC_transporter_permease_|_10005:11465_Reverse		di-tripeptide-proton ABC symporter
154	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11120_|_peptide_ABC_transporter_substrate-binding_protein_|_15526:17158_Forward		di-tripeptide-proton ABC symporter
155	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04735_|_peroxidase_|_52707:53663_Reverse		Dyp-type peroxidase family (iron-dependent), oxidative stress
156	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02205_|_PTS_mannose_transporter_subunit_IIAB_|_32596:33078_Forward		EIABCD PTS mannose/fructose?
157	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02210_|_PTS_fructose_transporter_subunit_IIC_|_33133:34041_Forward		EIABCD PTS mannose/fructose?
158	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02215_|_PTS_fructose_transporter_subunit_IID_|_34028:34840_Forward		EIABCD PTS mannose/fructose?
159	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11270_|_ATP_F0F1_synthase_subunit_alpha_|_36913:38447_Forward		energy production
160	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03215_|_ATP-dependent_exonuclease_|_7756:9534_Forward		exonuclease
161	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04995_|_ATP_synthase_F0_subunit_A_|_39594:41165_Forward		F0F1 ATPase, energy production
162	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05250_|_F0F1_ATP_synthase_subunit_A_|_34830:35543_Forward		F0F1 ATPase, energy production
163	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05260_|_ATP_synthase_F0_subunit_B_|_35838:36356_Forward		F0F1 ATPase, energy production
164	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05275_|_F0F1_ATP_synthase_subunit_gamma_|_38475:39410_Forward		F0F1 ATPase, energy production
165	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05280_|_F0F1_ATP_synthase_subunit_beta_|_39435:40865_Forward		F0F1 ATPase, energy production
166	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05285_|_F0F1_ATP_synthase_subunit_epsilon_|_40882:41316_Forward		F0F1 ATPase, energy production
167	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10325_|_fructokinase_|_600:1472_Reverse	2.7.1.56	Fructose degradation, Glycolysis
168	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00470_|_DeoR_family_transcriptional_regulator_|_98381:99346_Forward		FruR, fructose operon transcription regulator
169	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00630_|_galactose_mutarotase_|_129027:130067_Reverse		Galactose utilisation
170	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00640_|_UDP-glucose_4-epimerase_GalE_|_131578:132570_Reverse		Galactose utilisation
171	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00645_|_galactokinase_|_132589:133755_Reverse		Galactose utilisation
172	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00650_|_133895:134887_Forward		Galactose utilisation
173	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02585_|_universal_stress_protein_UspA_|_22761:23246_Reverse		General stress response
174	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03285_|_dnaK_|_molecular_chaperone_DnaK_|_21860:23695_Forward		General stress response
175	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11010_|_glucose_transporter_GlcU_|_48736:49601_Forward		glucose facilitator?
176	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04310_|_type_I_glyceraldehyde-3-phosphate_dehydrogenase_|_31702:32718_Forward		glycerol metabolism
177	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04315_|_phosphoglycerate_kinase_|_32818:34032_Forward		glycerol metabolism
178	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04675_|_propanediol_utilization_protein_|_39672:40016_Forward		glycerol metabolism
179	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10615_|_phosphate_acyltransferase_|_55088:56100_Forward		glycerol metabolism
180	**JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04595_|_microcompartment_protein_PduB_|_26702:27511_Forward**		glycerolipid metabolism
181	**JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04615_|_diol_dehydratase_reactivase_subunit_alpha_|_30493:32325_Forward**		glycerolipid metabolism
182	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04640_|_microcompartment_protein_PduM_|_34034:34543_Forward		glycerolipid metabolism
183	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04580_|_propanediol_utilization_protein_|_25091:25444_Reverse		glycerolipid metabolism
184	**JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04600_|_pduC_|_propanediol_dehydratase_|_27530:29194_Forward**		glycerolipid metabolism cobamide cofactor
185	**JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04605_|_propanediol_dehydratase_|_29225:29926_Forward**		glycerolipid metabolism cobamide cofactor
186	**JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04610_|_propanediol_dehydratase_|_29948:30466_Forward**		glycerolipid metabolism cobamide cofactor
187	**JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04620_|_propanediol_dehydratase_|_32328:32660_Forward**		glycerolipid metabolism cobamide cofactor
188	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11160_|_propanediol_utilization_protein_|_33371:34011_Forward		glycerolipid metabolism cobamide cofactor
189	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11165_|_ATP--cob(I)alamin_adenosyltransferase_|_35393:35866_Forward		glycerolipid metabolism cobamide cofactor
190	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08535_|_1,2-diacylglycerol_3-glucosyltransferase_|_19025:20227_Forward		glycerolipid metabolism.
191	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09030_|_CDP-diacylglycerol--glycerol-3-phosphate_3-phosphatidyltransferase_|_5:589_Reverse		glycerolipid metabolism.
192	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10060_|_phosphatidate_cytidylyltransferase_|_1335:2123_Reverse		glycerophospholipid metabolism
193	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00185_|_pyruvate_kinase_|_33229:34989_Reverse	2.7.1.4	glycolyse
194	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01880_|_phosphoketolase_|_39208:41571_Forward		Glycolysis
195	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01905_|_6-phosphogluconate_dehydrogenase_|_46285:47184_Forward		Glycolysis
196	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01910_|_gluconate_kinase_|_47221:48777_Forward		Glycolysis
197	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03510_|_pgi_|_glucose-6-phosphate_isomerase_|_65333:66679_Forward	5.3.1.9	Glycolysis
198	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07505_|_fructose-1,6-bisphosphate_aldolase,_class_II_|_29849:30712_Reverse	4.1.2.13	Glycolysis
199	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10575_|_alpha-glycerophosphate_oxidase_|_10818:12643_Forward	1.1.3.21	glycolysis

200	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05680_|_aldose_epimerase_|_18937:19812_Forward		glycolysis
201	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03490_|_pyruvate_oxidase_|_60204:62039_Reverse	1.2.3.3	Glycolysis end products, Pyruvate + phosphate + O(2) <=> acetyl phosphate + CO(2) + H(2)O(2)
202	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00490_|_phosphoglycerate_mutase_|_103856:104515_Reverse	5.4.2.1	Glycolysis heterolactic fermentation
203	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10740_|_phosphoglycerate_mutase_|_20583:21256_Forward	5.4.2.1	Glycolysis heterolactic fermentation
204	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04075_|_fructose_2,6-bisphosphatase_|_40154:40810_Forward	5.4.2.1	Glycolysis heterolactic fermentation or gluconeogenesis (il y en a 5 dans le génome de 23K)
205	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01900_|_transcriptional_regulator_|_45291:46133_Reverse		Glycolysis regulator?
206	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02240_|_agaS_|_tagatose-6-phosphate_ketose_isomerase_|_38846:40015_Forward		Glycolysis voie du tagatose
207	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02250_|_tagatose-bisphosphate_aldolase_|_41220:42206_Forward		Glycolysis voie du tagatose (galactose non PTS)
208	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09345_|_L-lactate_dehydrogenase_|_687:1664_Reverse	1.1.1.27	Glycolysis/fermentation
209	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11980_|_HAD_family_hydrolase_|_3925:4454_Forward		HAD_family_hydrolase
210	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11985_|_HAD_family_hydrolase_|_6571:7172_Forward		HAD_family_hydrolase
211	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10985_|_coproporphyrinogen_III_oxidase_|_18138:19275_Forward	1.3.99.22	heme biosynthesis (voie incomplete)
212	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02660_|_hemolysin_|_34503:35858_Forward		hemolysinC family Mg(2+)/Co(2+) transport protein
213	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06595_|_glucose-6-phosphate_dehydrogenase_|_15513:17006_Reverse	1.1.1.49	Heterolactic fermentation, pentose phosphate pathway
214	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03035_|_serine_protease_|_41422:42684_Reverse	3.4.21.-	HtrA, degradation of protein resistance to stress
215	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00190_|_6-phosphofructokinase_|_35067:36026_Reverse	2.7.1.11	http://www.genome.jp/kegg-bin/show_pathway?map00030
216	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00045_|_thymidylate_synthase_|_6229:7179_Reverse	2.1.1.45	http://www.genome.jp/kegg-bin/show_pathway?map00670 dUMP en dTMP thyA
217	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00765_|_X-Pro_dipeptidyl-peptidase_|_4090:6504_Reverse	3.4.14.11	Hydrolyzes Xaa-Pro-|- bonds to release unblocked, N-terminal dipeptides
218	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00120_|_peptidoglycan-binding_protein_LysM_|_23299:23958_Reverse		Hypothetical cell surface protein
219	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00610_|_tellurite_resistance_protein_TelA_|_124246:125451_Forward		Hypothetical, toxic anion resistance
220	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00545_|_114411:115214_Reverse		lactate_racemization_operon_protein
221	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06425_|_glycerol-3-phosphate_dehydrogenase_|_20087:21109_Forward	1.1.1.94	Lipid biosynthesis, oxidoreductase
222	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05420_|_lipid_kinase_|_12306:13331_Reverse		lipid kinase
223	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06420_|_prolipoprotein_diacylglyceryl_transferase_|_19232:20068_Forward	2.4.99.-	Lipoprotein (cell surface) biosynthesis prolipoprotein diacylglyceryl transferase
224	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05715_|_diacylglycerol_kinase_|_24943:25344_Forward	2.7.1.107	Lipoteichoic acid production (cell wall synthesis)
225	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10725_|_73616:74580_Reverse		LysR family transcriptional regulator, target unknown
226	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04715_|_membrane_protein_|_48093:48788_Forward		membrane protein
227	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06350_|_membrane_protein_|_6320:7456_Forward~~		membrane protein
228	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06790_|_mechanosensitive_ion_channel_protein_MscS_|_14977:15864_Reverse		membrane protein
229	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06600_|_Cro/Cl_family_transcriptional_regulator_|_17037:17684_Reverse		Metal-dependent transcriptional regulator
230	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11990_|_SAM-dependent_methyltransferase_|_7542:8272_Forward		~~methyl transferase of unknown function~~
231	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06265_|_hydroxymethylglutaryl-CoA_synthase_|_35173:36354_Reverse		mevalonate degradation (vers acetoacetate), mais autre enzyme 4.1.3.4 n'est pas surexprimée
232	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06270_|_hydroxymethylglutaryl-CoA_reductase,_degradative_|_36369:37637_Reverse	1.1.1.88	mevalonate degradation (vers acetoacetate), mais autre enzyme 4.1.3.4 n'est pas surexprimée
233	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06695_|_redox-sensing_transcriptional_repressor_Rex_|_40726:41376_Reverse		modulates transcription in response to the NADH/NAD(+) redox state, regulates cydAB in B. subtilis
234	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00900_|_N_utilization_substance_protein_B_|_30474:30905_Forward		N_utilization_substance_protein_B
235	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09690_|_N-acetylglucosamine-6-phosphate_deacetylase_|_5895:7034_Reverse	3.5.1.25	N-acetyl glucosamine degradation (PTS sugar) vers glycolysis
236	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05915_|_isochorismatase_|_19783:20337_Reverse	3.5.1.19	NAD salvage, Metabolism of coenzymes and prosthetic groups, Nicotinamide + H(2)O <=> nicotinate + NH(3)
237	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09535_|_nadD_|_nicotinate-nicotinamide_nucleotide_adenylyltransferase_|_5937:6578_Forward		nicotinate and nicotinamide metabolism.
238	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11325_|_NAD_synthetase_|_40957:41791_Reverse		nicotinate and nicotinamide metabolism.
239	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07875_|_aminotransferase_V_|_24838:25986_Reverse	2.8.1.7	NifS/IcsS protein homolog, AA (alanine) biosynthesis
240	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00295_|_nucleotide_pyrophosphohydrolase_|_62169:62462_Reverse		nucleotide_pyrophosphohydrolase
241	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10750_|_oligopeptidase_PepB_|_33596:35400_Reverse		oligopeptidase
242	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08290_|_D-ribose_ABC_transporter_substrate-binding_protein_|_24107:25063_Forward		operon ribose
243	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08295_|_ribokinase_|_25139:26047_Forward		operon ribose
244	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08300_|_26162:27166_Forward		operon ribose
245	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11730_|_sugar_ABC_transporter_permease_|_20810:21726_Forward		Operon ribose
246	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11750_|_D-ribose_transporter_ATP-binding_protein_|_21635:23125_Forward		operon ribose
247	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02530_|_2-Cys_peroxiredoxin_|_14825:15319_Forward		Oxidative stress response?
248	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09965_|_FMN_reductase_|_1784:3010_Reverse		oxidative stress?
249	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07360_|_NAD(P)-dependent_oxidoreductase_|_3358:4200_Reverse		oxidoreductase
250	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07550_|_oxidoreductase_|_37484:38476_Reverse		oxidoreductase
251	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08545_|_oxidoreductase_|_21342:22220_Forward		oxidoreductase
252	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09265_|_SDR_family_oxidoreductase_|_5739:6476_Reverse		oxidoreductase
253	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04280_|_NAD(FAD)-dependent_dehydrogenase_|_27141:28493_Forward		oxidoreductase
254	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05215_|_NrdH-redoxin_|_28441:28755_Forward		oxidoreductase
255	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05490_|_oxidoreductase_|_29989:30951_Forward		oxidoreductase
256	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09515_|_alcohol_dehydrogenase_|_2799:3734_Reverse		oxidoreductase

257	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10850_|_1,3-propanediol_dehydrogenase_|_1141:2308_Forward		oxidoreductase
258	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11470_|_acetaldehyde_dehydrogenase_|_18216:20809_Reverse		oxidoreductase
259	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09040_|_3-oxoacyl-ACP_reductase_|_1630:2361_Reverse		Oxidoreductase fatty acid synthesis
260	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07180_|_aldo/keto_reductase_|_41619:42470_Reverse		oxidoreductase NADPH-dependent
261	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06300_|_xylose_isomerase_|_40935:41759_Reverse		oxidoreductase xylose-> xylulose
262	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02555_|_2-dehydropantoate_2-reductase_|_18331:19269_Reverse	1.1.1.169	PanE ketopantoate reductase; catalyzes the NADPH reduction of ketopantoate to pantoate; functions in pantothenate (vitamin B5) biosynthesis
263	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02820_|_oligoendopeptidase_|_66887:68692_Forward		pepF oligopeptidase
264	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07455_|_type_I_methionyl_aminopeptidase_|_21211:22005_Reverse	3.4.11.18	PepM, methionine aminopeptidase, protein modification, chaperoning
265	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01385_|_peptidase_|_17884:18360_Forward		peptidase
266	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01050_|_peptide_ABC_transporter_ATP-binding_protein_|_60422:61483_Forward		peptide_ABC_transporter_ATP-binding_protein
267	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08785_|_undecaprenyl-diphosphatase_|_16200:17054_Reverse		peptidoglycan biosynthesis.
268	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08905_|_permease_|_14144:15598_Reverse		permease
269	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00960_|_phosphopantothenoylcysteine_decarboxylase_|_39267:40469_Forward		phosphopantothenoylcysteine_decarboxylase
270	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04415_|_phosphotransferase_|_54599:55384_Forward		phosphorylation
271	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04365_|_carboxypeptidase_|_42822:44903_Forward		polypeptide hydrolysis
272	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11630_|_spermidine/putrescine_ABC_transporter_ATP-binding_protein_|_23619:24709_Reverse		potA
273	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00245_|_obgE_|_GTPase_CgtA_|_50282:51574_Reverse		ppGpp-binding GTPase involved in cell partioning, DNA repair and ribosome assembly
274	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04190_|_aryl-alcohol_dehydrogenase_|_10066:11181_Reverse		production aa aromatic
275	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02470_|_GTPase_HflX_|_2785:4065_Forward		Protein fate or degradation
276	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10730_|_protease_|_6407:7635_Forward		proteolysis
277	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05670_|_ATP-dependent_protease_subunit_HslV_|_16936:17481_Forward		prtein degradation
278	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08500_|_PTS_mannose_transporter_subunit_EIIAB_|_13334:14305_Forward		PTS EIABCD mannose/sorbose/fructose family
279	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08505_|_PTS_mannose/fructose/sorbose_transporter_subunit_IIC_|_14354:15166_Forward		PTS EIABCD mannose/sorbose/fructose family
280	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08510_|_PTS_mannose_family_transporter_subunit_IID_|_15184:16095_Forward		PTS EIABCD mannose/sorbose/fructose family
281	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02175_|_PTS_sugar_transporter_subunit_IIA_|_25841:27157_Forward	2.7.1.69	PTS EIIA cellobiose
282	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07540_|_PTS_mannose_transporter_subunit_IIA_|_35529:35864_Reverse		PTS EIIA mannose
283	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06705_|_PTS_sugar_transporter_subunit_IIB_|_43605:43922_Reverse	2.7.1.69	PTS EIIB cellobiose
284	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06180_|_phosphocarrier_protein_HPr_|_17192:17458_Reverse	2.7.11.-	PTS general enzyme HPr, PTS sugar utilization
285	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11405_|_phosphoenolpyruvate-protein_phosphotransferase_|_15469:17192_Reverse	2.7.3.9	PTS general Enzyme I, PTS sugar utilization
286	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08395_|_PTS_N-acetylglucosamine_transporter_subunit_IIABC_|_15611:17623_Forward		PTS IIABC
287	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11105_|_neopullulanase_|_51726:53470_Reverse		pullulanase
288	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06525_|_nucleoside_hydrolase_|_43862:44809_Forward		purine and ribose degradation
289	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05475_|_phosphoribosylaminoimidazole_carboxylase_|_26885:28003_Reverse	4.1.1.21	Purine metabolism
290	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07295_|_deoxyadenosine_kinase_|_23164:23811_Forward	2.7.1.74, 2.7.1.76	Purine metabolism, ATP + deoxyadenosine <=> ADP + dAMP
291	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09385_|_hypoxanthine_phosphoribosyltransferase_|_11070:11615_Forward	2.4.2.8	Purine metabolism, IMP + diphosphate <=> hypoxanthine + 5-phospho-alpha-D-ribose 1-diphosphate,
292	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05220_|_thymidine_kinase_|_28924:29517_Forward	2.7.1.21	Purines pyrimidines metabolism
293	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05245_|_uracil_phosphoribosyltransferase_|_33934:34563_Forward	2.4.2.9	Purines pyrimidines metabolism; Salvage pathways of pyrimidine ribonucleotides; Nucleosides and nucleotides interconversion
294	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02695_|_41087:41845_Reverse	3.1.1.1	Putative carboxyesterase
295	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05005_|_41581:42357_Forward	3.1.1.1	Putative carboxyesterase
296	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07460_|_flavodoxin_|_22261:22713_Forward		Putative flavodoxin, electron transport
297	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08060_|_alpha/beta_hydrolase_|_31678:32532_Forward		~~putative Hydrolase of the alpha/beta superfamily, unknown function~~
298	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03300_|_haloacid_dehalogenase_|_27017:27790_Reverse		Putative hydrolase, haloacid dehalogenase family unknown function
299	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03650_|_haloacid_dehalogenase_|_22796:23638_Forward		Putative hydrolase, haloacid dehalogenase family unknown function
300	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06885_|_haloacid_dehalogenase_|_38641:39426_Forward		Putative hydrolase, haloacid dehalogenase family unknown function
301	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07605_|_haloacid_dehalogenase_|_8822:9709_Forward		Putative hydrolase, haloacid dehalogenase family unknown function
302	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07845_|_haloacid_dehalogenase_|_16989:17612_Reverse		Putative hydrolase, haloacid dehalogenase family unknown function
303	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00405_|_myo-inositol-1-monophosphatase_|_83536:84321_Reverse	3.1.3.25 / 3.1.3.-	Putative inositol monophosphatase / 5' nucleotidase (purine nucleoside monophosphate)
304	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11915_|_magnesium_transporter_|_9708:10660_Forward		Putative ion Mg(2+)/Co(2+) transport protein
305	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09105_|_multidrug_MFS_transporter_|_15262:16725_Reverse		Putative phosphotransferase involved in extracellular matrix synthesis
306	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11955_|_potassium_transporter_Kup_|_14749:16775_Forward		putative potassium transport system protein (kup)
307	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09045_|_zinc_protease_|_2361:3665_Reverse	3.4.24.-	Putative processing protease (protein trafficking?)
308	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04065_|_prolyl_aminopeptidase_|_38106:39008_Forward	3.4.11.5	Putative proline amino peptidase
309	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10530_|_short-chain_dehydrogenase_|_46313:47121_Reverse		putative protein

310	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04705_|_metal_ABC_transporter_permease_|_45539:46471_Forward		Putative zinc/iron ABC transporter
311	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04710_|_cobalt_ABC_transporter_ATP-binding_protein_|_46450:48096_Forward		Putative zinc/iron ABC transporter
312	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01845_|_metal_ABC_transporter_substrate-binding_protein_|_31014:31898_Forward		Putative zinc/iron ABC transporter 1 seule ssu surexprimée
313	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10755_|_metal_ABC_transporter_ATPase_|_50273:52957_Reverse		Putative zinc/iron ABC transporter 1 seule ssu surexprimée
314	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05325_|_glycine_cleavage_system_protein_H_|_46204:46506_Forward		Putative, Metabolism of amino acids and related molecules
315	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11330_|_nicotinate_phosphoribosyltransferase_|_41842:43307_Reverse	2.4.2.11	Pyridine nucleotide biosynthesis
316	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00495_|_pyridine_nucleotide-disulfide_oxidoreductase_|_104627:105961_Reverse		pyridine_nucleotide-disulfide_oxidoreductase
317	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00115_|_cytidylate_kinase_|_22569:23234_Reverse	2.7.4.25	Pyrimidine metabolism CMP vers CDP et dCMP vers dCDP
318	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09205_|_CTP_synthetase_|_13322:14914_Forward	6.3.4.2	pyrimidine ribonucleotides interconversion, Pyrimidine Nucleotide Biosynthesis
319	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11675_|_30016:32297_Reverse		redox?
320	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09575_|_two-component_sensor_histidine_kinase_|_12825:14330_Forward		regulation
321	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03055_|_PAS_domain-containing_sensor_histidine_kinase_|_46182:48080_Reverse		Regulator
322	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06145_|_two-component_sensor_histidine_kinase_|_7101:8165_Forward		Regulator
323	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06355_|_DNA-binding_response_regulator_|_7477:8184_Forward		Regulator
324	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06360_|_two-component_sensor_histidine_kinase_|_8177:9841_Forward		Regulator
325	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07445_|_transcriptional_regulator_|_18721:19713_Reverse		Regulator
326	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07485_|_TetR_family_transcriptional_regulator_|_26557:27120_Reverse		Regulator
327	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07700_|_two-component_sensor_histidine_kinase_|_26805:27878_Reverse		Regulator
328	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05025_|_MarR_family_transcriptional_regulator_|_46991:47482_Forward		Regulator MarR family
329	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10550_|_Crp/Fnr_family_transcriptional_regulator_|_117510:118174_Forward		regulator oxidative stress
330	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01825_|_two-component_sensor_histidine_kinase_|_25944:27140_Forward		Regulator two component system Phosphate regulon
331	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07175_|_MerR_family_transcriptional_regulator_|_41115:41486_Reverse		Regulator, oxidative stress response
332	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00195_|_dnaE_|_DNA_polymerase_III_subunit_alpha_|_36154:39498_Reverse Replication	2.7.7.7	Replication
333	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00655_|_DNA_topoisomerase_III_|_134931:137000_Reverse		Replication
334	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00935_|_DNA_repair_protein_RecN_|_35808:37499_Forward		Replication
335	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01375_|_helicase-exonuclease_AddAB_subunit_AddA_|_11036:14752_Forward		Replication
336	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02390_|_recombination_protein_RecR_|_67506:68102_Forward		Replication
337	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02410_|_DNA_polymerase_III_subunit_delta'_|_70050:71036_Forward		Replication
338	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02790_|_DNA_polymerase_III_subunit_beta_|_61402:62541_Reverse		Replication
339	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02795_|_chromosomal_replication_initiation_protein_DnaA_|_62719:64065_Reverse		Replication
340	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03670_|_ATP-dependent_DNA_helicase_|_25053:27341_Reverse		Replication
341	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04050_|_ATP-dependent_DNA_helicase_|_34607:36901_Reverse		Replication
342	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04385_|_3'-5'_exonuclease_|_49480:50445_Forward		Replication
343	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04545_|_ATP-dependent_DNA_helicase_RecQ_|_17841:19592_Forward		Replication
344	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05055_|_primosomal_protein_DnaI_|_53909:54829_Forward		Replication
345	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05725_|_DNA_repair_protein_RecO_|_26289:27107_Forward		Replication
346	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06650_|_DNA_mismatch_repair_protein_MutL_|_27684:29615_Reverse		Replication
347	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06655_|_DNA_mismatch_repair_protein_MutS_|_30074:32692_Reverse		Replication
348	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07545_|_helicase_|_36131:37471_Reverse		Replication
349	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09885_|_initiator_RepB_protein_|_4147:5082_Reverse		replication
350	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10595_|_primosomal_protein_DnaI_|_40482:42895_Forward		replication
351	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10675_|_ATP-dependent_helicase_|_7491:11043_Forward		replication
352	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10680_|_ATP-dependent_helicase_|_15776:17806_Forward		replication
353	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10885_|_recombinase_RecF_|_59340:60478_Reverse		replication
354	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10965_|_polC_|_DNA_polymerase_III_|_3756:7675_Forward		replication
355	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11235_|_DNA_polymerase_I_|_47648:50307_Forward		replication
356	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11485_|_ATP-dependent_DNA_helicase_RuvA_|_25875:26485_Reverse		replication
357	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11555_|_recombinase_RecJ_|_12840:15135_Reverse		replication
358	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12160_|_DNA_polymerase_III_subunit_epsilon_|_14979:15740_Forward		Replication
359	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02400_|_dTMP_kinase_|_69058:69702_Forward		replication

360	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08235_|_ribose_operon_repressor_|_12476:13483_Forward		repressor operon ribose
361	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00160_|_RibT_protein_|_29810:30178_Reverse		riboflavin biosynthesis
362	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01485_|_ribonucleotide-diphosphate_reductase_subunit_beta_|_36092:37057_Reverse		ribonucleotide-diphosphate_reductase_subunit_beta
363	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00175_|_DNA-binding_protein_|_31704:32588_Reverse		RNA processing
364	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07715_|_uridine_kinase_|_29281:29910_Reverse	2.7.1.48	salvage pathways of pyrimidine ribonucleotides
365	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00015_|_signal_peptidase_I_|_2305:2832_Reverse		secretion
366	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01780_|_S26_family_signal_peptidase_|_17556:18167_Reverse		Secretion
367	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00980_|_protein_phosphatase_|_45227:45973_Forward	3.1.3.16	serine/threonine phosphatase of unknown function
368	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01810_|_serine/threonine_protein_phosphatase_|_22521:23315_Reverse	3.1.3.48	Serine/tyrosine protein phosphatase
369	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11365_|_shikimate_kinase_|_44441:45000_Forward	2.7.1.71	shikimate metabolism biosynthèse de certains acides aminés aromatiques
370	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11770_|_sodium_ABC_transporter_permease_|_7880:9120_Forward		sodium_ABC_transporter_permease_
371	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10820_|_glycine/betaine_ABC_transporter_|_22539:23443_Forward		stress resistance (cold, osmotic stress?)
372	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10865_|_glycine/betaine_ABC_transporter_ATP-binding_protein_|_28107:29243_Forward		stress resistance (cold, osmotic stress?)
373	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00625_|_cold-shock_protein_|_128728:128928_Reverse		Stress response
374	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01515_|_cold-shock_protein_|_42113:42328_Reverse		Stress response
375	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05500_|_cold-shock_protein_|_31552:31752_Reverse		Stress response
376	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06910_|_general_stress_protein_|_42836:43213_Forward		Stress response
377	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07915_|_universal_stress_protein_UspA_|_34357:34848_Forward		stress response
378	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04125_|_sugar_ABC_transporter_permease_|_53586:54443_Reverse		Sugar ABC transporter
379	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04130_|_sugar_ABC_transporter_permease_|_54459:55808_Reverse		Sugar ABC transporter
380	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04135_|_sugar_ABC_transporter_substrate-binding_protein_|_55843:57093_Reverse		Sugar ABC transporter
381	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00700_|_PTS_glucitol_transporter_subunit_IIA_|_142648:144054_Forward		sugar metabolism
382	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00710_|_xylose_isomerase_|_145687:147030_Reverse		sugar metabolism
383	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00720_|_galactose_mutarotase_|_148564:149595_Forward		sugar metabolism
384	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09565_|_phosphogluconate_dehydrogenase_(NADP(+)-dependent,_decarboxylating)_|_10173:11594_Forward		sugar metabolism
385	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04100_|_maltose_phosphorylase_|_45861:48104_Reverse		sugar metabolism
386	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09495_|_207:1313_Forward		sugar metabolism, oxygen dependent
387	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00040_|_dihydrofolate_reductase_|_5720:6217_Reverse	1.5.1.3	synthèse tetrahydrofolate, cofactor thymidylate synthase dfrA
388	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00870_|_TetR_family_transcriptional_regulator_|_27358:27990_Forward		TetR family transcriptional regulator of unknown function
389	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03640_|_TetR_family_transcriptional_regulator_|_21757:22326_Forward		TetR family transcriptional regulator of unknown function
390	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03810_|_TetR_family_transcriptional_regulator_|_52686:53252_Reverse		TetR family transcriptional regulator of unknown function
391	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06490_|_thioredoxin_|_35572:36093_Reverse		Thioredoxine reductase/Redox
392	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06530_|_thiol_reductase_thioredoxin_|_12:323_Reverse		Thioredoxine reductase/Redox
393	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04430_|_thiol_reductase_thioredoxin_|_56604:56924_Forward		Thioredoxine/ redox
394	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00955_|_DNA-directed_RNA_polymerase_subunit_omega_|_38919:39176_Forward		Transcription
395	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02310_|_RNA_polymerase_subunit_sigma_|_53665:54261_Forward		Transcription
396	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07200_|_elongation_factor_P_|_1401:1964_Reverse		Transcription
397	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07710_|_transcription_elongation_factor_GreA_|_28774:29247_Reverse		Transcription
398	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11620_|_transcriptional_regulator_|_11873:13278_Reverse		transcription
399	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11965_|_transcription-repair_coupling_factor_|_2744:6261_Forward		transcription
400	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04475_|_DNA-binding_response_regulator_|_4164:4919_Forward		transcriptional regulator
401	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07090_|_transcriptional_regulator_|_28200:28931_Reverse		transcriptional regulator
402	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09035_|_XRE_family_transcriptional_regulator_|_617:1549_Reverse		transcriptional regulator
403	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10940_|_PhoP_family_transcriptional_regulator_|_48090:48802_Reverse		transcriptional regulator
404	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01535_|_bifunctional_pyr_operon_transcriptional_regulator/uracil_phosphoribosyltransferase_|_45758:46300_Forward		transcriptional regulator
405	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00170_|_transcriptional_repressor_|_31150:31668_Reverse		Transcriptional regulator for iron transport and metabolism
406	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03775_|_MarR_family_transcriptional_regulator_|_46956:47417_Forward		Transcriptional regulator MarR-type, unknown target
407	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06485_|_MarR_family_transcriptional_regulator_|_35051:35503_Forward		Transcriptional regulator MarR-type, unknown target
408	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03275_|_heat-inducible_transcriptional_repressor_HrcA_|_20173:21231_Forward		transcriptional regulator of heat-shock genes
409	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03785_|_pur_operon_repressor_|_48705:49541_Forward		transcriptional regulator of the purine biosynthesis operon
410	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02235_|_GntR_family_transcriptional_regulator_|_38095:38820_Forward		transcriptional regulator, gntR family, unknown target
411	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05160_|_GntR_family_transcriptional_regulator_|_15686:16414_Forward		transcriptional regulator, gntR family, unknown target

412	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07225_|_GntR_family_transcriptional_regulator_|_6893:7663_Forward		transcriptional regulator, gntR family, unknown target
413	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09685_|_GntR_family_transcriptional_regulator_|_5172:5873_Reverse		transcriptional regulator, gntR family, unknown target
414	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01875_|_GntR_family_transcriptional_regulator_|_38301:39032_Forward		transcriptional regulator, gntR family, unknown target
415	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09985_|_Rrf2_family_transcriptional_regulator_|_6067:6582_Reverse		Transcriptional regulator, unknown target
416	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00215_|_50S_ribosomal_protein_L32_|_42679:42861_Reverse		translation
417	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00315_|_tuf_|_elongation_factor_Tu_|_66147:67337_Reverse		translation
418	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00335_|_30S_ribosomal_protein_S20_|_71072:71326_Forward		translation
419	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00465_|_ribonuclease_J_|_96419:98107_Forward		Translation
420	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00480_|_exodeoxyribonuclease_V_subunit_alpha_|_100663:103179_Reverse		Translation
421	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00510_|_tRNA(5-methylaminomethyl-2-thiouridine)-_methyltransferase_|_108720:109829_Reverse		Translation
422	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00580_|_RNA-binding_protein_|_120957:121424_Reverse		Translation
423	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00620_|_tyrosine--tRNA_ligase_|_127125:128384_Reverse		Translation
424	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00885_|_50S_ribosomal_protein_L27_|_28824:29111_Forward		Translation
425	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00910_|_exodeoxyribonuclease_VII_large_subunit_|_31928:33277_Forward		Translation
426	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00975_|_16S_rRNA_(cytosine(967)-C(5))-methyltransferase_|_43862:45205_Forward		Translation
427	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01005_|_50S_ribosomal_protein_L28_|_50355:50540_Reverse		Translation
428	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01060_|_ribonuclease_III_|_62606:63295_Forward		Translation
429	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01105_|_rpsP_|_30S_ribosomal_protein_S16_|_74072:74347_Forward		Translation
430	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01110_|_RNA-binding_protein_|_74357:74599_Forward		Translation
431	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01120_|_tRNA_(guanine(37)-N(1))-methyltransferase_|_75184:75927_Forward		Translation
432	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01125_|_rplS_|_50S_ribosomal_protein_L19_|_76046:76393_Forward		Translation
433	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01170_|_aminoacyl-tRNA_deacylase_|_81018:81521_Reverse		Translation
434	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01195_|_ribosomal_protein_L11_methyltransferase_|_83578:84456_Forward		Translation
435	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01200_|_16S_rRNA_(uracil(1498)-N(3))-methyltransferase_|_84468:85223_Forward		Translation
436	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01390_|_asnC_|_asparagine--tRNA_ligase_|_18482:19780_Forward		Translation
437	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01430_|_RNA_methyltransferase_|_25723:26865_Forward		Translation
438	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02290_|_cysteine--tRNA_ligase_|_50390:51796_Forward		Translation
439	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02295_|_Mini-ribonuclease_3_|_51793:52197_Forward		Translation
440	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02300_|_23S_rRNA_(guanosine(2251)-2'-O)-methyltransferase_RlmB_|_52283:53089_Forward		Translation
441	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02305_|_DNA-binding_protein_|_53086:53619_Forward		Translation
442	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02315_|_50S_ribosomal_protein_L33_|_54325:54474_Forward		Translation
443	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02335_|_50S_ribosomal_protein_L11_|_56355:56780_Forward		Translation
444	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02350_|_50S_ribosomal_protein_L10_|_59149:59652_Forward		Translation
445	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02355_|_rplL_|_50S_ribosomal_protein_L7/L12_|_59704:60069_Forward		Translation
446	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02365_|_16S_rRNA_methyltransferase_|_62874:63485_Forward		Translation
447	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02375_|_tRNA-specific_adenosine_deaminase_|_64668:65171_Forward		Translation
448	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02425_|_rRNA_(cytidine-2'-O-)-methyltransferase_|_72286:73164_Forward		Translation
449	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02745_|_50S_ribosomal_protein_L9_|_50482:50934_Reverse		Translation
450	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02755_|_30S_ribosomal_protein_S18_|_53364:53603_Reverse		Translation
451	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02765_|_30S_ribosomal_protein_S6_|_54205:54501_Reverse		Translation
452	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02785_|_RNA-binding_S4_protein_|_60485:60712_Reverse		Translation
453	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02830_|_tRNA_modification_GTPase_|_69621:71009_Forward		Translation
454	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03180_|_tryptophan--tRNA_ligase_|_71350:72372_Reverse		Translation
455	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03255_|_ribosome-binding_factor_A_|_15807:16163_Forward		Translation
456	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03260_|_tRNA_pseudouridine(55)_synthase_|_16250:17167_Forward		Translation
457	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03675_|_tryptophan--tRNA_ligase_|_27705:28721_Forward		Translation
458	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03745_|_methionine--tRNA_ligase_|_39242:41287_Forward		Translation
459	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04360_|_RNA_pseudouridine_synthase_|_41770:42669_Reverse		Translation
460	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04435_|_tRNA-binding_protein_|_56942:57571_Forward		Translation
461	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04955_|_arginine--tRNA_ligase_|_29332:31023_Forward		Translation

462	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05235_|_translation_factor_Sua5_|_31496:32509_Forward		Translation
463	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05415_|_23S_rRNA_(uracil-5-)-methyltransferase_RumA_|_10857:12221_Reverse		Translation
464	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05425_|_aspartyl/glutamyl-tRNA_amidotransferase_subunit_B_|_13356:14786_Reverse		Translation
465	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05430_|_gatA_|_aspartyl/glutamyl-tRNA_amidotransferase_subunit_A_|_14786:16252_Reverse		Translation
466	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05435_|_glutamyl-tRNA_amidotransferase_|_16255:16551_Reverse		Translation
467	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05630_|_rbgA_|_ribosome_biogenesis_GTPase_YlqF_|_8192:9049_Forward		Translation
468	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05710_|_16S_rRNA_maturation_RNase_YbeY_|_24489:24965_Forward		Translation
469	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06055_|_50S_ribosomal_protein_L17_|_34302:34682_Forward		Translation
470	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06565_|_alaS_|_alanine--tRNA_ligase_|_6672:9311_Reverse		Translation
471	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06935_|_30S_ribosomal_protein_S2_|_2611:3405_Reverse		Translation
472	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07745_|_RNA_methyltransferase_|_36032:36793_Reverse		Translation
473	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07885_|_30S_ribosomal_protein_S4_|_28208:28795_Forward		translation
474	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09065_|_tRNA_(cytidine(34)-2'-O)-methyltransferase_|_8022:8531_Reverse		translation
475	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09215_|_rpmE2_|_50S_ribosomal_protein_L31_type_B_|_17119:17382_Forward		translation
476	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09350_|_aminoacyl-tRNA_hydrolase_|_1904:2461_Forward		translation
477	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09500_|_translation_initiation_factor_IF-3_|_1547:2050_Forward		translation
478	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09505_|_rpmI_|_50S_ribosomal_protein_L35_|_2084:2284_Forward		translation
479	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09510_|_50S_ribosomal_protein_L20_|_2361:2720_Forward		translation
480	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10135_|_ribonuclease_Y_|_1581:3143_Forward		translation
481	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10515_|_pseudouridine_synthase_|_27688:28436_Reverse		translation
482	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10700_|_pseudouridine_synthase_|_44674:45578_Forward		translation
483	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11475_|_tgt_|_queuine_tRNA-ribosyltransferase_|_21669:22810_Reverse		translation
484	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11480_|_S-adenosylmethionine_tRNA_ribosyltransferase_|_23810:24839_Reverse		translation
485	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11645_|_aspS_|_aspartate--tRNA_ligase_|_3233:5013_Reverse		translation
486	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11670_|_valS_|_valine--tRNA_ligase_|_19069:21716_Reverse		translation
487	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00310_|_tig_|_trigger_factor_|_64666:65961_Reverse		Translation
488	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09530_|_RNA-binding_protein_|_5603:5920_Forward		translation
489	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00300_|_YihA_family_ribosome_biogenesis_GTP-binding_protein_|_62462:63061_Reverse		Translation
490	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05660_|_gid_|_methylenetetrahydrofolate--tRNA-(uracil(54)-_C(5))-methyltransferase_(FADH(2)-oxidizing)_TrmFO_|_14545:15855_Forward		Translation
491	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05720_|_era_|_GTPase_Era_|_25378:26280_Forward		Translation
492	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06335_|_peptide_chain_release_factor_2_|_3461:4459_Forward		Translation
493	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10660_|_UDP-diphospho-muramoylpentapeptide_beta-N-acetylglucosaminyltransferase_|_96164:97262_Forward		Translation
494	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02595_|_protein-tyrosine-phosphatase_|_24522:25313_Forward		translation
495	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12075_|_UDP_pyrophosphate_synthase_|_2148:2908_Reverse		translation
496	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05015_|_SsrA-binding_protein_|_44719:45189_Forward		Translation, tmRNA-binding protein
497	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09400_|_tRNA-dihydrouridine_synthase_|_14873:15862_Forward		translation?
498	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09470_|_mRNA_interferase_PemK_|_11403:11768_Forward		translation? cell death?
499	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10110_|_sodium:cation_symporter_|_2459:3568_Reverse		transport cation
500	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03575_|_sodium:dicarboxylate_symporter_|_7424:8821_Forward		transport ions
501	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03845_|_Na+/proline_symporter_|_60059:61561_Forward		transport ions
502	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03610_|_peptide_transporter_|_15143:17080_Forward		Transport of peptides
503	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10760_|_sodium:proton_antiporter_|_53319:54496_Forward		transport proton
504	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07560_|_sodium:proton_antiporter_|_175:2292_Reverse		Transport, Na(+)/H(+) antiporter
505	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04975_|_transporter_|_33833:35272_Reverse		Transporter
506	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04690_|_glycerol_transporter_|_41469:42185_Forward		Transporter (facilitator) unknown substrate
507	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00445_|_ABC_transporter_|_92006:93763_Reverse		transporter ABC
508	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08745_|_transporter_|_5307:6944_Reverse		transporter?
509	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05765_|_peptidase_T_|_36060:37301_Forward	3.4.11.4	Tripeptide aminopeptidase PepT
510	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01240_|_16S_rRNA_(cytosine(1402)-N(4))-methyltransferase_|_90290:91249_Forward		Trnaslation
511	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09750_|_glycosyl_transferase_|_3740:4561_Forward	2.4.1.-	UDP-Glycosyltransferase/glycogen phosphorylase family Cell wall?
512	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05135_|_multidrug_ABC_transporter_ATP-binding_protein_|_8071:9801_Forward		Uncharacterized ABC transporter
513	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00070_|_hypothetical_protein_|_12143:13417_Reverse~~		unknown
514	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00075_|_DNA-binding_protein_|_13524:13799_Reverse~~		unknown

515	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00205_|_hypothetical_protein_|_39870:40742_Reverse		unknown
516	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00250_|_hypothetical_protein_|_51698:52792_Reverse		unknown
517	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00375_|_hypothetical_protein_|_78106:78402_Reverse		unknown
518	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00385_|_hypothetical_protein_|_78842:79885_Reverse		unknown
519	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00410_|_hypothetical_protein_|_84341:84622_Reverse		unknown
520	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00435_|_hypothetical_protein_|_90215:90892_Reverse		unknown
521	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00460_|_hypothetical_protein_|_96203:96415_Forward		unknown
522	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00475_|_GNAT_family_acetyltransferase_|_99403:100617_Reverse		Unknown
523	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00485_|_hypothetical_protein_|_103185:103844_Reverse		unknown
524	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00520_|_cysteine_desulfurase_|_110617:111783_Reverse		Unknown
525	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00540_|_hypothetical_protein_|_113959:114429_Reverse		unknown
526	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00570_|_hypothetical_protein_|_119345:120067_Forward		unknown
527	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00880_|_hypothetical_protein_|_28449:28790_Forward		unknown
528	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01015_|_hypothetical_protein_|_51289:52959_Forward		unknown
529	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01175_|_hypothetical_protein_|_81655:81837_Forward		unknown
530	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01185_|_hypothetical_protein_|_82529:82930_Forward		unknown
531	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01320_|_hypothetical_protein_|_108631:109311_Reverse		unknown
532	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01345_|_hypothetical_protein_|_2188:3150_Forward		unknown
533	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01405_|_penicillin-binding_protein_|_21258:23513_Reverse		unknown
534	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01435_|_hypothetical_protein_|_26972:27925_Forward		unknown
535	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01510_|_hypothetical_protein_|_41612:42022_Forward		unknown
536	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01615_|_hypothetical_protein_|_63242:64111_Forward		unknown
537	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01705_|_hypothetical_protein_|_86171:87961_Reverse		unknown
538	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01725_|_hypothetical_protein_|_351:821_Forward		unknown
539	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01840_|_hypothetical_protein_|_30259:30867_Forward		unknown
540	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01935_|_hypothetical_protein_|_55777:55995_Forward		unknown
541	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01955_|_hypothetical_protein_|_59989:61278_Forward		unknown
542	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01975_|_nucleotidyltransferase_|_62486:63244_Forward		unknown
543	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02050_|_hypothetical_protein_|_3548:5131_Forward		unknown
544	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02105_|_hypothetical_protein_|_11626:12849_Forward		unknown
545	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02360_|_hypothetical_protein_|_60238:62859_Forward		unknown
546	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02440_|_membrane_protein_|_75230:75760_Forward		unknown
547	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02480_|_hypothetical_protein_|_4683:8141_Reverse		unknown
548	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02565_|_hypothetical_protein_|_19951:20685_Reverse		unknown
549	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02575_|_hypothetical_protein_|_21531:22022_Reverse		unknown
550	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02610_|_hypothetical_protein_|_26944:27312_Reverse		unknown
551	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02645_|_hypothetical_protein_|_31503:31868_Forward		unknown
552	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02665_|_hypothetical_protein_|_35916:36779_Reverse		unknown
553	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02670_|_hypothetical_protein_|_36933:37298_Reverse		unknown
554	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02700_|_hypothetical_protein_|_41885:42562_Reverse		unknown
555	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02725_|_nucleoid_occlusion_protein_|_45612:46487_Reverse		unknown
556	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02750_|_hypothetical_protein_|_50958:52991_Reverse		unknown
557	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02935_|_hypothetical_protein_|_20280:21212_Reverse		unknown
558	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02990_|_hypothetical_protein_|_34476:35126_Forward		unknown
559	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03120_|_hypothetical_protein_|_59292:59657_Forward		unknown
560	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03165_|_hypothetical_protein_|_69665:70357_Forward		unknown
561	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03240_|_hypothetical_protein_|_12182:12481_Forward		unknown
562	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03465_|_membrane_protein_|_57264:57944_Reverse		unknown
563	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03590_|_hypothetical_protein_|_11865:12554_Forward		unknown
564	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03625_|_hypothetical_protein_|_18974:19825_Forward		unknown
565	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03720_|_hypothetical_protein_|_35635:36597_Forward		unknown
566	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03730_|_NUDIX_hydrolase_|_37052:37555_Forward		unknown
567	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03995_|_virion_core_protein_|_22733:23851_Reverse		unknown
568	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04040_|_GMP_synthetase_|_31479:33032_Reverse		unknown
569	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04265_|_hypothetical_protein_|_23960:24160_Forward		unknown
570	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04330_|_hypothetical_protein_|_36342:36719_Forward		unknown
571	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04335_|_hypothetical_protein_|_36872:38776_Forward		unknown
572	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04380_|_hypothetical_protein_|_46788:49490_Forward		unknown
573	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04395_|_hypothetical_protein_|_51595:51918_Reverse		unknown
574	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04400_|_histidine_triad_protein_|_51918:52349_Reverse		unknown
575	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04585_|_hypothetical_protein_|_25463:25996_Reverse		unknown
576	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04685_|_hypothetical_protein_|_40668:41441_Forward		unknown
577	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04695_|_hypothetical_protein_|_42289:44754_Forward		unknown
578	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05105_|_membrane_protein_|_4472:4654_Reverse		unknown
579	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05110_|_hypothetical_protein_|_4647:4967_Reverse		unknown
580	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05290_|_membrane_protein_|_41455:41685_Forward		unknown
581	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05360_|_type_II_A_CRISPR-associated_protein_Csn2_|_55113:55808_Forward		unknown
582	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05375_|_hypothetical_protein_|_1721:1915_Forward		unknown
583	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05505_|_hypothetical_protein_|_32065:32703_Forward		unknown
584	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05525_|_hypothetical_protein_|_34641:36806_Reverse		unknown
585	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05590_|_hypothetical_protein_|_51896:53692_Reverse		unknown
586	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05705_|_hypothetical_protein_|_23806:24249_Forward		unknown
587	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05875_|_hypothetical_protein_|_4905:6719_Forward		unknown
588	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06125_|_hypothetical_protein_|_2614:3399_Reverse		unknown
589	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06185_|_hypothetical_protein_|_17602:17793_Reverse		unknown
590	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06195_|_hypothetical_protein_|_20339:20638_Forward		unknown
591	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06200_|_hypothetical_protein_|_20952:22034_Forward		unknown
592	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06210_|_hypothetical_protein_|_24425:25756_Reverse		unknown
593	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06225_|_membrane_protein_|_27566:28039_Forward		unknown

594	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06230_|_hypothetical_protein_|_28813:29382_Reverse		unknown
595	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06395_|_hypothetical_protein_|_15133:16617_Forward		unknown
596	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06430_|_hypothetical_protein_|_21475:21885_Forward		unknown
597	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06445_|_hydrolase_|_25050:25688_Forward		unknown
598	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06470_|_RNase_adaptor_protein_RapZ_|_32093:32977_Forward		unknown
599	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06475_|_hypothetical_protein_|_32974:34008_Forward		unknown
600	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06480_|_DNA-binding_protein_WhiA_|_34011:34955_Forward		unknown
601	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06560_|_hypothetical_protein_|_6132:6392_Reverse		unknown
602	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06570_|_hypothetical_protein_|_9559:10542_Reverse		unknown
603	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06620_|_GNAT_family_acetyltransferase_|_23271:23786_Reverse		unknown
604	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06800_|_hypothetical_protein_|_16463:16948_Forward		unknown
605	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06865_|_cell_surface_protein_|_34614:35912_Reverse		unknown
606	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06875_|_5'-nucleotidase_|_36607:38028_Forward		unknown
607	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06880_|_hypothetical_protein_|_38003:38620_Forward		unknown
608	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06980_|_hypothetical_protein_|_10844:11086_Reverse		unknown
609	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06985_|_repressor_LexA_|_11222:11836_Forward		unknown
610	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07020_|_hypothetical_protein_|_17876:18313_Reverse		unknown
611	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07105_|_hypothetical_protein_|_29769:30143_Reverse		unknown
612	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07110_|_steroid-binding_protein_|_30336:30575_Forward		unknown
613	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07130_|_hypothetical_protein_|_33987:35771_Reverse		unknown
614	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07135_|_hypothetical_protein_|_35796:36608_Reverse		unknown
615	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07205_|_hypothetical_protein_|_2032:2820_Reverse		unknown
616	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07530_|_hypothetical_protein_|_34780:35199_Reverse		unknown
617	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07555_|_hypothetical_protein_|_38644:38988_Forward		unknown
618	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07575_|_hypothetical_protein_|_4191:4727_Forward		unknown
619	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07615_|_GNAT_family_acetyltransferase_|_10567:11130_Reverse		unknown
620	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07655_|_hypothetical_protein_|_18893:20005_Reverse		unknown
621	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07660_|_TIGR00159_family_protein_|_20002:20841_Reverse		unknown
622	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08100_|_hypothetical_protein_|_9859:10701_Forward		unknown
623	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08210_|_hypothetical_protein_|_7364:8224_Reverse		unknown
624	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08315_|_hypothetical_protein_|_1109:1324_Forward		unknown
625	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08550_|_hypothetical_protein_|_22292:22525_Forward		unknown
626	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08585_|_transcriptional_regulator_|_6055:6891_Reverse		unknown
627	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08595_|_hypothetical_protein_|_7921:8760_Reverse		unknown
628	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08690_|_hypothetical_protein_|_22796:23770_Forward		unknown
629	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09050_|_hypothetical_protein_|_3655:4926_Reverse		unknown
630	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09070_|_methyltransferase_|_8784:9620_Reverse		unknown
631	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09085_|_GNAT_family_acetyltransferase_|_11969:12424_Forward		unknown
632	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09090_|_AI-2E_family_transporter_|_12468:13613_Reverse		unknown
633	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09155_|_HD_domain-containing_protein_|_2747:4120_Reverse		unknown
634	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09185_|_hypothetical_protein_|_8147:10732_Forward		unknown
635	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09195_|_hypothetical_protein_|_12028:12468_Forward		unknown
636	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09225_|_membrane_protein_|_18087:18752_Forward		unknown
637	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09290_|_hypothetical_protein_|_8841:9278_Reverse		unknown
638	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09295_|_hypothetical_protein_|_9593:10234_Reverse		unknown
639	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09300_|_hypothetical_protein_|_10350:11021_Reverse		unknown
640	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09305_|_aquaporin_|_11251:11970_Forward		unknown
641	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09475_|_membrane_protein_|_11904:12428_Forward		unknown
642	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09490_|_hypothetical_protein_|_14561:15937_Reverse		unknown
643	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09560_|_hypothetical_protein_|_9452:10012_Forward		unknown
644	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09625_|_hypothetical_protein_|_7153:7524_Forward		unknown
645	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09815_|_rhomboid_family_intramembrane_serine_protease_|_5926:6591_Forward		unknown
646	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09830_|_sulfurtransferase_|_7849:8268_Forward		unknown
647	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09855_|_addiction_module_toxin,_RelE/StbE_family_protein_|_209:565_Forward		unknown
648	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09960_|_gamma-aminobutyrate_permease_|_18:1442_Reverse		unknown
649	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09990_|_hypothetical_protein_|_6588:7118_Reverse		unknown
650	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10190_|_hypothetical_protein_|_2489:2839_Reverse		unknown
651	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10330_|_hypothetical_protein_|_1559:2959_Reverse		unknown
652	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10380_|_hypothetical_protein_|_135:1373_Forward		unknown
653	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10385_|_transposase_|_107:1186_Reverse		unknown
654	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10565_|_hypothetical_protein_|_1606:2297_Forward		unknown
655	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10775_|_small_protein_|_61434:61562_Forward		unknown
656	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10835_|_nucleoid-associated_protein_|_67177:67484_Forward		unknown
657	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10990_|_histidine_kinase_|_28686:30118_Forward		unknown
658	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11005_|_alkaline_phosphatase_|_44360:46527_Reverse		unknown
659	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11015_|_hypothetical_protein_|_50853:52085_Forward		unknown
660	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11025_|_hypothetical_protein_|_56557:57242_Reverse		unknown
661	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11045_|_cell_surface_protein_|_10452:11758_Forward		unknown
662	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11060_|_hypothetical_protein_|_37908:38755_Forward		unknown
663	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11155_|_hypothetical_protein_|_32676:32939_Forward		unknown
664	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11315_|_hypothetical_protein_|_36879:37312_Reverse		unknown
665	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11465_|_hypothetical_protein_|_12911:14232_Reverse		unknown
666	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11585_|_hypothetical_protein_|_30316:34404_Reverse		unknown
667	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11605_|_hypothetical_protein_|_35234:35460_Reverse		unknown
668	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11640_|_hypothetical_protein_|_996:1864_Reverse		unknown
669	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11690_|_hypothetical_protein_|_16391:17616_Forward		unknown
670	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11815_|_hypothetical_protein_|_18187:18614_Reverse		unknown

671	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11940_|_hypothetical_protein_|_6879:7604_Reverse~~		unknown
672	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11995_|_hypothetical_protein_|_8281:9452_Forward		unknown
673	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12110_|_hypothetical_protein_|_206:2719_Reverse~~		unknown
674	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12155_|_hypothetical_protein_|_82033:82467_Forward~~		unknown
675	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12175_|_hypothetical_protein_|_72903:75893_Forward~~		unknown
676	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12185_|_hypothetical_protein_|_63:3638_Forward~~		unknown
677	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12265_|_hypothetical_protein_|_24241:25101_Forward~~		unknown
678	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12270_|_hypothetical_protein_|_44766:45590_Forward~~		unknown
679	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12285_|_hypothetical_protein_|_52525:53163_Forward~~		unknown
680	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12290_|_hypothetical_protein_|_53157:53909_Forward~~		unknown
681	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12295_|_hypothetical_protein_|_44104:46794_Reverse~~		unknown
682	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12350_|_hypothetical_protein_|_8773:9498_Reverse~~		unknown
683	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS12375_|_hypothetical_protein_|_2533:4461_Reverse~~		unknown
684	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01805_|_penicillin-binding_protein_|_21262:22452_Forward~~		unknown
685	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07290_|_hypothetical_protein_|_21498:22952_Reverse		unknown
686	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08515_|_hypothetical_protein_|_16201:16578_Forward		unknown
687	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10745_|_hypothetical_protein_|_27627:28472_Forward		unknown
688	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03050_|_hypothetical_protein_|_44849:46192_Reverse		unknown
689	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02905_|_amino_acid_permease_|_13753:15171_Reverse		voie arginine deiminase
690	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02910_|_arginine_deiminase_|_15263:16489_Reverse		voie arginine deiminase
691	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02915_|_carbamate_kinase_|_16548:17477_Reverse		voie arginine deiminase
692	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02920_|_ornithine_carbamoyltransferase_|_17493:18494_Reverse		voie arginine deiminase
693	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05480_|_xanthine_phosphoribosyltransferase_|_28392:28970_Reverse	2.4.2.22	Xanthine and xanthosine salvage, putine metabolism
694	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00905_|bifunctional_5,10-methylene-tetrahydrofolate_dehydrogenase/5,10-methylene-tetrahydrofolate_cyclohydrolase_|_31039:31935_Forward		
695	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03680_|_paraslipin_|_28956:29780_Reverse		
696	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05205_|_adenosylcobyric_acid_synthase_|_26165:26914_Reverse		
697	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07040_|_laaL_|_21611:22069_Reverse		
698	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07525_|_aryl-phospho-beta-D-glucosidase_|_32923:34368_Reverse		
699	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11460_|_oligoribonuclease_|_11954:12908_Reverse		
700	LACTOBACILLUS,VAGINALIS|AZGL01000004,1_cds_KRM48987,1_1005_[locus_tag=FC58_GL001036]_[protein=ribosomal_protein_L18]_[protein_id=KRM48987,1]_[location=complement(25760,,26125)]		translation
701	LACTOCOCCUS,LACTIS|AE005176,1_cds_AAK04600,1_502_[gene=hslA]_[locus_tag=L102317]_[protein=HU_like_DNA-binding_protein]_[protein_id=AAK04600,1]_[location=502338,,502613]		unknown
702	~~LACTOCOCCUS,LACTIS|AE005176,1_cds_AAK05337,1_1239_[gene=ymgG]_[locus_tag=L65637]_[protein=hypothetical_protein]_[protein_id=AAK05337,1]_[location=complement(1265891,,1266442)]~~		unknown
703	~~LACTOCOCCUS,LACTIS|AE005176,1_cds_AAK05339,1_1241_[gene=ymgI]_[locus_tag=L66407]_[protein=unknown_protein]_[protein_id=AAK05339,1]_[location=complement(1266661,,1267221)]~~		unknown
704	~~LACTOCOCCUS,LACTIS|AE005176,1_cds_AAK05340,1_1242_[gene=ymgJ]_[locus_tag=L67002]_[protein=hypothetical_protein]_[protein_id=AAK05340,1]_[location=complement(1267256,,1267498)]~~		unknown
705	LACTOCOCCUS,LACTIS|AE005176,1_cds_AAK06007,1_1909_[gene=fbaA]_[locus_tag=L0009]_[protein=fructose-bisphosphate_aldolase]_[protein_id=AAK06007,1]_[location=complement(1979402,,1980298)]		glycolysis
706	LACTOCOCCUS,LACTIS|AE005176,1_cds_AAK06344,1_2246_[gene=gapB]_[locus_tag=L0005]_[protein=glyceraldehyde_3-phosphate_dehydrogenase]_[protein_id=AAK06344,1]_[location=complement(2332466,,2333476)]	1.2.1.12	glycolysis
707	LACTOCOCCUS,LACTIS|AE005176,1_cds_AAK06359,1_2261_[gene=rpsG]_[locus_tag=L0384]_[protein=30S_ribosomal_protein_S7]_[protein_id=AAK06359,1]_[location=complement(2354564,,2355031)]		translation
708	LCA_LACTOBACILLUS_SAKEIRS06440_|_acetate_kinase_|_1274891:1276087_Reverse	2.7.2.1	acetate kinase 2, purines nucleosides degradation, pyruvate degradation
709	LCA_LACTOBACILLUS_SAKEIRS00920_|_acetate_kinase_|_191473:192657_Forward	2.7.2.1	ackA1 / Glycolyse, ribose catabolism
710	LCA_LACTOBACILLUS_SAKEIRS01775_|_carbamate_kinase_|_375713:376657_Forward	2.7.2.2	arcC - Degradation arginine
711	LCA_LACTOBACILLUS_SAKEIRS07900_|_pyrroline-5-carboxylate_reductase_|_1567048:1567854_Reverse	1.5.1.2	Arginine and proline metabolism
712	LCA_LACTOBACILLUS_SAKEIRS03125_|_arginine_repressor_|_617068:617535_Forward		ArgR family transcriptional regulator
713	LCA_LACTOBACILLUS_SAKEIRS07975_|_cell_division_protein_FtsH_|_1581251:1583341_Reverse	3.4.24.-	ATP-dependent zinc metallopeptidase
714	LCA_LACTOBACILLUS_SAKEIRS03580_|_chromosome_segregation_protein_SMC_|_708462:712022_Forward		Cell division
715	LCA_LACTOBACILLUS_SAKEIRS03820_|_arabinan_synthesis_protein_|_749472:750296_Forward		Cell division
716	LCA_LACTOBACILLUS_SAKEIRS04290_|_rod_shape-determining_protein_|_841470:842474_Forward		Cell division
717	LCA_LACTOBACILLUS_SAKEIRS06620_|_cell_division_protein_FtsI_|_1301490:1303589_Reverse		Cell division
718	LCA_LACTOBACILLUS_SAKEIRS00500_|_hypothetical_protein_|_100870:102144_Forward		cell surface protein of unknown function
719	LCA_LACTOBACILLUS_SAKEIRS00460_|_carboxylate--amine_ligase_|_94813:96066_Forward	6.3.1.12	Cell wall biogenesis/degradation
720	LCA_LACTOBACILLUS_SAKEIRS08215_|_1632935:1634323_Reverse		Cell wall biosynthesis
721	LCA_LACTOBACILLUS_SAKEIRS03235_|_UDP-N-acetylmuramate--L-alanine_ligase_|_639372:640703_Forward		Cell wall synthesis
722	LCA_LACTOBACILLUS_SAKEIRS07130_|_N-acetylmuramoyl-L-alanine_amidase_|_1411165:1413171_Reverse		Cell wall synthesis
723	LCA_LACTOBACILLUS_SAKEIRS07455_|_UTP--glucose-1-phosphate_uridylyltransferase_|_1475623:1476525_Reverse	2.7.7.9	Cell wall synthesis
724	LCA_LACTOBACILLUS_SAKEIRS07560_|_UDP-N-acetyl_glucosamine_2-epimerase_|_1494222:1495361_Reverse		Cell wall synthesis
725	LCA_LACTOBACILLUS_SAKEIRS05780_|_peptidase_M23_|_1146170:1146865_Reverse		Cell wall, Peptidoglycan-binding lysin domain
726	LCA_LACTOBACILLUS_SAKEIRS03180_|_peptidylprolyl_isomerase_|_629874:630782_Reverse	5.2.1.8	Chaperone, protein folding and stabilization
727	LCA_LACTOBACILLUS_SAKEIRS02735_|_ATP-dependent_Clp_protease_proteolytic_subunit_|_549884:550468_Reverse		ClpP protease, adaptation to atypical conditions
728	LCA_LACTOBACILLUS_SAKEIRS07755_|_cold-shock_protein_|_1533764:1533964_Reverse		cold shock protein
729	LCA_LACTOBACILLUS_SAKEIRS04015_|_2-deoxyribose-5-phosphate_aldolase_|_786849:787499_Forward	4.1.2.4	deoC voies des pentoses phosphates
730	LCA_LACTOBACILLUS_SAKEIRS09170_|_peroxidase_|_1821441:1822397_Forward		Dyp-type peroxidase family (iron-dependent), oxidative stress
731	LCA_LACTOBACILLUS_SAKEIRS02270_|_reactive_intermediate/imine_deaminase_|_457790:458158_Forward		endoribonuclease
732	LCA_LACTOBACILLUS_SAKEIRS03465_|_guanylate_kinase_|_682257:682868_Forward	2.7.4.8	Essential for recycling GMP, bases metabolism
733	LCA_LACTOBACILLUS_SAKEIRS03075_|_type_I_glyceraldehyde-3-phosphate_dehydrogenase_|_606702:607718_Forward	1.2.1.12	GapA glyceraldehyde 3-phosphate dehydrogenase glycolysis

734	LCA_LACTOBACILLUS_SAKEIRS05880_|_pgi_|_glucose-6-phosphate_isomerase_|_1161804:1163150_Reverse	5.3.1.9	Glycolysis
735	LCA_LACTOBACILLUS_SAKEIRS06595_|_glucokinase_|_1298719:1299690_Reverse	2.7.1.2	glycolysis
736	LCA_LACTOBACILLUS_SAKEIRS07590_|_fructose-1,6-bisphosphate_aldolase,_class_II_|_1499463:1500326_Reverse	4.1.2.13	Glycolysis
737	LCA_LACTOBACILLUS_SAKEIRS05925_|_pyruvate_oxidase_|_1170993:1172828_Forward	1.2.3.3	Glycolysis end products, Pyruvate + phosphate + O(2) <=> acetyl phosphate + CO(2) + H(2)O(2)
738	LCA_LACTOBACILLUS_SAKEIRS00960_|_gpmA_|_2,3-bisphosphoglycerate-dependent_phosphoglycerate_mutase_|_198757:199446_Forward	5.4.2.1	Glycolysis heterolactic fermentation or gluconeogenesis (il y en a 5 dans le génome de 23K)
739	LCA_LACTOBACILLUS_SAKEIRS08020_|_L-lactate_dehydrogenase_|_1593030:1594007_Forward	1.1.1.27	Glycolysis/fermentation
740	LCA_LACTOBACILLUS_SAKEIRS00650_|_GMP_synthetase_|_132817:134370_Forward	6.3.5.2	guaA synthese des nucleotides
741	LCA_LACTOBACILLUS_SAKEIRS01230_|_glyceraldehyde_3-phosphate_reductase_|_255502:256488_Forward		gylcolyse
742	LCA_LACTOBACILLUS_SAKEIRS01405_|_6-phosphogluconate_dehydrogenase_|_295038:295937_Forward	1.1.1.44	Heterolactic fermentation
743	LCA_LACTOBACILLUS_SAKEIRS06840_|_hydrolase_|_1347653:1348165_Reverse		~~Hydrolase of unknown function~~
744	LCA_LACTOBACILLUS_SAKEIRS04860_|_manganese-dependent_inorganic_pyrophosphatase_|_960467:961390_Reverse	3.6.1.1	manganese-dependent inorganic pyrophosphatase, phosphate metabolism
745	LCA_LACTOBACILLUS_SAKEIRS05955_|_membrane_protein_|_1175960:1176640_Forward		Membrane protein of unknown function
746	LCA_LACTOBACILLUS_SAKEIRS06855_|_insertase_|_1349511:1350500_Forward		Membrane protein of unknown function
747	LCA_LACTOBACILLUS_SAKEIRS01150_|_manganese_transporter_|_241472:243046_Forward		Mn(2+)/Fe(2+) transport protein
748	LCA_LACTOBACILLUS_SAKEIRS00845_|_membrane_protein_|_173993:174853_Forward		mtsB Abc transporter manganese
749	LCA_LACTOBACILLUS_SAKEIRS00840_|_manganese_transporter_|_173256:173996_Forward		mtsC ABC transporter manganese
750	LCA_LACTOBACILLUS_SAKEIRS02940_|_NADH_peroxidase_|_585192:586544_Forward	1.11.1.1	NADH peroxidase LSA0575 redox
751	LCA_LACTOBACILLUS_SAKEIRS01355_|_nucleoside_transporter_|_284050:285285_Forward		nucleoside permease
752	LCA_LACTOBACILLUS_SAKEIRS01515_|_peptide_transporter_|_322553:324490_Forward		oligopeptide transporter
753	LCA_LACTOBACILLUS_SAKEIRS00930_|_ribose_transporter_RbsU_|_193092:193976_Forward		operon ribose
754	LCA_LACTOBACILLUS_SAKEIRS00935_|_D-ribose_pyranase_|_193997:194392_Forward		operon ribose
755	LCA_LACTOBACILLUS_SAKEIRS00940_|_ribokinase_|_194412:195320_Forward		operon ribose
756	LCA_LACTOBACILLUS_SAKEIRS06665_|_eutD_|_phosphate_acetyltransferase_|_1313600:1314586_Reverse	2.3.1.8	Pathway: purine nucleobases degradation II (anaerobic)
757	LCA_LACTOBACILLUS_SAKEIRS01525_|_peptidase_C69_|_324882:326303_Forward	3.4.-.-	peptidase U34
758	LCA_LACTOBACILLUS_SAKEIRS03080_|_phosphoglycerate_kinase_|_607819:609033_Forward	2.7.2.3	PGK phosphoglycerate kinase glycolysis
759	LCA_LACTOBACILLUS_SAKEIRS01370_|_phosphoketolase_|_286496:288859_Forward	4.1.2.9	Phosphoketolase/ D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase
760	LCA_LACTOBACILLUS_SAKEIRS05220_|_obgE_|_GTPase_ObgE_|_1037400:1038692_Reverse		ppGpp-binding GTPase involved in cell partioning, DNA repair and ribosome assembly
761	LCA_LACTOBACILLUS_SAKEIRS06485_|_transcriptional_regulator_|_1281049:1281780_Reverse		Probable transcriptional regulatory , target unknown
762	LCA_LACTOBACILLUS_SAKEIRS05615_|_ATP_synthase_subunit_gamma_|_1115551:1116498_Reverse	3.6.3.14	Produces ATP from ADP in the presence of a proton gradient across the membrane F0F1 ATPase
763	LCA_LACTOBACILLUS_SAKEIRS05625_|_ATP_synthase_subunit_delta_|_1118074:1118616_Reverse	3.6.3.14	Produces ATP from ADP in the presence of a proton gradient across the membrane F0F1 ATPase
764	LCA_LACTOBACILLUS_SAKEIRS05630_|_ATP_synthase_subunit_B_|_1118603:1119124_Reverse	3.6.3.14	Produces ATP from ADP in the presence of a proton gradient across the membrane F0F1 ATPase
765	LCA_LACTOBACILLUS_SAKEIRS05640_|_F0F1_ATP_synthase_subunit_A_|_1119418:1120131_Reverse	3.6.3.14	Produces ATP from ADP in the presence of a proton gradient across the membrane F0F1 ATPase
766	LCA_LACTOBACILLUS_SAKEIRS01680_|_PTS_sugar_transporter_subunit_IIB_|_353900:354217_Forward	2.7.1.69	PTS EIIB cellobiose
767	LCA_LACTOBACILLUS_SAKEIRS07260_|_phosphocarrier_protein_HPr_|_1439647:1439913_Reverse	2.7.11.-	PTS general enzyme HPr, PTS sugar utilization
768	LCA_LACTOBACILLUS_SAKEIRS07255_|_phosphoenolpyruvate--protein_phosphotransferase_|_1437923:1439647_Reverse	2.7.3.9	PTS general Enzyme I, PTS sugar utilization
769	LCA_LACTOBACILLUS_SAKEIRS03970_|_1-(5-phosphoribosyl)-5-amino-4-imidazole-_carboxylate_carboxylase_|_779645:780427_Forward		PurE like, purine metabolism
770	LCA_LACTOBACILLUS_SAKEIRS06275_|_pyrH_|_UMP_kinase_|_1246332:1247057_Reverse	2.7.4.22	purine and pyrimidine metabolism; pyrimidine ribonucleotides interconversion
771	LCA_LACTOBACILLUS_SAKEIRS09050_|_deoxyadenosine_kinase_|_1795859:1796515_Forward	2.7.1.74 , 2.7.1.76	Purine metabolism, ATP + deoxyadenosine <=> ADP + dAMP
772	LCA_LACTOBACILLUS_SAKEIRS02530_|_ribonuclease_Y_|_506382:507947_Forward	3.1.4.16	Putative 2',3'-cyclic-nucleotide 2'-phosphodiesterase. RNA degradation
773	LCA_LACTOBACILLUS_SAKEIRS02440_|_hypothetical_protein_|_488035:489060_Reverse	3.1.1.31	Putative 6-phosphogluconolactonase produit 6P-gluconate Utilisation des suches
774	LCA_LACTOBACILLUS_SAKEIRS07185_|_adaptor_protein_MecA_|_1421409:1422098_Reverse		putative adaptor protein controlling oligomerization of the AAA+ protein ClpC, Role: control, adaptation
775	LCA_LACTOBACILLUS_SAKEIRS06820_|_aminodeoxychorismate_lyase_|_1342235:1343386_Reverse	4.1.3.38	putative aminodeoxychorismate lyase family protein, 4-amino-4-deoxychorismate <=> 4-aminobenzoate + pyruvate
776	LCA_LACTOBACILLUS_SAKEIRS03805_|_cell_division_protein_SepF_|_747929:748363_Forward		putative cell division
777	LCA_LACTOBACILLUS_SAKEIRS01500_|_short-chain_dehydrogenase_|_319948:320589_Reverse		putative protein
778	LCA_LACTOBACILLUS_SAKEIRS06600_|_hypothetical_protein_|_1299687:1299920_Reverse		Putative transcription factor of unknown function
779	LCA_LACTOBACILLUS_SAKEIRS06980_|_MarR_family_transcriptional_regulator_|_1374739:1375230_Reverse		Putative transcriptional regulator, MarR family, unknown target
780	LCA_LACTOBACILLUS_SAKEIRS07295_|_hypothetical_protein_|_1446610:1447950_Reverse		Putative transporter, unknown substrate
781	LCA_LACTOBACILLUS_SAKEIRS09415_|_ABC_transporter_ATP-binding_protein_|_1874495:1876119_Reverse		Putative transporter, unknown substrate
782	LCA_LACTOBACILLUS_SAKEIRS00915_|_MarR_family_transcriptional_regulator_|_190786:191328_Forward		Regulator ackA?
783	LCA_LACTOBACILLUS_SAKEIRS00010_|_DNA_polymerase_III_subunit_beta_|_1734:2873_Forward		replication
784	LCA_LACTOBACILLUS_SAKEIRS00040_|_single-stranded_DNA-binding_protein_|_9683:10195_Forward		Replication
785	LCA_LACTOBACILLUS_SAKEIRS02525_|_DNA_recombination/repair_protein_RecA_|_504928:505995_Forward		Replication/recombination
786	LCA_LACTOBACILLUS_SAKEIRS07625_|_helicase_|_1505389:1506735_Reverse		Replication/transcription
787	LCA_LACTOBACILLUS_SAKEIRS07990_|_RNA-binding_protein_|_1585478:1585921_Reverse	2.7.7.8	S1 RNA binding domain protein
788	LCA_LACTOBACILLUS_SAKEIRS01805_|_preprotein_translocase_subunit_YajC_|_383023:383379_Forward		Secretion
789	LCA_LACTOBACILLUS_SAKEIRS02560_|_protein_translocase_subunit_SecA_|_512874:515237_Forward		Secretion
790	LCA_LACTOBACILLUS_SAKEIRS09445_|_hypothetical_protein_|_1882883:1883662_Reverse		Secretion system?
791	LCA_LACTOBACILLUS_SAKEIRS04215_|_universal_stress_protein_UspA_|_823411:823905_Reverse		stress repsonse
792	LCA_LACTOBACILLUS_SAKEIRS00185_|_universal_stress_protein_UspA_|_36687:37160_Forward		stress response
793	LCA_LACTOBACILLUS_SAKEIRS01155_|_universal_stress_protein_UspA_|_243064:243498_Forward		Stress response
794	LCA_LACTOBACILLUS_SAKEIRS03220_|_thiol_reductase_thioredoxin_|_636150:636470_Forward		Thioredoxine LSA0634 Redox
795	LCA_LACTOBACILLUS_SAKEIRS03090_|_eno_|_enolase_|_609932:611227_Forward	5.3.1.1	TPI Triose phosphate isomerase glycolysis

796	LCA_LACTOBACILLUS_SAKEIRS06445_|_DNA_methyltransferase_|_1276109:1277119_Reverse		Transcription
797	LCA_LACTOBACILLUS_SAKEIRS06220_|_transcription_termination/antitermination_protein_NusA_|_1229357:1230574_Reverse		Transcription; transcription elongation factor NusA
798	LCA_LACTOBACILLUS_SAKEIRS07520_|_transcriptional_regulator_|_1487955:1488926_Reverse		Transcriptional regulator, unknown target
799	LCA_LACTOBACILLUS_SAKEIRS00035_|_30S_ribosomal_protein_S6_|_9348:9644_Forward		Translation
800	LCA_LACTOBACILLUS_SAKEIRS00045_|_30S_ribosomal_protein_S18_|_10226:10465_Forward		Translation
801	LCA_LACTOBACILLUS_SAKEIRS03390_|_50S_ribosomal_protein_L21_|_672083:672391_Forward		Translation
802	LCA_LACTOBACILLUS_SAKEIRS03405_|_elongation_factor_P_|_673365:673922_Forward		Translation
803	LCA_LACTOBACILLUS_SAKEIRS03620_|_RNA-binding_protein_|_719456:719698_Forward		Translation
804	LCA_LACTOBACILLUS_SAKEIRS03825_|_ileS_|_isoleucine--tRNA_ligase_|_750528:753314_Forward	6.1.1.5	Translation
805	LCA_LACTOBACILLUS_SAKEIRS04245_|_30S_ribosomal_protein_S4_|_829520:830125_Reverse		Translation
806	LCA_LACTOBACILLUS_SAKEIRS04345_|_aspS_|_aspartate--tRNA_ligase_|_852574:854346_Forward		Translation
807	LCA_LACTOBACILLUS_SAKEIRS04940_|_gid_|_tRNA_(uracil-5-)-methyltransferase_|_980403:981714_Reverse		Translation
808	LCA_LACTOBACILLUS_SAKEIRS05190_|_50S_ribosomal_protein_L32_|_1029820:1030002_Reverse		Translation
809	LCA_LACTOBACILLUS_SAKEIRS05295_|_tig_|_trigger_factor_|_1056051:1057346_Reverse		Translation
810	LCA_LACTOBACILLUS_SAKEIRS05300_|_tuf_|_elongation_factor_Tu_|_1057557:1058747_Reverse		Translation
811	LCA_LACTOBACILLUS_SAKEIRS05310_|_RNase_J_family_beta-CASP_ribonuclease_|_1060006:1061724_Reverse		Translation
812	LCA_LACTOBACILLUS_SAKEIRS05320_|_30S_ribosomal_protein_S20_|_1062501:1062755_Forward		Translation
813	LCA_LACTOBACILLUS_SAKEIRS06270_|_ribosome_recycling_factor_|_1245772:1246329_Reverse		Translation
814	LCA_LACTOBACILLUS_SAKEIRS06280_|_elongation_factor_Ts_|_1247193:1248068_Reverse		Translation
815	LCA_LACTOBACILLUS_SAKEIRS06925_|_50S_ribosomal_protein_L20_|_1362034:1362393_Reverse		Translation
816	LCA_LACTOBACILLUS_SAKEIRS07000_|_ribonuclease_R_|_1378741:1381095_Reverse		Translation
817	LCA_LACTOBACILLUS_SAKEIRS07045_|_arginine--tRNA_ligase_|_1389483:1391174_Reverse		Translation
818	LCA_LACTOBACILLUS_SAKEIRS08115_|_rpmE2_|_50S_ribosomal_protein_L31_type_B_|_1611937:1612200_Reverse		Translation
819	LCA_LACTOBACILLUS_SAKEIRS08265_|_methionine--tRNA_ligase_|_1641460:1643505_Reverse		Translation
820	LCA_LACTOBACILLUS_SAKEIRS08305_|_rplL_|_50S_ribosomal_protein_L7/L12_|_1648874:1649242_Reverse		Translation
821	LCA_LACTOBACILLUS_SAKEIRS08355_|_50S_ribosomal_protein_L33_|_1658051:1658200_Reverse		Translation
822	LCA_LACTOBACILLUS_SAKEIRS08575_|_30S_ribosomal_protein_S9_|_1705186:1705578_Reverse		Translation
823	LCA_LACTOBACILLUS_SAKEIRS08695_|_50S_ribosomal_protein_L30_|_1728860:1729045_Reverse		Translation
824	LCA_LACTOBACILLUS_SAKEIRS08715_|_30S_ribosomal_protein_S8_|_1730543:1730941_Reverse		Translation
825	LCA_LACTOBACILLUS_SAKEIRS08740_|_50S_ribosomal_protein_L29_|_1732793:1732987_Reverse		Translation
826	LCA_LACTOBACILLUS_SAKEIRS08770_|_50S_ribosomal_protein_L23_|_1735697:1735981_Reverse		Translation
827	LCA_LACTOBACILLUS_SAKEIRS08775_|_50S_ribosomal_protein_L4_|_1735981:1736604_Reverse		Translation
828	LCA_LACTOBACILLUS_SAKEIRS08785_|_30S_ribosomal_protein_S10_|_1737304:1737612_Reverse		Translation
829	LCA_LACTOBACILLUS_SAKEIRS09450_|_rnpA_|_ribonuclease_P_protein_component_|_1883731:1884093_Reverse		Translation
830	LCA_LACTOBACILLUS_SAKEIRS09455_|_50S_ribosomal_protein_L34_|_1884159:1884299_Reverse		Translation
831	LCA_LACTOBACILLUS_SAKEIRS07320_|_hypothetical_protein_|_1452214:1452783_Reverse		Translation, putative tRNA binding factor
832	LCA_LACTOBACILLUS_SAKEIRS03985_|_glycerol_transporter_|_781764:782477_Forward		Transporter (facilitator) unknown substrate
833	LCA_LACTOBACILLUS_SAKEIRS00660_|_integrase_|_135980:136900_Reverse		transposase
834	LCA_LACTOBACILLUS_SAKEIRS01005_|_integrase_|_210685:211605_Forward		~~Transposase~~
835	LCA_LACTOBACILLUS_SAKEIRS05740_|_integrase_|_1141271:1142191_Reverse		transposase
836	LCA_LACTOBACILLUS_SAKEIRS00855_|_amidase_|_175881:176387_Forward		Uncharacterized isochorismatase family protein
837	~~LCA_LACTOBACILLUS_SAKEIRS00050_|_hypothetical_protein_|_10683:12716_Forward~~		unknown
838	LCA_LACTOBACILLUS_SAKEIRS01170_|_hypothetical_protein_|_245582:246370_Forward		Unknown
839	LCA_LACTOBACILLUS_SAKEIRS01980_|_hypothetical_protein_|_419971:420408_Forward		Unknown
840	~~LCA_LACTOBACILLUS_SAKEIRS02675_|_hypothetical_protein_|_535680:536081_Forward~~		Unknown
841	LCA_LACTOBACILLUS_SAKEIRS03240_|_membrane_protein_|_640800:641501_Forward		~~Unknown~~
842	LCA_LACTOBACILLUS_SAKEIRS03395_|_hypothetical_protein_|_672406:672747_Forward		Unknown
843	LCA_LACTOBACILLUS_SAKEIRS03530_|_hypothetical_protein_|_695401:697071_Forward		Unknown
844	LCA_LACTOBACILLUS_SAKEIRS03965_|_hypothetical_protein_|_778359:779633_Forward		~~Unknown~~
845	LCA_LACTOBACILLUS_SAKEIRS03975_|_hypothetical_protein_|_780424:781248_Forward		~~Unknown~~
846	LCA_LACTOBACILLUS_SAKEIRS03980_|_hypothetical_protein_|_781230:781700_Forward		~~Unknown~~
847	LCA_LACTOBACILLUS_SAKEIRS03990_|_hypothetical_protein_|_782498:782803_Forward		~~Unknown~~
848	LCA_LACTOBACILLUS_SAKEIRS03995_|_TIGR00268_family_protein_|_782828:783664_Forward		~~Unknown~~
849	LCA_LACTOBACILLUS_SAKEIRS04810_|_hypothetical_protein_|_950590:951459_Forward		Unknown
850	LCA_LACTOBACILLUS_SAKEIRS04995_|_hypothetical_protein_|_990582:991427_Reverse		~~Unknown~~
851	~~LCA_LACTOBACILLUS_SAKEIRS07635_|_hypothetical_protein_|_1507960:1508304_Forward~~		~~Unknown~~
852	LCA_LACTOBACILLUS_SAKEIRS08035_|_hypothetical_protein_|_1596722:1597402_Forward		Unknown
853	LCA_LACTOBACILLUS_SAKEIRS08085_|_membrane_protein_|_1606162:1606728_Reverse		~~Unknown~~
854	~~LCA_LACTOBACILLUS_SAKEIRS08470_|_hypothetical_protein_|_1682910:1683128_Reverse~~		~~Unknown~~
855	LCA_LACTOBACILLUS_SAKEIRS09140_|_hypothetical_protein_|_1815066:1815596_Reverse		~~Unknown~~
856	LCA_LACTOBACILLUS_SAKEIRS09545_|_hypothetical_protein_|_1471062:1471496_Reverse		~~Unknown~~
857	LCA_LACTOBACILLUS_SAKEIRS03285_|_CHAP_domain-containing_protein_|_649323:650597_Forward		Unknown cell surface protein
858	LCA_LACTOBACILLUS_SAKEIRS04390_|_hypothetical_protein_|_861331:861774_Forward		Unknown or translation
859	LCA_LACTOBACILLUS_SAKEIRS02370_|_hypothetical_protein_|_475428:475661_Forward		unkwnown
860	~~LCA_LACTOBACILLUS_SAKEIRS02640_|_hypothetical_protein_|_530102:531577_Forward~~		unkwnown
861	LCA_LACTOBACILLUS_SAKEIRS03160_|_hypothetical_protein_|_624469:624813_Forward		unkwnown
862	LCA_LACTOBACILLUS_SAKEIRS05225_|_hypothetical_protein_|_1038851:1039945_Reverse		unkwnown
863	LCA_LACTOBACILLUS_SAKEIRS01985_|_dipeptidase_|_420820:421917_Reverse	3.4.13.9	Xaa Pro dipeptidase PepQ
864	LCA_LACTOBACILLUS_SAKEIRS07735_|_xanthine_phosphoribosyltransferase_|_1530643:1531230_Reverse	2.4.2.22	Xanthine and xanthosine salvage, putine metabolism
865	LCA_LACTOBACILLUS_SAKEIRS08080_|_zinc_metalloprotease_HtpX_|_1605249:1606148_Reverse		Zn-dependent protease with chaperone function, stress response
866	~~LCRIS_LACTOBACILLUS_CRISPATUS_RS02865_|_integrase_|_544887:545807_Reverse~~		~~Unknown~~
867	~~LHE_LACTOBACILLUS_HELVETICUS_RS12800_|_transposase_|_276037:276707_Reverse~~		~~Unknown~~
868	~~RT94_PSEUDOMONAS_VIRIDIFLAVA_RS24855_|_RT94_PSEUDOMONAS_VIRIDIFLAVA_RS24855_|_hypothetical_protein_|_63909:64787_Reverse~~		~~Unknown~~
869	~~SFRI_SHEWANELLA_FRIGIDIMARINA_RS05600_|_SFRI_SHEWANELLA_FRIGIDIMARINA_RS05600_|_raiA_ribosome-associated_inhibitor_A_|_1282848:1283204_Forward~~		~~Unknown~~

Differentially expressed genes up-regulated in UB condition.

	UBvsCup _ descriptions	ec number	remarques
1	~~ACINETOBACTER,JUNII|KB849655,1_cds_ENV65579,1_2876_[protein=hypothetical_protein]_[protein_id=ENV65579,1]_[location=complement(1853605,,1854057)]~~		
2	~~AYI71_LACTOBACILLUS_ORIS_RS08120_|_AYI71_LACTOBACILLUS_ORIS_RS08120_|_protease_|_1624179:1624682_Reverse~~		
3	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS02735_|_queT_transporter_family_protein_|_642717:643205_Forward		hypothetical Membrane protein
4	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS03365_|_Na+/H+_antiporter_NhaC_|_785652:787052_Forward		Na(+)/H(+) antiporter NhaC or Arginine/ornithine antiporter ArcD
5	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS11580_|_DNA_helicase_UvrD_|_2471406:2473698_Reverse		Putative ATP-dependent DNA helicase (replication, transcription)
6	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS14500_|_MFS_transporter_|_3091994:3093241_Reverse		Putative metabolite transport protein
7	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS09305_|_DeoR_family_transcriptional_regulator_|_2003950:2004702_Forward		regulation operon fructose
8	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS09100_|_haloacid_dehalogenase_|_1960211:1960924_Forward		Uncharacterized HAD-hydrolase
9	BN424_CARNOBACTERIUM_MALTAROMATICUM_RS03470_|_QacE_family_quaternary_ammonium_compound_efflux_SMR_transporter_|_806468:806791_Forward		uncharacterized protein
10	~~BN424_CARNOBACTERIUM_MALTAROMATICUM_RS09925_|_hypothetical_protein_|_2138520:2139344_Reverse~~		~~Unknown~~
11	BR52_CARNOBACTERIUM_DIVERGENS_RS01095_|_Na+/H+_antiporter_NhaC_|_231526:232932_Forward		[Carnobacterium divergens V41 WGS CDIV41] nhaC | Na(+)/H(+) antiporter NhaC putative
12	BR52_CARNOBACTERIUM_DIVERGENS_RS02995_|_604720:605040_Forward		50S RNA-binding protein, translation?
13	BR52_CARNOBACTERIUM_DIVERGENS_RS08580_|_GNAT_family_acetyltransferase_|_1776641:1777093_Forward	2.3.1.-	Acetyltransferase, unknown substrate
14	BR52_CARNOBACTERIUM_DIVERGENS_RS02840_|_bifunctional_acetaldehyde-CoA/alcohol_dehydrogenase_|_571790:574393_Forward	1.1.1.1/1.2.1.10	ADH Aldehyde-alcohol dehydrogenase 2 [Includes: Alcohol dehydrogenase ; Acetaldehyde dehydrogenase] Acetaldehyde + CoA + NAD(+) <=> acetyl-CoA + NADH and An alcohol + NAD(+) <=> an aldehyde or ketone + NADH
15	BR52_CARNOBACTERIUM_DIVERGENS_RS03150_|_alpha/beta_hydrolase_|_635545:636474_Forward		Alpha/beta hydrolase, unknown specificity, unknown substrate
16	BR52_CARNOBACTERIUM_DIVERGENS_RS02180_|_amino_acid_permease_|_433016:434338_Forward		aminoacid permease. Unknown substrate
17	BR52_CARNOBACTERIUM_DIVERGENS_RS03705_|_GTP-binding_protein_|_747417:749261_Forward		bipA | GTPase homolog, Ribosome fixation, Translation
18	BR52_CARNOBACTERIUM_DIVERGENS_RS00375_|_eutD_|_phosphate_acetyltransferase_|_76506:77486_Forward	2.3.1.8	Carnobacterium divergens V41 WGS CDIV41] pta | Phosphate acetyltransferase Acetyl-CoA + phosphate <=> CoA + acetyl phosphate, heterolactic fermentation
19	BR52_CARNOBACTERIUM_DIVERGENS_RS00210_|_transporter_|_41826:43259_Reverse		CDIV41_v1_140053 [Carnobacterium divergens V41 WGS CDIV41] ycaM | putative transporter unknown substrate
20	BR52_CARNOBACTERIUM_DIVERGENS_RS00335_|_glutamine_ABC_transporter_permease_|_69051:70493_Reverse		CDIV41_v1_140081 [Carnobacterium divergens V41 WGS CDIV41] glnP | Amino ABC transporter, permease , 3-TM region,His/Glu/Gln/Arg/opine family domain protein
21	BR52_CARNOBACTERIUM_DIVERGENS_RS02435_|_hypothetical_protein_|_487002:489878_Reverse		Cell wall peptidoglycan synthesis, Transglycosylase family protein
22	BR52_CARNOBACTERIUM_DIVERGENS_RS03535_|_membrane_protein_|_709468:710337_Forward		conserved membrane protein of unknown function
23	~~BR52_CARNOBACTERIUM_DIVERGENS_RS08750_|_membrane_protein_|_1807077:1807574_Reverse~~		~~conserved membrane protein of unknown function~~
24	~~BR52_CARNOBACTERIUM_DIVERGENS_RS09915_|_membrane_protein_|_2066304:2067227_Reverse~~		~~conserved membrane protein of unknown function~~
25	~~BR52_CARNOBACTERIUM_DIVERGENS_RS09920_|_membrane_protein_|_2067227:2067937_Reverse~~		~~conserved membrane protein of unknown function~~
26	BR52_CARNOBACTERIUM_DIVERGENS_RS08355_|_transcriptional_regulator_|_1720277:1720585_Reverse		czrA | transcriptional regulator (multiple metal-sensing ArsR-SmtB transcriptional repressors family)
27	BR52_CARNOBACTERIUM_DIVERGENS_RS04920_|_peptide_ABC_transporter_permease_|_1000998:1002458_Forward		dtpT | di-tripeptide-proton ABC symporter
28	BR52_CARNOBACTERIUM_DIVERGENS_RS10420_|_flavocytochrome_c_|_2181371:2182897_Reverse		FMN-binding_protein
29	BR52_CARNOBACTERIUM_DIVERGENS_RS07515_|_glucose_transporter_GlcU_|_1543245:1544096_Reverse		Glucose uptake protein GlcU, glucose transport
30	BR52_CARNOBACTERIUM_DIVERGENS_RS03865_|_glycine/betaine_ABC_transporter_ATP-binding_protein_|_782766:783959_Reverse		glycine/betaine_ABC_transporter_ATP-binding_protein, osmotic cold shock stress response, catalyzes the osmotically controlled import of the compatible solutes glycine betaine and proline betaine
31	BR52_CARNOBACTERIUM_DIVERGENS_RS00995_|_sodium:dicarboxylate_symporter_|_209259:210653_Forward		L-cystine uptake protein TcyP
32	BR52_CARNOBACTERIUM_DIVERGENS_RS00355_|_72663:73652_Forward	1.1.1.28	ldhD | D-lactate dehydrogenase | 1.1.1.28 |Glycolysis CDIV41_v1_140085 [Carnobacterium divergens V41 WGS CDIV41]
33	BR52_CARNOBACTERIUM_DIVERGENS_RS09910_|_pyroglutamyl-peptidase_I_|_2065620:2066267_Reverse	3.4.19.3	Les 4 genes font 1 opéron mais 3 inconnues, pcp, pyrrolidone-carboxylate peptidase , Release of an N-terminal pyroglutamyl group from a polypeptide, the second amino acid generally not being Pro
34	BR52_CARNOBACTERIUM_DIVERGENS_RS06500_|_MFS_transporter_|_1328162:1329595_Reverse		Major Facilitator Superfamily
35	BR52_CARNOBACTERIUM_DIVERGENS_RS08585_|_peptidase_|_1777117:1777776_Forward	3.4.-.-	membrane protein, protease family protein
36	BR52_CARNOBACTERIUM_DIVERGENS_RS08510_|_NAD(+)_synthetase_|_1756836:1757666_Reverse	6.3.5.1	nadE | ammonium-dependent NAD+ synthetase the enzyme that catalyzes the final reaction in the biosynthesis of NAD, ATP + deamido-NAD(+) + NH(3) <=> AMP + diphosphate + NAD(+)

37	BR52_CARNOBACTERIUM_DIVERGENS_RS06640_|_guanine_permease_|_1363383:1364684_Forward		pbuO hypoxanthine/guanine permease regulated by PurR
38	BR52_CARNOBACTERIUM_DIVERGENS_RS04605_|_glycerol-3-phosphate_acyltransferase_|_930079:930672_Reverse		plsY | acylphosphate:glycerol-3-phosphate acyltransferase, metabolism of lipids
39	BR52_CARNOBACTERIUM_DIVERGENS_RS02280_|_DNA-binding_response_regulator_|_452977:453717_Reverse		Putative accessory gene regulator A, AgrA family, unknown target
40	BR52_CARNOBACTERIUM_DIVERGENS_RS02985_|_haloacid_dehalogenase_|_603013:603546_Forward		Putative hydrolase, haloacid dehalogenase family unknown function
41	~~BR52_CARNOBACTERIUM_DIVERGENS_RS05540_|_membrane_protein_|_1138530:1139144_Reverse~~		~~putative membrane protein of unknow function~~
42	BR52_CARNOBACTERIUM_DIVERGENS_RS07270_|_yibE/F-like_family_protein_|_1492505:1493632_Reverse		Putative transporter, unknown substrate
43	BR52_CARNOBACTERIUM_DIVERGENS_RS11030_|_single-stranded_DNA-binding_protein_|_2310854:2311429_Reverse		replication
44	BR52_CARNOBACTERIUM_DIVERGENS_RS03390_|_50S_ribosomal_protein_L21_|_679657:679965_Forward		translation
45	BR52_CARNOBACTERIUM_DIVERGENS_RS06375_|_50S_ribosomal_protein_L20_|_1300913:1301272_Reverse		translation
46	BR52_CARNOBACTERIUM_DIVERGENS_RS06380_|_50S_ribosomal_protein_L35_|_1301391:1301591_Reverse		translation
47	BR52_CARNOBACTERIUM_DIVERGENS_RS06385_|_translation_initiation_factor_IF-3_|_1301622:1302143_Reverse		translation
48	BR52_CARNOBACTERIUM_DIVERGENS_RS07700_|_translation_factor_Sua5_|_1573057:1574073_Reverse		translation
49	BR52_CARNOBACTERIUM_DIVERGENS_RS08860_|_50S_ribosomal_protein_L31_|_1825395:1825658_Reverse		translation
50	BR52_CARNOBACTERIUM_DIVERGENS_RS09965_|_50S_ribosomal_protein_L10_|_2076021:2076521_Reverse		translation
51	BR52_CARNOBACTERIUM_DIVERGENS_RS11035_|_30S_ribosomal_protein_S6_|_2311482:2311781_Reverse		translation
52	BR52_CARNOBACTERIUM_DIVERGENS_RS07705_|_protein-(glutamine-N5)_methyltransferase,_release_factor-specific_|_1574098:1574949_Reverse		Translation, Metabolism of coenzymes and prosthetic groups, prmC | glutamine methylase of release factor 1, Class I release factors bind to ribosomes in response to stop codons and trigger peptidyl-tRNA hydrolysis
53	BR52_CARNOBACTERIUM_DIVERGENS_RS11845_|_porin_|_2488321:2488989_Forward		transport?
54	BR52_CARNOBACTERIUM_DIVERGENS_RS05905_|_gamma-aminobutyrate_permease_|_1206584:1208059_Forward		Transporter, lysP | Lysine-specific permease
55	~~BR52_CARNOBACTERIUM_DIVERGENS_RS03395_|_hypothetical_protein_|_679983:680315_Forward~~		Unknown
56	BR52_CARNOBACTERIUM_DIVERGENS_RS08240_|_hypothetical_protein_|_1695543:1696046_Forward		Unknown
57	~~BR52_CARNOBACTERIUM_DIVERGENS_RS10005_|_FMN-binding_protein_|_2082543:2083478_Reverse~~		~~Unknown~~
58	~~BR52_CARNOBACTERIUM_DIVERGENS_RS04740_|_hypothetical_protein_|_960154:961083_Forward~~		~~Unknown~~
59	~~BR52_CARNOBACTERIUM_DIVERGENS_RS11695_|_hypothetical_protein_|_2455563:2456708_Reverse~~		~~Unknown~~
60	~~BR52_CARNOBACTERIUM_DIVERGENS_RS12620_|_hypothetical_protein_|_1955598:1959860_Reverse~~		~~Unknown~~
61	~~BR52_CARNOBACTERIUM_DIVERGENS_RS09905_|_butirosin_biosynthesis_protein_BtrG_|_2065165:2065620_Reverse~~		~~Unknown function~~
62	BR52_CARNOBACTERIUM_DIVERGENS_RS05895_|_MarR_family_transcriptional_regulator_|_1203549:1203926_Forward		ytcD | putative transcriptional regulator (MarD family) Unknown target
63	FD34_LACTOBACILLUS_PONTIS_RS06280_|_1,3-propanediol_dehydrogenase_|_68496:69668_Forward		
64	GJA_Janthinobacterium agaricidamnosum_RS22270_|_transcriptional_regulator_|_5168697:5168921_Reverse		Transcriptional regulator
65	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07720_|_ABC_transporter_substrate-binding_protein_|_29991:31136_Reverse		ABC transporter
66	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08050_|_manganese_transporter_|_29567:31135_Forward		ABC transporter (Mn?) Manque 1 ss u qui n'est pas surexprimée
67	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06025_|_adenylate_kinase_|_31227:31883_Forward		ATP genaration
68	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09390_|_cell_division_protein_FtsH_|_11704:13815_Forward		Cell division
69	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08555_|_glycerol_phosphate_lipoteichoic_acid_synthase_|_22734:24797_Forward		Cell wall synthesis, exported glycerol phosphate lipoteichoic acid synthetase and anion-binding protein
70	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04390_|_peptidylprolyl_isomerase_|_50494:51399_Reverse	5.2.1.8	Chaperone, protein folding and stabilization
71	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00310_|_tig_|_trigger_factor_|_64666:65961_Reverse		Chaperoning, translation, Trigger Factor (TF) represents the only ribosome-associated chaperone known in bacteria; Involved in protein export. Acts as a chaperone by maintaining the newly synthesized secretory and non-secretory proteins in an open conformation
72	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00525_|_112050:112886_Reverse		conversion L to D lactate, enf of glycolysis? or cell wall biosynthesis
73	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00555_|_116007:117281_Reverse		conversion L to D lactate, enf of glycolysis? or cell wall biosynthesis
74	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11265_|_ATP_synthase_subunit_delta_|_36343:36884_Forward		F0F1 ATPase energy production
75	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11270_|_ATP_F0F1_synthase_subunit_alpha_|_36913:38447_Forward		F0F1 ATPase energy production
76	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05250_|_F0F1_ATP_synthase_subunit_A_|_34830:35543_Forward		F0F1 ATPase energy production
77	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05260_|_ATP_synthase_F0_subunit_B_|_35838:36356_Forward		F0F1 ATPase, energy production
78	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11010_|_glucose_transporter_GlcU_|_48736:49601_Forward		glucose facilitator?

79	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04315_|_phosphoglycerate_kinase_|_32818:34032_Forward		glycerol metabolism
80	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08305_|_gpmA_|_phosphoglyceromutase_|_27288:27977_Forward	5.4.2.1	Glycolysis heterolactic fermentation or gluconeogenesis (il y en a 5 dans le génome de 23K)
81	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09345_|_L-lactate_dehydrogenase_|_687:1664_Reverse	1.1.1.27	Glycolysis/fermentation
82	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05020_|_cell_surface_protein_|_45453:46526_Forward		Membrane protein
83	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01940_|_manganese_transporter_|_56279:57676_Forward		Mn transport
84	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02850_|_manganese_ABC_transporter_substrate-binding_protein_|_1362:2303_Reverse		Mn transport
85	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS01870_|_nucleoside_permease_|_36908:38143_Forward		nucleoside permease
86	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07360_|_NAD(P)-dependent_oxidoreductase_|_3358:4200_Reverse		oxidoreductase
87	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08510_|_PTS_mannose_family_transporter_subunit_IID_|_15184:16095_Forward		PTS EiiD mannose/sorbose/fructose family
88	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00550_|_1-(5-phosphoribosyl)-5-amino-4-imidazole-_carboxylate_carboxylase_|_115214:115993_Reverse		PurE like, purine metabolism
89	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06925_|_pyrH_|_UMP_kinase_|_757:1482_Reverse	2.7.4.22	purine and pyrimidine metabolism; pyrimidine ribonucleotides interconversion
90	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10135_|_1581:3143_Forward	3.1.4.16	Putative 2',3'-cyclic-nucleotide 2'-phosphodiesterase. RNA degradation
91	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11955_|_potassium_transporter_Kup_|_14749:16775_Forward		putative potassium transport system protein (kup)
92	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04065_|_prolyl_aminopeptidase_|_38106:39008_Forward	3.4.11.5	Putative proline amino peptidase
93	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09885_|_initiator_RepB_protein_|_4147:5082_Reverse		Replication
94	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02760_|_single-stranded_DNA-binding_protein_|_53643:54165_Reverse		Replication or transcription
95	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS08055_|_universal_stress_protein_UspA_|_31160:31585_Forward		stress repsonse
96	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05500_|_cold-shock_protein_|_31552:31752_Reverse		Stress response
97	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09565_|_phosphogluconate_dehydrogenase_(NADP(+)-dependent,_decarboxylating)_|_10173:11594_Forward		sugar metabolism
98	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS04280_|_NAD(FAD)-dependent_dehydrogenase_|_27141:28493_Forward		sugar metabolism
99	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07385_|_dTDP-glucose_4,6-dehydratase_|_7782:8810_Reverse		sugar metabolism
100	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07390_|_dTDP-4-dehydrorhamnose_3,5-epimerase_|_8829:9410_Reverse		sugar metabolism
101	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09200_|_DNA-directed_RNA_polymerase_subunit_delta_|_12512:13120_Forward		Transcription
102	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07090_|_transcriptional_regulator_|_28200:28931_Reverse		Transcriptional regulator
103	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00215_|_50S_ribosomal_protein_L32_|_42679:42861_Reverse		Translation
104	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00315_|_tuf_|_elongation_factor_Tu_|_66147:67337_Reverse		Translation
105	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00875_|_50S_ribosomal_protein_L21_|_28126:28434_Forward		Translation
106	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02070_|_50S_ribosomal_protein_L13_|_7507:7953_Forward		Translation
107	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02350_|_50S_ribosomal_protein_L10_|_59149:59652_Forward		Translation
108	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02355_|_50S_ribosomal_protein_L7/L12_|_59704:60069_Forward		Translation
109	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02765_|_30S_ribosomal_protein_S6_|_54205:54501_Reverse		Translation
110	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02800_|_50S_ribosomal_protein_L34_|_64635:64775_Forward		Translation
111	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS03250_|_translation_initiation_factor_IF-2_|_12806:15784_Forward		Translation
112	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05060_|_threonine--tRNA_ligase_|_55149:57119_Forward		Translation
113	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05700_|_30S_ribosomal_protein_S21_|_23606:23782_Forward		Translation
114	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05895_|_30S_ribosomal_protein_S12_|_15512:15925_Forward		Translation
115	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05935_|_50S_ribosomal_protein_L23_|_22294:22578_Forward		Translation
116	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05945_|_30S_ribosomal_protein_S19_|_23538:23819_Forward		Translation
117	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06010_|_50S_ribosomal_protein_L30_|_29224:29409_Forward		Translation
118	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06030_|_infA_|_translation_initiation_factor_IF-1_|_32072:32290_Forward		Translation
119	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06040_|_30S_ribosomal_protein_S13_|_32472:32837_Forward		Translation
120	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06920_|_197:754_Reverse		Translation
121	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06930_|_elongation_factor_Ts_|_1621:2496_Reverse		Translation
122	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS07885_|_30S_ribosomal_protein_S4_|_28208:28795_Forward		Translation
123	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09215_|_50S_ribosomal_protein_L31_type_B_|_17119:17382_Forward		Translation

124	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS09375_|_9115:9561_Forward		Translation
125	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06020_|_preprotein_translocase_subunit_SecY_|_29877:31172_Forward		Translation/secretion
126	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS10520_|_transporter_|_40875:42409_Reverse		Transport
127	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00075_|_DNA-binding_protein_|_13524:13799_Reverse~~		~~Unknown~~
128	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00530_|_hypothetical_protein_|_112903:113208_Reverse~~		~~Unknown~~
129	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS00880_|_hypothetical_protein_|_28449:28790_Forward~~		~~Unknown~~
130	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS02520_|_hypothetical_protein_|_13226:14404_Reverse~~		~~Unknown~~
131	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06210_|_hypothetical_protein_|_24425:25756_Reverse~~		~~Unknown~~
132	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11045_|_cell_surface_protein_|_10452:11758_Forward~~		~~Unknown~~
133	~~JCM11249_LACTOBACILLUS_FUCHUENSIS_RS11690_|_hypothetical_protein_|_16391:17616_Forward~~		~~Unknown~~
134	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS05480_|_xanthine_phosphoribosyltransferase_|_28392:28970_Reverse	2.4.2.22	Xanthine and xanthosine salvage, putine metabolism
135	JCM11249_LACTOBACILLUS_FUCHUENSIS_RS06160_|_anaerobic_ribonucleoside_triphosphate_reductase_|_10392:12608_Reverse		Zn anaerobic reductase
136	LACTOBACILLUS,VAGINALIS|AZGL01000017,1_cds_KRM48403,1_386_[locus_tag=FC58_GL000396]_[protein=30S_ribosomal_protein_S4]_[protein_id=KRM48403,1]_[location=complement(7004,,7693)]		
137	LCA_LACTOBACILLUS_SAKEI_RS07165_|_GTP_pyrophosphokinase_|_1418194:1418868_Reverse	2.7.6.5	(p)ppGpp synthetase; (p)ppGpp) are involved in regulating growth and several different stress responses
138	LCA_LACTOBACILLUS_SAKEI_RS01720_|_metallophosphoesterase_|_362960:363766_Forward		60% identical to 2'3' and 3'5' cyclic nucleotide monophosphates phosphodiesterase involved in biofilm formation of B. subtilis
139	LCA_LACTOBACILLUS_SAKEI_RS04000_|_cysteine_desulfurase_|_783792:784958_Forward	2.8.1.7	AA (alanine) biosynthesis
140	LCA_LACTOBACILLUS_SAKEI_RS03230_|_asparagine_synthetase_B_|_637303:639207_Forward	6.3.5.4	AA (asparagine) biosynthesis
141	LCA_LACTOBACILLUS_SAKEI_RS07205_|_ABC_transporter_permease_|_1424669:1426768_Reverse		ABC transport system, permease component, unknown substrate
142	LCA_LACTOBACILLUS_SAKEI_RS01690_|_multidrug_ABC_transporter_ATP-binding_protein_|_354719:356665_Reverse		ABC transporter
143	LCA_LACTOBACILLUS_SAKEI_RS00840_|_manganese_transporter_|_173256:173996_Forward		ABC transporter (Mn?) Manque 1 ss u qui n'est pas surexprimée
144	LCA_LACTOBACILLUS_SAKEI_RS00845_|_membrane_protein_|_173993:174853_Forward		ABC transporter (Mn?) Manque 1 ss u qui n'est pas surexprimée
145	LCA_LACTOBACILLUS_SAKEI_RS04660_|_multidrug_ABC_transporter_ATP-binding_protein_|_919307:920179_Forward		ABC transporter substrate unknown
146	LCA_LACTOBACILLUS_SAKEI_RS00590_|_118452:119210_Forward		ABC transporter unknown substrate
147	LCA_LACTOBACILLUS_SAKEI_RS00595_|_permease_|_119225:121045_Forward		ABC transporter unknown substrate
148	LCA_LACTOBACILLUS_SAKEI_RS05720_|_multidrug_ABC_transporter_ATP-binding_protein_|_1136044:1137921_Reverse		ABC transporter unknown substrate
149	LCA_LACTOBACILLUS_SAKEI_RS04900_|_putrescine/spermidine_ABC_transporter_ATP-binding_protein_|_972085:972744_Reverse		ABC transporter, unknown substrate 1seul des 2 gènes upregulated
150	LCA_LACTOBACILLUS_SAKEI_RS06440_|_acetate_kinase_|_1274891:1276087_Reverse	2.7.2.1	acetate kinase 2, purines nucleosides degradation, pyruvate degradation
151	LCA_LACTOBACILLUS_SAKEI_RS04910_|_alpha-acetolactate_decarboxylase_|_973885:974604_Reverse	4.1.1.5	acetolactate decarboxylase, acetoin biosynthesis, potential spoilage, pyruvate degradation
152	LCA_LACTOBACILLUS_SAKEI_RS04915_|_acetolactate_synthase_|_974638:976320_Reverse	2.2.1.6	acetolactate synthase, acetoin biosynthesis, potential spoilage, pyruvate degradation
153	LCA_LACTOBACILLUS_SAKEI_RS03280_|_acyltransferase_|_647279:649096_Forward		Acetyl transferase of unknown function
154	LCA_LACTOBACILLUS_SAKEI_RS04070_|_GNAT_family_acetyltransferase_|_799904:801115_Forward		Acetyltransferase of unknown function
155	LCA_LACTOBACILLUS_SAKEI_RS03545_|_acyl_carrier_protein_|_700879:701121_Forward		Acyl carrier, lipid metabolism
156	LCA_LACTOBACILLUS_SAKEI_RS07145_|_copper_homeostasis_protein_CutC_|_1414641:1415270_Reverse		Adaptations to atypical conditions
157	LCA_LACTOBACILLUS_SAKEI_RS06335_|_adenine_phosphoribosyltransferase_|_1257177:1257695_Reverse	2.4.2.7	adenine_phosphoribosyltransferase, adenine adenosine salvage pathway. purine pyrimidine metabolism
158	LCA_LACTOBACILLUS_SAKEI_RS08055_|_alanine_racemase_|_1598851:1599993_Reverse	5.1.1.1	Alanine biosynthesis
159	LCA_LACTOBACILLUS_SAKEI_RS07020_|_transporter_|_1384031:1385464_Forward		amino acid/polyamine transport protein
160	LCA_LACTOBACILLUS_SAKEI_RS05180_|_glutamate/gamma-aminobutyrate_family_transporter_YjeM_|_1027429:1028961_Reverse		amino acid/polyamine transporter
161	LCA_LACTOBACILLUS_SAKEI_RS01715_|_amino_acid_permease_|_360954:362705_Forward		Aminoacid permease
162	LCA_LACTOBACILLUS_SAKEI_RS01065_|_aminopeptidase_N_|_221085:223616_Forward	3.4.11.2	Aminopeptidase PepN
163	LCA_LACTOBACILLUS_SAKEI_RS08050_|_hypothetical_protein_|_1598503:1598769_Reverse		antitoxin inactivating the upstream endoribonuclease which overexpression in lethal
164	LCA_LACTOBACILLUS_SAKEI_RS07900_|_pyrroline-5-carboxylate_reductase_|_1567048:1567854_Reverse	1.5.1.2	Arginine and proline metabolism
165	LCA_LACTOBACILLUS_SAKEI_RS03125_|_arginine_repressor_|_617068:617535_Forward		ArgR family transcriptional regulator
166	LCA_LACTOBACILLUS_SAKEI_RS01650_|_L-asparaginase_|_347803:348777_Reverse	3.5.1.1	Asparagine degradation
167	LCA_LACTOBACILLUS_SAKEI_RS04570_|_hypothetical_protein_|_897612:901356_Forward		ATP-dependent exoDNAse (exonuclease V) DNA recombination
168	LCA_LACTOBACILLUS_SAKEI_RS08065_|_DEAD/DEAH_box_helicase_|_1600484:1602076_Reverse		ATP-dependent RNA helicase; cold shock, RNA modification

169	LCA_LACTOBACILLUS_SAKEI_RS03155_|_penicillin-binding_protein_1A_|_622364:624445_Forward		bifunctional glycolsyltransferase/transpeptidase penicillin binding protein 2A peptidoglycan biosynthesis
170	LCA_LACTOBACILLUS_SAKEI_RS01440_|_aspartate_4-decarboxylase_|_304048:305655_Forward	2.6.1.1/4.1.1.12	Bifunctional? Amino acid metabolisms
171	LCA_LACTOBACILLUS_SAKEI_RS02495_|_cell_division_protein_FtsK_|_497267:499636_Forward		Cell division
172	LCA_LACTOBACILLUS_SAKEI_RS02570_|_cell_division_ATP-binding_protein_FtsE_|_516554:517240_Forward		Cell division
173	LCA_LACTOBACILLUS_SAKEI_RS03580_|_chromosome_segregation_protein_SMC_|_708462:712022_Forward		Cell division
174	LCA_LACTOBACILLUS_SAKEI_RS03755_|_division/cell_wall_cluster_transcriptional_repressor_MraZ_|_736911:737342_Forward		Cell division
175	LCA_LACTOBACILLUS_SAKEI_RS03765_|_cell_division_protein_FtsL_|_738319:738699_Forward		Cell division
176	LCA_LACTOBACILLUS_SAKEI_RS03795_|_cell_division_protein_FtsA_|_745337:746647_Forward		Cell division
177	LCA_LACTOBACILLUS_SAKEI_RS03800_|_cell_division_protein_FtsZ_|_746675:747913_Forward		Cell division
178	LCA_LACTOBACILLUS_SAKEI_RS03820_|_arabinan_synthesis_protein_|_749472:750296_Forward		Cell division
179	LCA_LACTOBACILLUS_SAKEI_RS04250_|_septation_ring_formation_regulator_EzrA_|_830498:832210_Forward		Cell division
180	LCA_LACTOBACILLUS_SAKEI_RS04290_|_841470:842474_Forward		Cell division
181	LCA_LACTOBACILLUS_SAKEI_RS04295_|_842632:843492_Forward		Cell division
182	LCA_LACTOBACILLUS_SAKEI_RS04300_|_843494:844024_Forward		Cell division
183	LCA_LACTOBACILLUS_SAKEI_RS04305_|_septum_site-determining_protein_|_844041:844712_Forward		Cell division
184	LCA_LACTOBACILLUS_SAKEI_RS04310_|_septum_site-determining_protein_MinD_|_844714:845508_Forward		Cell division
185	LCA_LACTOBACILLUS_SAKEI_RS04615_|_cell_cycle_protein_GpsB_|_911372:911752_Forward		Cell division
186	LCA_LACTOBACILLUS_SAKEI_RS05135_|_site-specific_tyrosine_recombinase_XerD_|_1016756:1017640_Reverse		Cell division
187	LCA_LACTOBACILLUS_SAKEI_RS05570_|_cell_division_protein_FtsW_|_1108754:1109953_Reverse		Cell division
188	LCA_LACTOBACILLUS_SAKEI_RS05590_|_1110786:1111775_Reverse		Cell division
189	LCA_LACTOBACILLUS_SAKEI_RS06620_|_cell_division_protein_FtsI_|_1301490:1303589_Reverse		Cell division
190	LCA_LACTOBACILLUS_SAKEI_RS07975_|_cell_division_protein_FtsH_|_1581251:1583341_Reverse		Cell division
191	LCA_LACTOBACILLUS_SAKEI_RS04935_|_tyrosine_recombinase_XerC_|_979424:980335_Reverse		Cell division, site-specific tyrosine recombinase for chromosome partitioning
192	LCA_LACTOBACILLUS_SAKEI_RS00900_|_hypothetical_protein_|_186239:187585_Reverse		cell surface protein
193	LCA_LACTOBACILLUS_SAKEI_RS00500_|_hypothetical_protein_|_100870:102144_Forward		cell surface protein of unknown function
194	LCA_LACTOBACILLUS_SAKEI_RS00520_|_cell_surface_protein_|_103786:104262_Forward		cell surface protein of unknown function
195	LCA_LACTOBACILLUS_SAKEI_RS01490_|_cell_surface_protein_|_317566:319107_Forward		cell surface protein with cysteine proteinase domain
196	LCA_LACTOBACILLUS_SAKEI_RS00905_|_lipoprotein_precursor_|_187871:188863_Reverse		cell surface protein, prolipoprotein
197	~~LCA_LACTOBACILLUS_SAKEI_RS09080_|_cell_surface_protein_|_1802276:1802998_Reverse~~		~~Cell surface protein, unlnown function~~
198	LCA_LACTOBACILLUS_SAKEI_RS05090_|_peptidoglycan-binding_protein_LysM_|_1009695:1010396_Reverse		Cell wall
199	LCA_LACTOBACILLUS_SAKEI_RS08595_|_cell_surface_protein_|_1707594:1708571_Reverse		Cell wall
200	LCA_LACTOBACILLUS_SAKEI_RS00240_|_D-alanine--D-alanine_ligase_|_44293:45345_Reverse	6.3.2.4	Cell wall (peptidoglycan) biosynthesis
201	LCA_LACTOBACILLUS_SAKEI_RS00460_|_carboxylate--amine_ligase_|_94813:96066_Forward	6.3.1.12	Cell wall biogenesis/degradation
202	LCA_LACTOBACILLUS_SAKEI_RS00465_|_aspartate_racemase_|_96075:96779_Forward	5.1.1.13	Cell wall biogenesis/degradation
203	LCA_LACTOBACILLUS_SAKEI_RS01315_|_D-alanyl-D-alanine_carboxypeptidase_|_274535:275821_Forward	3.4.16.4	Cell wall biogenesis/degradation DacA
204	LCA_LACTOBACILLUS_SAKEI_RS01325_|_lipoprotein_precursor_|_277534:278157_Forward	6.3.2.13	Cell wall biogenesis/degradation peptidoglycan biosynthesis MurE
205	LCA_LACTOBACILLUS_SAKEI_RS01895_|_D-alanine--poly(phosphoribitol)_ligase_|_403918:405444_Forward	6.1.1.13	Cell wall biosynthesis
206	LCA_LACTOBACILLUS_SAKEI_RS01900_|_D-alanyl-lipoteichoic_acid_biosynthesis_protein_DltB_|_405437:406645_Forward	6.1.1.13	Cell wall biosynthesis
207	LCA_LACTOBACILLUS_SAKEI_RS01905_|_D-alanine--poly(phosphoribitol)_ligase_subunit_2_|_406675:406911_Forward	6.1.1.13	Cell wall biosynthesis
208	LCA_LACTOBACILLUS_SAKEI_RS01910_|_D-alanyl-lipoteichoic_acid_biosynthesis_protein_DltD_|_406913:408184_Forward	6.1.1.13	Cell wall biosynthesis
209	LCA_LACTOBACILLUS_SAKEI_RS03770_|_penicillin-binding_protein_|_738696:740834_Forward		Cell wall biosynthesis
210	LCA_LACTOBACILLUS_SAKEI_RS03775_|_phospho-N-acetylmuramoyl-pentapeptide-_transferase_|_740867:741829_Forward		Cell wall biosynthesis
211	LCA_LACTOBACILLUS_SAKEI_RS03785_|_UDP-N-acetylglucosamine--N-acetylmuramyl-_(pentapeptide)_pyrophosphoryl-undecaprenol_N-acetylglucosamine_transferase_|_743225:744325_Forward		Cell wall biosynthesis
212	LCA_LACTOBACILLUS_SAKEI_RS07845_|_teichoic_acid/polysaccharide_biosynthesis_protein_|_1552964:1553839_Reverse		Cell wall biosynthesis

213	LCA_LACTOBACILLUS_SAKEI_RS07850_|_teichoic_acid/polysaccharide_export_protein_|_1553850:1555391_Reverse		Cell wall biosynthesis
214	LCA_LACTOBACILLUS_SAKEI_RS08120_|_UDP-N-acetylglucosamine_1-carboxyvinyltransferase_|_1612355:1613614_Reverse		Cell wall biosynthesis
215	LCA_LACTOBACILLUS_SAKEI_RS08215_|_1632935:1634323_Reverse		Cell wall biosynthesis
216	LCA_LACTOBACILLUS_SAKEI_RS09085_|_surface_polysaccharide_deacetylase_|_1803704:1805110_Forward		Cell wall biosynthesis
217	LCA_LACTOBACILLUS_SAKEI_RS04600_|_penicillin-binding_protein_1A_|_907714:910017_Reverse	2.4.1.129	Cell wall peptidoglycan synthesis
218	LCA_LACTOBACILLUS_SAKEI_RS02535_|_undecaprenyl-phosphate_alpha-N-acetylglucosaminyl_1-phosphate_transferase_|_508103:509191_Forward	2.7.8.13	Cell wall synthesis
219	LCA_LACTOBACILLUS_SAKEI_RS03235_|_UDP-N-acetylmuramate--L-alanine_ligase_|_639372:640703_Forward		Cell wall synthesis
220	LCA_LACTOBACILLUS_SAKEI_RS04335_|_N-acetylmuramoyl-L-alanine_amidase_|_849559:850881_Reverse		Cell wall synthesis
221	LCA_LACTOBACILLUS_SAKEI_RS05595_|_UDP-N-acetylglucosamine_1-carboxyvinyltransferase_|_1111924:1113225_Reverse	2.5.1.7	Cell wall synthesis
222	LCA_LACTOBACILLUS_SAKEI_RS05675_|_UDP-N-acetylmuramyl_peptide_synthase_|_1126340:1127689_Forward		Cell wall synthesis
223	LCA_LACTOBACILLUS_SAKEI_RS07130_|_N-acetylmuramoyl-L-alanine_amidase_|_1411165:1413171_Reverse		Cell wall synthesis
224	LCA_LACTOBACILLUS_SAKEI_RS07455_|_UTP--glucose-1-phosphate_uridylyltransferase_|_1475623:1476525_Reverse	2.7.7.9	Cell wall synthesis
225	LCA_LACTOBACILLUS_SAKEI_RS07560_|_UDP-N-acetyl_glucosamine_2-epimerase_|_1494222:1495361_Reverse		Cell wall synthesis
226	LCA_LACTOBACILLUS_SAKEI_RS07820_|_teichoic_acid/polysaccharide_glycosyl_transferase_group_1_|_1547354:1548430_Reverse		Cell wall synthesis
227	LCA_LACTOBACILLUS_SAKEI_RS07830_|_teichoic_acid/polysaccharide_export_protein_|_1548846:1550261_Reverse		Cell wall synthesis
228	LCA_LACTOBACILLUS_SAKEI_RS07835_|_CDP-glycerol--glycerophosphate_glycerophosphotransferase_|_1550263:1551408_Reverse	2.7.8.-	Cell wall synthesis
229	LCA_LACTOBACILLUS_SAKEI_RS08070_|_UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-_alanine_ligase_|_1602350:1603726_Reverse		Cell wall synthesis
230	LCA_LACTOBACILLUS_SAKEI_RS06020_|_alkaline_phosphatase_|_1189948:1192125_Forward		Cell wall synthesis, exported glycerol phosphate lipoteichoic acid synthetase and anion-binding protein
231	LCA_LACTOBACILLUS_SAKEI_RS07510_|_exopolysaccharide_biosynthesis_protein_|_1486375:1487121_Reverse	2.7.10.2	Cell wall, EPS biosynthesis
232	LCA_LACTOBACILLUS_SAKEI_RS05780_|_peptidase_M23_|_1146170:1146865_Reverse		Cell wall, Peptidoglycan-binding lysin domain
233	LCA_LACTOBACILLUS_SAKEI_RS05820_|_peptidase_M23_|_1150395:1151120_Reverse		Cell wall, Peptidoglycan-binding lysin domain
234	LCA_LACTOBACILLUS_SAKEI_RS03070_|_SorC_family_transcriptional_regulator_|_605626:606663_Forward		CggR transcriptional regulator of gapA
235	LCA_LACTOBACILLUS_SAKEI_RS03180_|_peptidylprolyl_isomerase_|_629874:630782_Reverse	5.2.1.8	Chaperone, protein folding and stabilization
236	LCA_LACTOBACILLUS_SAKEI_RS06145_|_molecular_chaperone_DnaJ_|_1213558:1214709_Reverse		Chaperoning , adaptation to atypical conditions (other genes from the operon (DnaK, GroES, GroEL?)
237	LCA_LACTOBACILLUS_SAKEI_RS02085_|_peptidyl-prolyl_cis-trans_isomerase_|_443641:444225_Forward	5.2.1.8	Chaperoning folding
238	LCA_LACTOBACILLUS_SAKEI_RS02735_|_ATP-dependent_Clp_protease_proteolytic_subunit_|_549884:550468_Reverse		ClpP protease, adaptation to atypical conditions
239	LCA_LACTOBACILLUS_SAKEI_RS06965_|_dephospho-CoA_kinase_|_1370499:1371107_Reverse	2.7.1.24	coenzyme A biosynthesis
240	LCA_LACTOBACILLUS_SAKEI_RS05350_|_coaD_|_phosphopantetheine_adenylyltransferase_|_1068486:1068983_Reverse	2.7.7.3	Coenzyme A biosynthesis, phosphopantetheine adenylyltransferase, 4 étapes: 6.3.2.5; 4.1.1.36; 2.7.1.24
241	LCA_LACTOBACILLUS_SAKEI_RS00220_|_cyclopropane-fatty-acyl-phospholipid_synthase_|_41152:42339_Reverse	2.1.1.79	cyclopropane-fatty-acyl-phospholipid synthase
242	LCA_LACTOBACILLUS_SAKEI_RS05085_|_cytidylate_kinase_|_1008969:1009628_Reverse	2.7.4.14	cytidylate kinase, purines pyrimidines metabolism
243	LCA_LACTOBACILLUS_SAKEI_RS01410_|_gluconate_kinase_|_295975:297531_Forward	2.7.1.12	D-gluconate vers 6P-D-gluconate
244	LCA_LACTOBACILLUS_SAKEI_RS06745_|_TIGR00159_family_protein_|_1329163:1330002_Reverse	2.7.7.85	Diadenylate cyclase, 2 ATP <=> 2 diphosphate + cyclic di-3',5'-adenylate
245	LCA_LACTOBACILLUS_SAKEI_RS00910_|_peptidase_C69_|_189076:190500_Forward	3.4.-.-	Dipeptidase
246	LCA_LACTOBACILLUS_SAKEI_RS04505_|_peptidase_U34_|_883107:884543_Reverse	3.4.-.-	Dipeptidase
247	LCA_LACTOBACILLUS_SAKEI_RS02025_|_dipeptidase_PepV_|_431474:432877_Reverse	3.4.13.3	Dipeptidase PePV
248	LCA_LACTOBACILLUS_SAKEI_RS07920_|_peptide_ABC_transporter_permease_|_1569369:1570829_Reverse		di-tripeptide-proton ABC symporter
249	LCA_LACTOBACILLUS_SAKEI_RS08465_|_divalent_metal_cation_transporter_|_1681251:1682627_Reverse		Divalent metal cation transporter MntH
250	LCA_LACTOBACILLUS_SAKEI_RS01600_|_hypothetical_protein_|_341984:342292_Forward		DNA binding protein, replication?
251	LCA_LACTOBACILLUS_SAKEI_RS01880_|_endonuclease_MutS2_|_400730:403093_Forward		DNA mismatch repair, replication
252	LCA_LACTOBACILLUS_SAKEI_RS08390_|_DNA_repair_protein_RadA_|_1664119:1665483_Reverse		DNA repair
253	LCA_LACTOBACILLUS_SAKEI_RS04285_|_hypothetical_protein_|_840626:841306_Forward		DNA repair or phosphate metabolism?
254	LCA_LACTOBACILLUS_SAKEI_RS06865_|_DNA-binding_response_regulator_|_1352199:1352885_Reverse		DNA-binding_response_regulator, unknown target
255	LCA_LACTOBACILLUS_SAKEI_RS09170_|_peroxidase_|_1821441:1822397_Forward		Dyp-type peroxidase family (iron-dependent), oxidative stress
256	LCA_LACTOBACILLUS_SAKEI_RS08045_|_PemK_family_transcriptional_regulator_|_1598123:1598509_Reverse		endoribonuclease that inactivates cellular mRNAs by cleaving at specific sites
257	LCA_LACTOBACILLUS_SAKEI_RS03465_|_guanylate_kinase_|_682257:682868_Forward	2.7.4.8	Essential for recycling GMP, bases metabolism

258	LCA_LACTOBACILLUS_SAKEI_RS05605_|_F0F1_ATP_synthase_subunit_epsilon_|_1113647:1114081_Reverse	3.6.3.14	F0F1 ATP ase energy production
259	LCA_LACTOBACILLUS_SAKEI_RS05610_|_ATP_synthase_subunit_beta_|_1114097:1115527_Reverse	3.6.3.14	F0F1 ATP ase energy production
260	LCA_LACTOBACILLUS_SAKEI_RS05615_|_ATP_synthase_subunit_gamma_|_1115551:1116498_Reverse	3.6.3.14	F0F1 ATP ase energy production
261	LCA_LACTOBACILLUS_SAKEI_RS05625_|_ATP_synthase_subunit_delta_|_1118074:1118616_Reverse	3.6.3.14	F0F1 ATP ase energy production
262	LCA_LACTOBACILLUS_SAKEI_RS05630_|_ATP_synthase_subunit_B_|_1118603:1119124_Reverse	3.6.3.14	F0F1 ATP ase energy production
263	LCA_LACTOBACILLUS_SAKEI_RS05640_|_F0F1_ATP_synthase_subunit_A_|_1119418:1120131_Reverse	3.6.3.14	F0F1 ATP ase energy production
264	LCA_LACTOBACILLUS_SAKEI_RS04105_|_3-oxoacyl-ACP_synthase_III_|_805683:806663_Forward	2.3.1.41	Fatty acid biosynthesis
265	LCA_LACTOBACILLUS_SAKEI_RS04125_|_808772:810004_Forward	2.3.1.41	Fatty acid biosynthesis
266	LCA_LACTOBACILLUS_SAKEI_RS06135_|_1212343:1213110_Reverse	6.3.4.15	Fattyacid biosynthesis, biotin-carboxyl carrier protein assembly
267	LCA_LACTOBACILLUS_SAKEI_RS04805_|_hypothetical_protein_|_948613:950328_Reverse		fibronectin/fibrinogene-binding protein, Adaptation to atypical conditions
268	LCA_LACTOBACILLUS_SAKEI_RS04275_|_glutamate_synthase_|_838606:839907_Forward	6.3.2.17	Folylpolyglutamate synthase, folate polyglutamylation, folate synthesis
269	LCA_LACTOBACILLUS_SAKEI_RS08210_|_1631162:1632133_Reverse	2.7.6.1	From ribose degradation to purine metabolism ATP + D-ribose 5-phosphate <=> AMP + 5-phospho-alpha-D-ribose 1-diphosphate
270	LCA_LACTOBACILLUS_SAKEI_RS05235_|_1-phosphofructokinase_|_1040897:1041814_Forward	2.7.1.56	Fructose degradation, Glycolysis
271	LCA_LACTOBACILLUS_SAKEI_RS05230_|_DeoR_family_transcriptional_regulator_|_1040145:1040897_Forward		FruR, fructose operon transcription regulator
272	LCA_LACTOBACILLUS_SAKEI_RS06345_|_UDP-glucose_4-epimerase_GalE_|_1260176:1261168_Forward	5.1.3.2	Galactose degradation
273	LCA_LACTOBACILLUS_SAKEI_RS01605_|_342318:342914_Forward		Gap repair replication
274	LCA_LACTOBACILLUS_SAKEI_RS03075_|_type_I_glyceraldehyde-3-phosphate_dehydrogenase_|_606702:607718_Forward	1.2.1.12	GapA glyceraldehyde 3-phosphate dehydrogenase glycolysis
275	LCA_LACTOBACILLUS_SAKEI_RS02685_|_phosphoglucomutase_|_537274:538998_Forward	5.4.2.2	glucose et glucose-1P degradation
276	LCA_LACTOBACILLUS_SAKEI_RS06005_|_glucose_transporter_GlcU_|_1186810:1187676_Reverse		Glucose transporter
277	LCA_LACTOBACILLUS_SAKEI_RS04230_|_825971:828250_Forward	6.3.2.2, 6.3.2.3	Gluthation biosynthesis, Synthesizes glutathione from L-glutamate and L-cysteine via gamma-L-glutamyl-L-cysteine, oxidative stress response
278	LCA_LACTOBACILLUS_SAKEI_RS05155_|_pyruvate_kinase_|_1019787:1021547_Reverse	2.7.1.40	Glycolysis
279	LCA_LACTOBACILLUS_SAKEI_RS05160_|_6-phosphofructokinase_|_1021630:1022589_Reverse	2.7.1.11	Glycolysis
280	LCA_LACTOBACILLUS_SAKEI_RS05880_|_pgi_|_glucose-6-phosphate_isomerase_|_1161804:1163150_Reverse	5.3.1.9	Glycolysis
281	LCA_LACTOBACILLUS_SAKEI_RS06595_|_glucokinase_|_1298719:1299690_Reverse	2.7.1.2	glycolysis
282	LCA_LACTOBACILLUS_SAKEI_RS07590_|_fructose-1,6-bisphosphate_aldolase,_class_II_|_1499463:1500326_Reverse	4.1.2.13	Glycolysis
283	LCA_LACTOBACILLUS_SAKEI_RS05925_|_pyruvate_oxidase_|_1170993:1172828_Forward	1.2.3.3	Glycolysis end products, Pyruvate + phosphate + O(2) <=> acetyl phosphate + CO(2) + H(2)O(2)
284	LCA_LACTOBACILLUS_SAKEI_RS04055_|_phosphoglycerate_mutase_|_795997:796656_Forward	5.4.2.1	Glycolysis heterolactic fermentation
285	LCA_LACTOBACILLUS_SAKEI_RS00470_|_2,3-bisphosphoglycerate-dependent_phosphoglycerate_mutase_|_96789:97496_Forward	5.4.2.1	Glycolysis heterolactic fermentation or gluconeogenesis
286	LCA_LACTOBACILLUS_SAKEI_RS00610_|_fructose_2,6-bisphosphatase_|_123831:124487_Reverse	5.4.2.1	Glycolysis heterolactic fermentation or gluconeogenesis (il y en a 5 dans le génome de 23K)
287	LCA_LACTOBACILLUS_SAKEI_RS00960_|_gpmA_|_2,3-bisphosphoglycerate-dependent_phosphoglycerate_mutase_|_198757:199446_Forward	5.4.2.1	Glycolysis heterolactic fermentation or gluconeogenesis (il y en a 5 dans le génome de 23K)
288	LCA_LACTOBACILLUS_SAKEI_RS08020_|_L-lactate_dehydrogenase_|_1593030:1594007_Forward	1.1.1.27	Glycolysis/fermentation
289	LCA_LACTOBACILLUS_SAKEI_RS04185_|_inosine-uridine_preferring_nucleoside_hydrolase_|_818364:819338_Reverse	3.2.2.1	Guanine guanosine salvage
290	LCA_LACTOBACILLUS_SAKEI_RS06170_|_coproporphyrinogen_III_oxidase_|_1219358:1220497_Reverse	1.3.99.22	heme biosynthesis (voie incomplete)
291	LCA_LACTOBACILLUS_SAKEI_RS01405_|_6-phosphogluconate_dehydrogenase_|_295038:295937_Forward	1.1.1.44	Heterolactic fermentation
292	LCA_LACTOBACILLUS_SAKEI_RS03510_|_692835:693497_Forward	5.1.3.1	heterolactic fermentation pentose phosphate pathway
293	LCA_LACTOBACILLUS_SAKEI_RS01820_|_glucose-6-phosphate_dehydrogenase_|_387082:388578_Forward	1.1.1.49	Heterolactic fermentation, pentose phosphate pathway
294	LCA_LACTOBACILLUS_SAKEI_RS06870_|_phosphogluconate_dehydrogenase_(NADP(+)-dependent,_decarboxylating)_|_1353144:1354565_Reverse	1.1.1.44	heterolactic fermentation, pentose phosphate pathway
295	LCA_LACTOBACILLUS_SAKEI_RS06835_|_HxlR_family_transcriptional_regulator_|_1347218:1347574_Reverse		HTH-type transcriptional regulator unknown target
296	LCA_LACTOBACILLUS_SAKEI_RS00395_|_serine_protease_|_78181:79404_Forward	3.4.21.-	HtrA, degradation of protein resistance to stress
297	~~LCA_LACTOBACILLUS_SAKEI_RS06840_|_hydrolase_|_1347653:1348165_Reverse~~		~~Hydrolase of unknown function~~
298	~~LCA_LACTOBACILLUS_SAKEI_RS08500_|_serine_hydrolase_|_1690589:1691572_Reverse~~		~~Hydrolase, unknown function~~
299	LCA_LACTOBACILLUS_SAKEI_RS04905_|_hydroxymethylpyrimidine/phosphomethylpyrimidine_kinase_|_972913:973710_Forward	2.7.4.7	hydroxymethylpyrimidine / phosphomethylpyrimidine kinase, thiamine metabolism
300	~~LCA_LACTOBACILLUS_SAKEI_RS01830_|_hypothetical_protein_|_389902:391224_Forward~~		~~Hypothetical~~
301	LCA_LACTOBACILLUS_SAKEI_RS04670_|_hypothetical_protein_|_921116:921769_Forward		hypothetical

302	LCA_LACTOBACILLUS_SAKEI_RS03900_|_tellurite_resistance_protein_TelA_|_768747:769943_Reverse		Hypothetical, toxic anion resistance
303	LCA_LACTOBACILLUS_SAKEI_RS02670_|_glycerol-3-phosphate_dehydrogenase_(NAD(P)(+))_|_534539:535561_Forward	1.1.1.94	Lipid biosynthesis, oxidoreductase
304	LCA_LACTOBACILLUS_SAKEI_RS04405_|_diacylglycerol_kinase_|_863501:863902_Forward	2.7.1.107/2.7.1.66	Lipid or membrane synthesis
305	LCA_LACTOBACILLUS_SAKEI_RS04675_|_lipoate--protein_ligase_A_|_921979:922992_Reverse	6.3.1.20	Lipoate is used as an essential cofactor by many enzyme complexes involved in oxidative metabolism inclusing pyruvate dehydrogenase Lipoate biosynthesis voir si 2.8.1.8 lipoyl syntase existe aussi.
306	LCA_LACTOBACILLUS_SAKEI_RS02665_|_prolipoprotein_diacylglyceryl_transferase_|_533694:534521_Forward	2.4.99.-	Lipoprotein (cell surface) biosynthesis prolipoprotein diacylglyceryl transferase
307	LCA_LACTOBACILLUS_SAKEI_RS07675_|_diacylglycerol_kinase_|_1514794:1515822_Reverse	2.7.1.107	Lipoteichoic acid production (cell wall synthesis)
308	LCA_LACTOBACILLUS_SAKEI_RS04865_|_LysR_family_transcriptional_regulator_|_961443:962408_Reverse		LysR family transcriptional regulator, target unknown
309	LCA_LACTOBACILLUS_SAKEI_RS04860_|_manganese-dependent_inorganic_pyrophosphatase_|_960467:961390_Reverse	3.6.1.1	manganese-dependent inorganic pyrophosphatase, phosphate metabolism
310	LCA_LACTOBACILLUS_SAKEI_RS05710_|_mannose-6-phosphate_isomerase_|_1133860:1134834_Reverse	5.3.1.8	Mannose degradation, Glycolysis
311	LCA_LACTOBACILLUS_SAKEI_RS09000_|_alpha-galactosidase_|_1781915:1784089_Reverse	3.2.1.22	Melibiose (sugar) degradation
312	LCA_LACTOBACILLUS_SAKEI_RS01245_|_membrane_protein_|_258072:258539_Reverse		Membran protein of unknown function
313	LCA_LACTOBACILLUS_SAKEI_RS07105_|_transporter_|_1402309:1403946_Reverse		Membran protein of unknown function
314	LCA_LACTOBACILLUS_SAKEI_RS02075_|_membrane_protein_|_441802:442455_Forward		Membrane homeostasis DedA membrane protein
315	LCA_LACTOBACILLUS_SAKEI_RS05150_|_membrane_protein_|_1019157:1019618_Reverse		Membrane protein of unknown function
316	~~LCA_LACTOBACILLUS_SAKEI_RS05750_|_CAAX_amino_protease_|_1142886:1143542_Forward~~		~~Membrane protein of unknown function~~
317	LCA_LACTOBACILLUS_SAKEI_RS05955_|_membrane_protein_|_1175960:1176640_Forward		Membrane protein of unknown function
318	LCA_LACTOBACILLUS_SAKEI_RS05960_|_membrane_protein_|_1176700:1177407_Forward		Membrane protein of unknown function
319	LCA_LACTOBACILLUS_SAKEI_RS06000_|_hypothetical_protein_|_1184101:1186644_Reverse		Membrane protein of unknown function
320	~~LCA_LACTOBACILLUS_SAKEI_RS06265_|_phage_infection_protein_|_1242967:1245714_Forward~~		~~Membrane protein of unknown function~~
321	LCA_LACTOBACILLUS_SAKEI_RS06855_|_insertase_|_1349511:1350500_Forward		Membrane protein of unknown function
322	~~LCA_LACTOBACILLUS_SAKEI_RS09025_|_ABC_transporter_|_1787269:1790439_Reverse~~		~~Membrane protein, unknown~~
323	LCA_LACTOBACILLUS_SAKEI_RS04370_|_hypothetical_protein_|_857410:859275_Forward		Membren protein of unknown function
324	LCA_LACTOBACILLUS_SAKEI_RS02580_|_membrane_protein_|_518311:519444_Forward		Membrene protein cell division?
325	LCA_LACTOBACILLUS_SAKEI_RS03955_|_cobalt_transporter_CbiM_|_776354:777361_Reverse		Metal transport protein
326	LCA_LACTOBACILLUS_SAKEI_RS01815_|_Cro/Cl_family_transcriptional_regulator_|_386400:387062_Forward		Metal-dependent transcriptional regulator
327	~~LCA_LACTOBACILLUS_SAKEI_RS06885_|_SAM-dependent_methyltransferase_|_1356469:1357200_Reverse~~		~~methyl transferase of unknown function~~
328	LCA_LACTOBACILLUS_SAKEI_RS02430_|_S-adenosylmethionine_synthase_|_485247:486446_Forward	2.5.1.6	MetK catalyzes the formation of S-adenosylmethionine from methionine and ATP
329	LCA_LACTOBACILLUS_SAKEI_RS07365_|_hydroxymethylglutaryl-CoA_reductase,_degradative_|_1460261:1461529_Reverse	1.1.1.88	mevalonate degradation (vers acetoacetate), mais autre enzyme 4.1.3.4 n'est pas surexprimée
330	LCA_LACTOBACILLUS_SAKEI_RS04560_|_mevalonate_kinase_|_892840:893805_Reverse	2.7.1.36	Mevalonate kinase pathway mevalonate synhtesis
331	LCA_LACTOBACILLUS_SAKEI_RS01150_|_manganese_transporter_|_241472:243046_Forward		Mn(2+)/Fe(2+) transport protein
332	LCA_LACTOBACILLUS_SAKEI_RS01695_|_356863:357510_Forward		modulates transcription in response to the NADH/NAD(+) redox state, regulates cydAB in B. subtilis
333	LCA_LACTOBACILLUS_SAKEI_RS01560_|_MATE_family_efflux_transporter_|_332622:333989_Forward		Na(+) antiporter (drug efflux pump)
334	LCA_LACTOBACILLUS_SAKEI_RS07890_|_N-acetylglucosamine-6-phosphate_deacetylase_|_1564739:1565878_Reverse	3.5.1.25	N-acetyl glucosamine degradation (PTS sugar) vers glycolysis
335	LCA_LACTOBACILLUS_SAKEI_RS02000_|_glucosamine-6-phosphate_deaminase_|_426022:426729_Forward	3.5.99.6	N-Acetyl-glucosamine degradation, carbon nitrogen metabolism
336	LCA_LACTOBACILLUS_SAKEI_RS08790_|_isochorismatase_|_1737948:1738499_Forward	3.5.1.19	NAD salvage, Metabolism of coenzymes and prosthetic groups, Nicotinamide + H(2)O <=> nicotinate + NH(3)
337	LCA_LACTOBACILLUS_SAKEI_RS02940_|_NADH_peroxidase_|_585192:586544_Forward	1.11.1.1	NADH peroxidase LSA0575 redox
338	LCA_LACTOBACILLUS_SAKEI_RS04255_|_aminotransferase_V_|_832370:833518_Forward	2.8.1.7	NifS/IcsS protein homolog, AA (alanine) biosynthesis
339	LCA_LACTOBACILLUS_SAKEI_RS01355_|_nucleoside_transporter_|_284050:285285_Forward		nucleoside permease
340	LCA_LACTOBACILLUS_SAKEI_RS01345_|_oligoendopeptidase_F_|_280719:282524_Reverse	3.4.24.-	oligoendopeptidase PepF1
341	LCA_LACTOBACILLUS_SAKEI_RS02510_|_3-oxoacyl-ACP_reductase_|_502399:503130_Forward		Oxidoreductase fatty acid synthesis
342	LCA_LACTOBACILLUS_SAKEI_RS00150_|_2,5-diketo-D-gluconic_acid_reductase_|_29633:30484_Reverse	1.1.1.274	oxidoreductase of aldo/keto reductase family EC number putative
343	LCA_LACTOBACILLUS_SAKEI_RS04100_|_805217:805672_Forward	4.2.1.59	Palmitate (fatty acids) biosynthesis
344	LCA_LACTOBACILLUS_SAKEI_RS00200_|_2-dehydropantoate_2-reductase_|_38535:39473_Forward	1.1.1.169	PanE ketopantoate reductase; catalyzes the NADPH reduction of ketopantoate to pantoate; functions in pantothenate (vitamin B5) biosynthesis

345	LCA_LACTOBACILLUS_SAKEI_RS08060_|_holo-ACP_synthase_|_1599997:1600350_Reverse	2.7.8.7	Pantothenate and CoA biosynthesis
346	~~LCA_LACTOBACILLUS_SAKEI_RS07195_|_hypothetical_protein_|_1422996:1423736_Reverse~~		~~partial gene (mutation?) with identity to histidine kinase (regulator)~~
347	LCA_LACTOBACILLUS_SAKEI_RS09505_|_814723:815424_Forward	6.3.4.15	Pathway biotin-carboxyl carrier protein assembly
348	LCA_LACTOBACILLUS_SAKEI_RS08400_|_1666291:1666974_Forward	5.3.1.6	Pentose phosphate pathway (ribose degradation)
349	LCA_LACTOBACILLUS_SAKEI_RS07530_|_type_I_methionyl_aminopeptidase_|_1490240:1491037_Reverse	3.4.11.18	PepM, methionine aminopeptidase, protein modification, chaperoning
350	LCA_LACTOBACILLUS_SAKEI_RS05215_|_acyltransferase_|_1035333:1037261_Reverse		peptidoglycan O-acetyltransferase, cell wall, OatA
351	LCA_LACTOBACILLUS_SAKEI_RS00475_|_penicillin-binding_protein_|_97477:98628_Forward	3.4.16.4	Peptodoglycan biosysnthesis
352	LCA_LACTOBACILLUS_SAKEI_RS03080_|_phosphoglycerate_kinase_|_607819:609033_Forward	2.7.2.3	PGK phosphoglycerate kinase glycolysis
353	LCA_LACTOBACILLUS_SAKEI_RS02600_|_phosphate_ABC_transporter_permease_subunit_PstC_|_522788:523711_Forward		Phosphate ABC transporter
354	LCA_LACTOBACILLUS_SAKEI_RS02610_|_phosphate_ABC_transporter_ATP-binding_protein_|_524605:525414_Forward		Phosphate ABC transporter
355	LCA_LACTOBACILLUS_SAKEI_RS02615_|_phosphate_ABC_transporter_ATP-binding_protein_|_525435:526193_Forward		Phosphate ABC transporter
356	LCA_LACTOBACILLUS_SAKEI_RS04395_|_phosphate_starvation_protein_PhoH_|_862069:863037_Forward		phosphate starvation induced protein of unknown function
357	LCA_LACTOBACILLUS_SAKEI_RS01370_|_phosphoketolase_|_286496:288859_Forward	4.1.2.9	Phosphoketolase/ D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase
358	LCA_LACTOBACILLUS_SAKEI_RS03730_|_GTP_pyrophosphokinase_|_732689:734920_Forward	2.7.6.5	ppGpp biosynthesis
359	LCA_LACTOBACILLUS_SAKEI_RS05220_|_obgE_|_GTPase_ObgE_|_1037400:1038692_Reverse		ppGpp-binding GTPase involved in cell partioning, DNA repair and ribosome assembly
360	LCA_LACTOBACILLUS_SAKEI_RS06485_|_transcriptional_regulator_|_1281049:1281780_Reverse		Probable transcriptional regulatory , target unknown
361	LCA_LACTOBACILLUS_SAKEI_RS05290_|_ATP-dependent_Clp_protease_ATP-binding_subunit_ClpX_|_1054588:1055841_Reverse		Protease, stress response
362	LCA_LACTOBACILLUS_SAKEI_RS07150_|_undecaprenyl-diphosphatase_|_1415510:1416346_Reverse	3.6.1.27	Protect, drug resistance
363	LCA_LACTOBACILLUS_SAKEI_RS00565_|_GTPase_HflX_|_111469:112740_Reverse		Protein fate or degradation
364	~~LCA_LACTOBACILLUS_SAKEI_RS06695_|_GNAT_family_acetyltransferase_|_1318784:1319347_Reverse~~		~~protein of unknown function, acetyltransferase family protein~~
365	LCA_LACTOBACILLUS_SAKEI_RS02300_|_PTS_mannose_transporter_subunit_EIIAB_|_463668:464645_Forward		PTS EIIABCD mannose
366	LCA_LACTOBACILLUS_SAKEI_RS02305_|_PTS_mannose/fructose/sorbose_transporter_subunit_IIC_|_464680:465486_Forward		PTS EIIABCD mannose
367	LCA_LACTOBACILLUS_SAKEI_RS02310_|_PTS_mannose_transporter_subunit_IID_|_465505:466416_Forward		PTS EIIABCD mannose
368	LCA_LACTOBACILLUS_SAKEI_RS01680_|_PTS_sugar_transporter_subunit_IIB_|_353900:354217_Forward	2.7.1.69	PTS EIIB cellobiose
369	LCA_LACTOBACILLUS_SAKEI_RS07260_|_phosphocarrier_protein_HPr_|_1439647:1439913_Reverse	2.7.11.-	PTS general enzyme HPr, PTS sugar utilization
370	LCA_LACTOBACILLUS_SAKEI_RS07255_|_phosphoenolpyruvate--protein_phosphotransferase_|_1437923:1439647_Reverse	2.7.3.9	PTS general Enzyme I, PTS sugar utilization
371	LCA_LACTOBACILLUS_SAKEI_RS00305_|_adenylosuccinate_synthetase_|_59513:60793_Forward	6.3.4.4	PurA, adenylosuccinate synthetase, purines adenosine nucleotide biosynthesis
372	LCA_LACTOBACILLUS_SAKEI_RS03970_|_1-(5-phosphoribosyl)-5-amino-4-imidazole-_carboxylate_carboxylase_|_779645:780427_Forward		PurE like, purine metabolism
373	LCA_LACTOBACILLUS_SAKEI_RS06275_|_pyrH_|_UMP_kinase_|_1246332:1247057_Reverse	2.7.4.22	purine and pyrimidine metabolism; pyrimidine ribonucleotides interconversion
374	LCA_LACTOBACILLUS_SAKEI_RS07240_|_anaerobic_ribonucleoside_triphosphate_reductase_|_1432875:1435091_Reverse	1.17.4.2	purine biosynthesis
375	LCA_LACTOBACILLUS_SAKEI_RS07725_|_adenylosuccinate_lyase_|_1528041:1529333_Reverse	4.3.2.2	Purine metabolism
376	LCA_LACTOBACILLUS_SAKEI_RS07730_|_phosphoribosylaminoimidazole_carboxylase_|_1529389:1530501_Reverse	4.1.1.21	Purine metabolism
377	LCA_LACTOBACILLUS_SAKEI_RS09050_|_deoxyadenosine_kinase_|_1795859:1796515_Forward	2.7.1.74, 2.7.1.76	Purine metabolism, ATP + deoxyadenosine <=> ADP + dAMP
378	LCA_LACTOBACILLUS_SAKEI_RS07980_|_hypoxanthine_phosphoribosyltransferase_|_1583430:1583975_Reverse	2.4.2.8	Purine metabolism, IMP + diphosphate <=> hypoxanthine + 5-phospho-alpha-D-ribose 1-diphosphate,
379	LCA_LACTOBACILLUS_SAKEI_RS01615_|_thymidylate_kinase_|_343329:343973_Forward	2.7.4.9	Purine pyrimidine metabolism
380	LCA_LACTOBACILLUS_SAKEI_RS05670_|_thymidine_kinase_|_1125569:1126162_Reverse	2.7.1.21	Purines pyrimidines metabolism
381	LCA_LACTOBACILLUS_SAKEI_RS05645_|_uracil_phosphoribosyltransferase_|_1120440:1121069_Reverse	2.4.2.9	Purines pyrimidines metabolism; Salvage pathways of pyrimidine ribonucleotides; Nucleosides and nucleotides interconversion
382	LCA_LACTOBACILLUS_SAKEI_RS02530_|_506382:507947_Forward	3.1.4.16	Putative 2',3'-cyclic-nucleotide 2'-phosphodiesterase. RNA degradation
383	LCA_LACTOBACILLUS_SAKEI_RS02440_|_hypothetical_protein_|_488035:489060_Reverse	3.1.1.31	Putative 6-phosphogluconolactonase produit 6P-gluconate Utilisation des suches
384	LCA_LACTOBACILLUS_SAKEI_RS07185_|_adaptor_protein_MecA_|_1421409:1422098_Reverse		putative adaptor protein controlling oligomerization of the AAA+ protein ClpC, Role: control, adaptation
385	LCA_LACTOBACILLUS_SAKEI_RS06820_|_aminodeoxychorismate_lyase_|_1342235:1343386_Reverse	4.1.3.38	putative aminodeoxychorismate lyase family protein, 4-amino-4-deoxychorismate <=> 4-aminobenzoate + pyruvate
386	LCA_LACTOBACILLUS_SAKEI_RS05330_|_DNA_internalization-related_competence_protein_ComEC/Rec2_|_1063849:1066116_Reverse		Putative bacterial type II secretion/competence system, protein ComEC-like
387	LCA_LACTOBACILLUS_SAKEI_RS07005_|_lipase_|_1381111:1381869_Reverse	3.1.1.1	Putative carboxyesterase
388	LCA_LACTOBACILLUS_SAKEI_RS04625_|_D-3-phosphoglycerate_dehydrogenase_|_913496:914449_Forward	1.1.1.95	Putative D-3-phosphoglycerate dehydrogenase Serine biosynthesis
389	LCA_LACTOBACILLUS_SAKEI_RS03190_|_histidine_triad_protein_|_631277:631705_Reverse		Putative diadenosine polyphosphate hydrolase. Unknown function

390	LCA_LACTOBACILLUS_SAKEI_RS08340_|_lipase_|_1656016:1656873_Reverse		Putative esterase, unknown substrate
391	LCA_LACTOBACILLUS_SAKEI_RS08005_|_sugar_transporter_|_1586862:1588445_Reverse		putative exporter, unknown substrate
392	LCA_LACTOBACILLUS_SAKEI_RS07535_|_flavodoxin_|_1491300:1491752_Forward		Putative flavodoxin, electron transport
393	LCA_LACTOBACILLUS_SAKEI_RS02040_|_cell_surface_protein_|_435713:437041_Reverse	3.1.4.46	Putative Glycerophosphoryl diester phosphodiesterase
394	LCA_LACTOBACILLUS_SAKEI_RS07555_|_glucosyl_transferase_family_2_|_1493231:1494163_Forward		Putative glycosyltransferase CsbB, cell wall? Controled by stress in B. subtilis
395	LCA_LACTOBACILLUS_SAKEI_RS00310_|_guanine_permease_|_60964:62274_Forward		Putative Guanine/hypoxanthine permease pbuG
396	~~LCA_LACTOBACILLUS_SAKEI_RS06305_|_alpha/beta_hydrolase_|_1250954:1251889_Reverse~~		~~putative Hydrolase of the alpha/beta superfamily, unknown function~~
397	LCA_LACTOBACILLUS_SAKEI_RS06895_|_HD_domain-containing_protein_|_1357569:1358171_Reverse		Putative hydrolase of unknown function
398	LCA_LACTOBACILLUS_SAKEI_RS04205_|_haloacid_dehalogenase_|_821726:822376_Forward		Putative hydrolase, haloacid dehalogenase family unknown function
399	LCA_LACTOBACILLUS_SAKEI_RS04515_|_haloacid_dehalogenase_|_885030:885836_Reverse		Putative hydrolase, haloacid dehalogenase family unknown function
400	LCA_LACTOBACILLUS_SAKEI_RS06060_|_haloacid_dehalogenase_|_1199176:1199940_Forward		Putative hydrolase, haloacid dehalogenase family unknown function
401	LCA_LACTOBACILLUS_SAKEI_RS06675_|_haloacid_dehalogenase_|_1315482:1316369_Forward		Putative hydrolase, haloacid dehalogenase family unknown function
402	LCA_LACTOBACILLUS_SAKEI_RS06915_|_haloacid_dehalogenase_|_1360296:1360826_Reverse		Putative hydrolase, haloacid dehalogenase family unknown function
403	LCA_LACTOBACILLUS_SAKEI_RS05385_|_myo-inositol-1-monophosphatase_|_1074562:1075359_Reverse	3.1.3.25/ 3.1.3.-	Putative inositol monophosphatase / 5' nucleotidase (purine nucleoside monophosphate)
404	LCA_LACTOBACILLUS_SAKEI_RS02475_|_magnesium_transporter_|_494068:495021_Reverse		Putative ion Mg(2+)/Co(2+) transport protein
405	~~LCA_LACTOBACILLUS_SAKEI_RS07700_|_CamS_family_sex_pheromone_protein_|_1520008:1521135_Reverse~~		~~putative lipoprotein of unknown function~~
406	LCA_LACTOBACILLUS_SAKEI_RS05580_|_membrane_protein_insertion_efficiency_factor_YidD_|_1110307:1110555_Reverse		putative membrane protein insertion efficiency factor, secretion? Cell division?
407	LCA_LACTOBACILLUS_SAKEI_RS08385_|_twitching_motility_protein_PilT_|_1662976:1664088_Reverse		Putative membrane protein possibly involved in RNA binding
408	LCA_LACTOBACILLUS_SAKEI_RS01835_|_phosphoesterase_|_391228:392181_Forward		putative nanoRNase (oligoribonuclease), 3',5'-bisphosphate nucleotidase
409	LCA_LACTOBACILLUS_SAKEI_RS06540_|_FMN_reductase_|_1289947:1290504_Forward	1.6.5.2	Putative oxidoreductase
410	LCA_LACTOBACILLUS_SAKEI_RS01575_|_hypothetical_protein_|_335565:338186_Forward	2.3.2.3	Putative Phosphatidylglycerol lysyltransferase
411	LCA_LACTOBACILLUS_SAKEI_RS07505_|_multidrug_MFS_transporter_|_1485657:1486352_Reverse		Putative phosphotransferase involved in extracellular matrix synthesis
412	LCA_LACTOBACILLUS_SAKEI_RS05825_|_potassium_transporter_Kup_|_1151363:1153405_Reverse		putative potassium transport system protein (kup)
413	LCA_LACTOBACILLUS_SAKEI_RS02505_|_zinc_protease_|_501095:502399_Forward	3.4.24.-	Putative processing protease (protein trafficking?)
414	LCA_LACTOBACILLUS_SAKEI_RS00620_|_prolyl_aminopeptidase_|_125526:126428_Reverse	3.4.11.5	Putative proline amino peptidase
415	LCA_LACTOBACILLUS_SAKEI_RS04380_|_hypothetical_protein_|_860078:860920_Reverse		Putative pyruvate, phosphate dikinase regulatory protein 1, sugar metabolism
416	LCA_LACTOBACILLUS_SAKEI_RS07990_|_1585478:1585921_Reverse		putative RNA degradation protein
417	LCA_LACTOBACILLUS_SAKEI_RS08165_|_membrane_protein_|_1622233:1622964_Reverse		putative stress adaptation transporter
418	LCA_LACTOBACILLUS_SAKEI_RS06775_|_hypothetical_protein_|_1335081:1335299_Reverse		Putative stress response protein
419	~~LCA_LACTOBACILLUS_SAKEI_RS06590_|_sulfurtransferase_|_1298281:1298676_Reverse~~		~~Putative sulfur transferase of unknown function~~
420	LCA_LACTOBACILLUS_SAKEI_RS07550_|_membrane_protein_|_1492807:1493229_Forward		Putative teichoic acid glycosylation protein, cell wall?
421	LCA_LACTOBACILLUS_SAKEI_RS06600_|_hypothetical_protein_|_1299687:1299920_Reverse		Putative transcription factor of unknown function
422	LCA_LACTOBACILLUS_SAKEI_RS02515_|_XRE_family_transcriptional_regulator_|_503212:504135_Forward		Putative transcription regulator unknown function
423	LCA_LACTOBACILLUS_SAKEI_RS06160_|_HrcA_family_transcriptional_regulator_|_1217325:1218383_Reverse		Putative transcriptional regulator of heat-shock genes
424	LCA_LACTOBACILLUS_SAKEI_RS06980_|_MarR_family_transcriptional_regulator_|_1374739:1375230_Reverse		Putative transcriptional regulator, MarR family, unknown target
425	LCA_LACTOBACILLUS_SAKEI_RS07295_|_hypothetical_protein_|_1446610:1447950_Reverse		Putative transporter, unknown substrate
426	LCA_LACTOBACILLUS_SAKEI_RS09030_|_drug_ABC_exporter,_membrane-spanning_subunit_|_1790636:1791370_Reverse		Putative transporter, unknown substrate
427	LCA_LACTOBACILLUS_SAKEI_RS09035_|_drug_ABC_exporter,_ATP-binding_subunit_|_1791383:1792252_Reverse		Putative transporter, unknown substrate
428	LCA_LACTOBACILLUS_SAKEI_RS09415_|_ABC_transporter_ATP-binding_protein_|_1874495:1876119_Reverse		Putative transporter, unknown substrate
429	LCA_LACTOBACILLUS_SAKEI_RS01330_|_metal_ABC_transporter_substrate-binding_protein_|_278307:279194_Forward		Putative zinc/iron ABC transporter 1 seule ssu surexprimée
430	LCA_LACTOBACILLUS_SAKEI_RS05565_|_glycine_cleavage_system_protein_H_|_1108427:1108735_Reverse		Putative, Metabolism of amino acids and related molecules
431	LCA_LACTOBACILLUS_SAKEI_RS02285_|_dihydroorotate_oxidase_|_460281:461222_Forward	1.3.3.1/1.3.98.1	PyrD de novo biosynthesis of pyrimidine nucleotides
432	LCA_LACTOBACILLUS_SAKEI_RS07805_|_nicotinate_phosphoribosyltransferase_|_1544046:1545509_Reverse	2.4.2.11	Pyridine nucleotide biosynthesis
433	LCA_LACTOBACILLUS_SAKEI_RS08125_|_CTP_synthetase_|_1613960:1615552_Reverse	6.3.4.2	pyrimidine ribonucleotides interconversion, Pyrimidine Nucleotide Biosynthesis
434	LCA_LACTOBACILLUS_SAKEI_RS04065_|_hypothetical_protein_|_797333:799861_Forward	3.1.11.5	Recombination

435	LCA_LACTOBACILLUS_SAKEI_RS00640_|_ATP-dependent_DNA_helicase_|_129322:131613_Forward		Recombination/Replication ?
436	LCA_LACTOBACILLUS_SAKEI_RS07220_|_histidine_kinase_|_1428346:1429414_Forward		Regulation, unknown target, two-component system sensor histidine kinase
437	LCA_LACTOBACILLUS_SAKEI_RS00375_|_PAS_domain-containing_sensor_histidine_kinase_|_73161:75059_Forward		Regulator (two component system)
438	LCA_LACTOBACILLUS_SAKEI_RS01990_|_catabolite_control_protein_A_|_422134:423135_Forward		Regulator catabolite repression
439	LCA_LACTOBACILLUS_SAKEI_RS00605_|_LacI_family_transcriptional_regulator_|_122821:123780_Forward		Regulator LacI family
440	LCA_LACTOBACILLUS_SAKEI_RS00615_|_MarR_family_transcriptional_regulator_|_124702:125160_Forward		Regulator MarR family
441	LCA_LACTOBACILLUS_SAKEI_RS00915_|_MarR_family_transcriptional_regulator_|_190786:191328_Forward		Regulator MarR family
442	LCA_LACTOBACILLUS_SAKEI_RS08345_|_transcription_termination/antitermination_protein_NusG_|_1657216:1657764_Reverse		Regulator of elongation (transcription)
443	LCA_LACTOBACILLUS_SAKEI_RS05010_|_ABC_transporter_ATP-binding_protein_|_993280:995169_Reverse		regulator or transporter, unknown substrate
444	LCA_LACTOBACILLUS_SAKEI_RS02585_|_DNA-binding_response_regulator_|_519464:520177_Forward		Regulator two component system Phosphate regulon
445	LCA_LACTOBACILLUS_SAKEI_RS02590_|_two-component_sensor_histidine_kinase_|_520164:521825_Forward		Regulator two component system Phosphate regulon
446	LCA_LACTOBACILLUS_SAKEI_RS06045_|_two-component_sensor_histidine_kinase_|_1196352:1197788_Reverse		Regulator, two component system (histidine kinase is not upregulated
447	LCA_LACTOBACILLUS_SAKEI_RS00005_|_chromosomal_replication_initiator_protein_DnaA_|_210:1556_Forward		Replication
448	LCA_LACTOBACILLUS_SAKEI_RS00010_|_DNA_polymerase_III_subunit_beta_|_1734:2873_Forward		Replication
449	LCA_LACTOBACILLUS_SAKEI_RS00020_|_DNA_recombination_protein_RecF_|_3350:4477_Forward		Replication
450	LCA_LACTOBACILLUS_SAKEI_RS00025_|_gyrB_|_DNA_gyrase_subunit_B_|_4499:6490_Forward		Replication
451	LCA_LACTOBACILLUS_SAKEI_RS00040_|_single-stranded_DNA-binding_protein_|_9683:10195_Forward		Replication
452	LCA_LACTOBACILLUS_SAKEI_RS00060_|_13320:14717_Forward	3.6.1.-	Replication
453	LCA_LACTOBACILLUS_SAKEI_RS01595_|_DNA_polymerase_III_subunit_gamma/tau_|_340242:341957_Forward	2.7.7.7	Replication
454	LCA_LACTOBACILLUS_SAKEI_RS01625_|_DNA_polymerase_III_subunit_delta'_|_344321:345313_Forward	2.7.7.7	Replication
455	LCA_LACTOBACILLUS_SAKEI_RS01745_|_Holliday_junction_ATP-dependent_DNA_helicase_RuvA_|_369905:370516_Forward		Replication
456	LCA_LACTOBACILLUS_SAKEI_RS01750_|_Holliday_junction_DNA_helicase_RuvB_|_370529:371536_Forward		Replication
457	LCA_LACTOBACILLUS_SAKEI_RS03480_|_primosomal_protein_N'_|_684438:686855_Forward		Replication
458	LCA_LACTOBACILLUS_SAKEI_RS03845_|_DNA_topoisomerase_III_|_755103:757175_Forward		Replication
459	LCA_LACTOBACILLUS_SAKEI_RS04435_|_DNA_primase_|_869440:871323_Forward		Replication
460	LCA_LACTOBACILLUS_SAKEI_RS04565_|_ATP-dependent_helicase/deoxyribonuclease_subunit_B_|_894068:897628_Forward		Replication
461	LCA_LACTOBACILLUS_SAKEI_RS04575_|_ATP-dependent_helicase_|_901422:904256_Forward		Replication
462	LCA_LACTOBACILLUS_SAKEI_RS04605_|_Holliday_junction_resolvase_RecU_|_910024:910656_Reverse		Replication
463	LCA_LACTOBACILLUS_SAKEI_RS05165_|_dnaE_|_DNA_polymerase_III_subunit_alpha_|_1022722:1026054_Reverse		Replication
464	LCA_LACTOBACILLUS_SAKEI_RS06975_|_DNA_polymerase_I_|_1371955:1374615_Reverse		Replication
465	LCA_LACTOBACILLUS_SAKEI_RS07705_|_DNA_ligase_(NAD(+))_LigA_|_1521218:1523251_Reverse		Replication
466	LCA_LACTOBACILLUS_SAKEI_RS07710_|_ATP-dependent_DNA_helicase_PcrA_|_1523766:1526015_Reverse		Replication
467	LCA_LACTOBACILLUS_SAKEI_RS01725_|_DNA_mismatch_repair_protein_MutS_|_363788:366391_Forward		Replication and DNA repair
468	LCA_LACTOBACILLUS_SAKEI_RS01730_|_DNA_mismatch_repair_protein_MutL_|_366415:368373_Forward		Replication and DNA repair
469	LCA_LACTOBACILLUS_SAKEI_RS00075_|_nucleoid_occlusion_protein_|_17517:18416_Forward		Replication parB
470	LCA_LACTOBACILLUS_SAKEI_RS07330_|_1453927:1454733_Reverse		Replication, recombination regulator RecX
471	LCA_LACTOBACILLUS_SAKEI_RS02525_|_DNA_recombination/repair_protein_RecA_|_504928:505995_Forward		Replication/recombination
472	LCA_LACTOBACILLUS_SAKEI_RS03535_|_ATP-dependent_DNA_helicase_RecG_|_697651:699699_Forward		Replication/transcription
473	LCA_LACTOBACILLUS_SAKEI_RS04880_|_DNA_topoisomerase_IV_|_966009:968426_Reverse		Replication/transcription
474	LCA_LACTOBACILLUS_SAKEI_RS04945_|_DNA_topoisomerase_I_|_981736:983820_Reverse		Replication/transcription
475	LCA_LACTOBACILLUS_SAKEI_RS05095_|_ATP-dependent_DNA_helicase_RecQ_|_1010465:1011910_Reverse		Replication/transcription
476	LCA_LACTOBACILLUS_SAKEI_RS07625_|_helicase_|_1505389:1506735_Reverse		Replication/transcription
477	LCA_LACTOBACILLUS_SAKEI_RS04710_|_927727:929898_Reverse	1.17.4.1	ribonucleoside diphosphate reductase subunit alpha, 3 subunits, in operon, but only one upregulated. Catalyzes the rate-limiting step in dNTP synthesis
478	LCA_LACTOBACILLUS_SAKEI_RS00930_|_193092:193976_Forward		Ribose operon

479	LCA_LACTOBACILLUS_SAKEI_RS00935_|_D-ribose_pyranase_|_193997:194392_Forward		Ribose operon
480	LCA_LACTOBACILLUS_SAKEI_RS00940_|_194412:195320_Forward		Ribose operon
481	LCA_LACTOBACILLUS_SAKEI_RS00945_|_LacI_family_transcriptional_regulator_|_195385:196407_Forward		Ribose operon
482	LCA_LACTOBACILLUS_SAKEI_RS01840_|_DEAD/DEAH_box_helicase_|_392226:393581_Forward	3.6.4.13	RNA helicases utilize the energy from ATP hydrolysis to unwind RNA
483	LCA_LACTOBACILLUS_SAKEI_RS03440_|_cell_division_protein_FtsJ_|_678340:679170_Forward		RNA processing
484	LCA_LACTOBACILLUS_SAKEI_RS05145_|_S1_RNA-binding_protein_|_1018225:1019136_Reverse		RNA processing
485	LCA_LACTOBACILLUS_SAKEI_RS03470_|_DNA-directed_RNA_polymerase_subunit_omega_|_682873:683130_Forward	2.7.7.6	RpoZ, transcription
486	LCA_LACTOBACILLUS_SAKEI_RS06815_|_uridine_kinase_|_1341521:1342156_Reverse	2.7.1.48	salvage pathways of pyrimidine ribonucleotides
487	LCA_LACTOBACILLUS_SAKEI_RS01805_|_preprotein_translocase_subunit_YajC_|_383023:383379_Forward		Secretion
488	LCA_LACTOBACILLUS_SAKEI_RS02560_|_protein_translocase_subunit_SecA_|_512874:515237_Forward		Secretion
489	LCA_LACTOBACILLUS_SAKEI_RS02565_|_peptide_chain_release_factor_2_|_515425:516423_Forward		Secretion
490	LCA_LACTOBACILLUS_SAKEI_RS03585_|_signal_recognition_particle-docking_protein_FtsY_|_712033:713496_Forward		Secretion
491	LCA_LACTOBACILLUS_SAKEI_RS03605_|_DNA-binding_protein_|_717276:717617_Forward		Secretion
492	LCA_LACTOBACILLUS_SAKEI_RS03610_|_signal_recognition_particle_protein_|_717642:719078_Forward		Secretion
493	LCA_LACTOBACILLUS_SAKEI_RS04745_|_signal_peptidase_II_|_935242:935697_Forward		Secretion
494	LCA_LACTOBACILLUS_SAKEI_RS09445_|_hypothetical_protein_|_1882883:1883662_Reverse		Secretion system?
495	LCA_LACTOBACILLUS_SAKEI_RS03500_|_protein_kinase_|_689929:691857_Forward	2.7.11.1	serine/threonine kinase of unknown function
496	LCA_LACTOBACILLUS_SAKEI_RS03495_|_protein_phosphatase_|_689186:689932_Forward	3.1.3.16	serine/threonine phosphatase of unknown function
497	LCA_LACTOBACILLUS_SAKEI_RS01295_|_serine/threonine_protein_phosphatase_|_269925:270722_Reverse	3.1.3.48	Serine/tyrosine protein phosphatase
498	LCA_LACTOBACILLUS_SAKEI_RS06340_|_1257719:1260031_Reverse		single-stranded-DNA-specific exonuclease RecJ, DNA recombination and repair
499	LCA_LACTOBACILLUS_SAKEI_RS01480_|_L-cystine_transporter_tcyP_|_314604:316001_Forward		sodium-cystine symporter
500	LCA_LACTOBACILLUS_SAKEI_RS00185_|_universal_stress_protein_UspA_|_36687:37160_Forward		Stress response
501	LCA_LACTOBACILLUS_SAKEI_RS00205_|_universal_stress_protein_A_|_39487:39954_Forward		Stress response
502	LCA_LACTOBACILLUS_SAKEI_RS01155_|_universal_stress_protein_UspA_|_243064:243498_Forward		Stress response
503	LCA_LACTOBACILLUS_SAKEI_RS01705_|_co-chaperone_GroES_|_358364:358648_Forward		Stress response
504	LCA_LACTOBACILLUS_SAKEI_RS01710_|_groEL_|_molecular_chaperone_GroEL_|_358702:360327_Forward		Stress response
505	LCA_LACTOBACILLUS_SAKEI_RS01975_|_general_stress_protein_|_419531:419965_Forward		Stress response
506	LCA_LACTOBACILLUS_SAKEI_RS02090_|_general_stress_protein_|_444373:444750_Forward		Stress response
507	LCA_LACTOBACILLUS_SAKEI_RS03410_|_alkaline-shock_protein_|_673968:674411_Forward		Stress response
508	LCA_LACTOBACILLUS_SAKEI_RS08445_|_glycine/betaine_ABC_transporter_|_1676360:1677202_Reverse		Stress response (osmoprotection)
509	LCA_LACTOBACILLUS_SAKEI_RS00795_|_alkaline-shock_protein_|_160758:161186_Forward		Stress response protein
510	LCA_LACTOBACILLUS_SAKEI_RS07270_|_ATP-dependent_Clp_protease_ATP-binding_subunit_|_1440481:1442646_Forward		Stress response, ATPase/chaperone ClpE, specificity factor for ClpP protease
511	LCA_LACTOBACILLUS_SAKEI_RS05745_|_cold-shock_protein_|_1142484:1142684_Reverse		Stress response, cold shok protein CspC
512	LCA_LACTOBACILLUS_SAKEI_RS06250_|_1239741:1241018_Reverse		Stress response; inner membrane zinc metalloprotease required for the extracytoplasmic stress response mediated by sigma(E) (YaeL)
513	LCA_LACTOBACILLUS_SAKEI_RS00865_|_sodium:solute_symporter_|_176864:178279_Forward		sugar:cation symporter
514	LCA_LACTOBACILLUS_SAKEI_RS03385_|_TetR_family_transcriptional_regulator_|_671315:671935_Forward		TetR family transcriptional regulator of unknown function
515	LCA_LACTOBACILLUS_SAKEI_RS03515_|_thiamine_pyrophosphokinase_|_693484:694134_Forward	2.7.6.2	thiamine salvage cofactor synthesis
516	LCA_LACTOBACILLUS_SAKEI_RS03220_|_thiol_reductase_thioredoxin_|_636150:636470_Forward		Thioredoxine LSA0634 Redox
517	LCA_LACTOBACILLUS_SAKEI_RS02080_|_Ferredoxin--NADP_reductase_2_|_442510:443496_Reverse	1.8.1.9	Thioredoxine reductase/Redox
518	LCA_LACTOBACILLUS_SAKEI_RS01885_|_thiol_reductase_thioredoxin_|_403233:403544_Forward		Thioredoxine/ redox
519	LCA_LACTOBACILLUS_SAKEI_RS05005_|_thymidylate_synthase_|_992134:993084_Reverse	2.1.1.45	thymidylate synthase, purines pyrimidines metabolism
520	LCA_LACTOBACILLUS_SAKEI_RS03090_|_eno_|_enolase_|_609932:611227_Forward	5.3.1.1	TPI Triose phosphate isomerase glycolysis
521	LCA_LACTOBACILLUS_SAKEI_RS04440_|_871347:872456_Forward		Transcription
522	LCA_LACTOBACILLUS_SAKEI_RS08010_|_transcription-repair_coupling_factor_|_1588482:1592003_Reverse		Transcription

523	LCA_LACTOBACILLUS_SAKEI_RS08360_|_DNA-directed_RNA_polymerase_subunit_sigma_|_1658326:1658886_Reverse		Transcription
524	LCA_LACTOBACILLUS_SAKEI_RS05440_|_hypothetical_protein_|_1087273:1087485_Forward		Transcription, omega 1 subunit of RNA polymerase
525	LCA_LACTOBACILLUS_SAKEI_RS06955_|_helicase_DnaB_|_1368080:1369459_Reverse		Transcription/replication
526	LCA_LACTOBACILLUS_SAKEI_RS01860_|_crossover_junction_endodeoxyribonuclease_RuvA_|_397932:398375_Forward		Transcription/translation
527	LCA_LACTOBACILLUS_SAKEI_RS06220_|_transcription_termination/antitermination_protein_NusA_|_1229357:1230574_Reverse		Transcription; transcription elongation factor NusA
528	LCA_LACTOBACILLUS_SAKEI_RS05140_|_Fur_family_transcriptional_regulator_|_1017727:1018170_Reverse		Transcriptional regulator for iron transport and metabolism
529	LCA_LACTOBACILLUS_SAKEI_RS01350_|_LytR_family_transcriptional_regulator_|_282718:283905_Forward		Transcriptional regulator LytR family, transcriptional attenuator
530	LCA_LACTOBACILLUS_SAKEI_RS08240_|_MarR_family_transcriptional_regulator_|_1637883:1638281_Reverse		Transcriptional regulator MarR-type, unknown target
531	LCA_LACTOBACILLUS_SAKEI_RS08230_|_pur_operon_repressor_|_1635725:1636561_Reverse		transcriptional regulator of the purine biosynthesis operon
532	LCA_LACTOBACILLUS_SAKEI_RS01390_|_transcriptional_regulator_|_292531:292842_Reverse		Transcriptional regulator of unknown function
533	LCA_LACTOBACILLUS_SAKEI_RS03960_|_Crp/Fnr_family_transcriptional_regulator_|_777460:778125_Reverse		Transcriptional regulator, Anaerobic regulatory protein
534	LCA_LACTOBACILLUS_SAKEI_RS07885_|_GntR_family_transcriptional_regulator_|_1564019:1564720_Reverse		transcriptional regulator, gntR family, unknown target
535	LCA_LACTOBACILLUS_SAKEI_RS07520_|_transcriptional_regulator_|_1487955:1488926_Reverse		Transcriptional regulator, unknown target
536	LCA_LACTOBACILLUS_SAKEI_RS09135_|_1814544:1815059_Reverse		Transcriptional regulator, unknown target
537	LCA_LACTOBACILLUS_SAKEI_RS00035_|_30S_ribosomal_protein_S6_|_9348:9644_Forward		Translation
538	LCA_LACTOBACILLUS_SAKEI_RS00045_|_30S_ribosomal_protein_S18_|_10226:10465_Forward		Translation
539	LCA_LACTOBACILLUS_SAKEI_RS00055_|_50S_ribosomal_protein_L9_|_12737:13189_Forward		Translation
540	LCA_LACTOBACILLUS_SAKEI_RS00400_|_23S_rRNA_(pseudouridine(1915)-N(3))-methyltransferase_RlmH_|_79985:80464_Forward		Translation
541	LCA_LACTOBACILLUS_SAKEI_RS01175_|_elongation_factor_P_|_246457:247020_Forward		Translation
542	LCA_LACTOBACILLUS_SAKEI_RS01375_|_glutamate--tRNA_ligase_|_288994:290481_Forward		Translation
543	LCA_LACTOBACILLUS_SAKEI_RS01755_|_tRNA_preQ1(34)_S-adenosylmethionine_ribosyltransferase-isomerase_QueA_|_371551:372582_Forward		Translation
544	LCA_LACTOBACILLUS_SAKEI_RS02485_|_tRNA_(cytidine(34)-2'-O)-methyltransferase_|_496199:496708_Forward		Translation
545	LCA_LACTOBACILLUS_SAKEI_RS03225_|_DSBA_oxidoreductase_|_636489:637118_Forward		Translation
546	LCA_LACTOBACILLUS_SAKEI_RS03390_|_50S_ribosomal_protein_L21_|_672083:672391_Forward		Translation
547	LCA_LACTOBACILLUS_SAKEI_RS03400_|_50S_ribosomal_protein_L27_|_672776:673063_Forward		Translation
548	LCA_LACTOBACILLUS_SAKEI_RS03405_|_elongation_factor_P_|_673365:673922_Forward		Translation
549	LCA_LACTOBACILLUS_SAKEI_RS03505_|_691897:692811_Forward		Translation
550	LCA_LACTOBACILLUS_SAKEI_RS03520_|_50S_ribosomal_protein_L28_|_694487:694672_Reverse		Translation
551	LCA_LACTOBACILLUS_SAKEI_RS03615_|_30S_ribosomal_protein_S16_|_719171:719446_Forward		Translation
552	LCA_LACTOBACILLUS_SAKEI_RS03620_|_719456:719698_Forward		Translation
553	LCA_LACTOBACILLUS_SAKEI_RS03630_|_tRNA_(guanosine(37)-N1)-methyltransferase_TrmD_|_720284:721024_Forward		Translation
554	LCA_LACTOBACILLUS_SAKEI_RS03635_|_50S_ribosomal_protein_L19_|_721143:721490_Forward		Translation
555	LCA_LACTOBACILLUS_SAKEI_RS03760_|_737357:738313_Forward		Translation
556	LCA_LACTOBACILLUS_SAKEI_RS03825_|_ileS_|_isoleucine--tRNA_ligase_|_750528:753314_Forward		Translation
557	LCA_LACTOBACILLUS_SAKEI_RS04010_|_tRNA(5-methylaminomethyl-2-thiouridine)-_methyltransferase_|_785587:786690_Forward		Translation
558	LCA_LACTOBACILLUS_SAKEI_RS04245_|_30S_ribosomal_protein_S4_|_829520:830125_Reverse		Translation
559	LCA_LACTOBACILLUS_SAKEI_RS04260_|_tRNA_sulfurtransferase_ThiI_|_833543:834760_Forward		Translation
560	LCA_LACTOBACILLUS_SAKEI_RS04270_|_valS_|_valine--tRNA_ligase_|_835833:838481_Forward		Translation
561	LCA_LACTOBACILLUS_SAKEI_RS04340_|_histidine--tRNA_ligase_|_851270:852574_Forward		Translation
562	LCA_LACTOBACILLUS_SAKEI_RS04345_|_aspS_|_aspartate--tRNA_ligase_|_852574:854346_Forward		Translation
563	LCA_LACTOBACILLUS_SAKEI_RS04360_|_membrane_protein_|_855486:856364_Forward		Translation
564	LCA_LACTOBACILLUS_SAKEI_RS04385_|_30S_ribosomal_protein_S21_|_861130:861306_Forward		Translation
565	LCA_LACTOBACILLUS_SAKEI_RS04420_|_glycine--tRNA_ligase_subunit_alpha_|_865906:866823_Forward		Translation
566	LCA_LACTOBACILLUS_SAKEI_RS04425_|_glycine--tRNA_ligase_subunit_beta_|_866816:868897_Forward		Translation
567	LCA_LACTOBACILLUS_SAKEI_RS04750_|_935690:936595_Forward		Translation

568	LCA_LACTOBACILLUS_SAKEI_RS04940_|_gid_|_tRNA_(uracil-5-)-methyltransferase_|_980403:981714_Reverse		Translation
569	LCA_LACTOBACILLUS_SAKEI_RS04970_|_ribosome_biogenesis_GTPase_YlqF_|_987185:988033_Reverse		Translation
570	LCA_LACTOBACILLUS_SAKEI_RS05080_|_30S_ribosomal_protein_S1_|_1007663:1008874_Reverse		Translation
571	LCA_LACTOBACILLUS_SAKEI_RS05190_|_50S_ribosomal_protein_L32_|_1029820:1030002_Reverse		Translation
572	LCA_LACTOBACILLUS_SAKEI_RS05295_|_tig_|_trigger_factor_|_1056051:1057346_Reverse		Translation
573	LCA_LACTOBACILLUS_SAKEI_RS05300_|_tuf_|_elongation_factor_Tu_|_1057557:1058747_Reverse		Translation
574	LCA_LACTOBACILLUS_SAKEI_RS05310_|_1060006:1061724_Reverse		Translation
575	LCA_LACTOBACILLUS_SAKEI_RS05315_|_30S_ribosomal_protein_S15_|_1061953:1062222_Reverse		Translation
576	LCA_LACTOBACILLUS_SAKEI_RS05320_|_30S_ribosomal_protein_S20_|_1062501:1062755_Forward		Translation
577	LCA_LACTOBACILLUS_SAKEI_RS05355_|_1068996:1069550_Reverse		Translation
578	LCA_LACTOBACILLUS_SAKEI_RS05445_|_1087490:1089178_Forward		Translation
579	LCA_LACTOBACILLUS_SAKEI_RS05655_|_translation_factor_Sua5_|_1122560:1123588_Reverse		Translation
580	LCA_LACTOBACILLUS_SAKEI_RS05665_|_peptide_chain_release_factor_1_|_1124465:1125562_Reverse		Translation
581	LCA_LACTOBACILLUS_SAKEI_RS06180_|_tRNA_pseudouridine_synthase_B_|_1221469:1222386_Reverse		Translation
582	LCA_LACTOBACILLUS_SAKEI_RS06205_|_translation_initiation_factor_IF-2_|_1225910:1228723_Reverse		Translation
583	LCA_LACTOBACILLUS_SAKEI_RS06210_|_50S_ribosomal_protein_L7ae_|_1228741:1229046_Reverse		Translation
584	LCA_LACTOBACILLUS_SAKEI_RS06225_|_1230593:1231065_Reverse		Translation
585	LCA_LACTOBACILLUS_SAKEI_RS06245_|_proline--tRNA_ligase_|_1238006:1239715_Reverse		Translation
586	LCA_LACTOBACILLUS_SAKEI_RS06270_|_1245772:1246329_Reverse		Translation
587	LCA_LACTOBACILLUS_SAKEI_RS06280_|_elongation_factor_Ts_|_1247193:1248068_Reverse		Translation
588	LCA_LACTOBACILLUS_SAKEI_RS06405_|_elongation_factor_4_|_1268339:1270177_Reverse		Translation
589	LCA_LACTOBACILLUS_SAKEI_RS06615_|_50S_ribosomal_protein_L33_|_1301232:1301381_Reverse		Translation
590	LCA_LACTOBACILLUS_SAKEI_RS06810_|_transcription_elongation_factor_GreA_|_1341026:1341499_Reverse		Translation
591	LCA_LACTOBACILLUS_SAKEI_RS06825_|_phenylalanine--tRNA_ligase_subunit_beta_|_1343472:1345892_Reverse		Translation
592	LCA_LACTOBACILLUS_SAKEI_RS06830_|_pheS_|_phenylalanine--tRNA_ligase_subunit_alpha_|_1345896:1346942_Reverse		Translation
593	LCA_LACTOBACILLUS_SAKEI_RS06845_|_23S_rRNA_methyltransferase_|_1348279:1349040_Reverse		Translation
594	LCA_LACTOBACILLUS_SAKEI_RS06890_|_1357197:1357556_Reverse		Translation
595	LCA_LACTOBACILLUS_SAKEI_RS06910_|_1359167:1360303_Reverse		Translation
596	LCA_LACTOBACILLUS_SAKEI_RS06925_|_50S_ribosomal_protein_L20_|_1362034:1362393_Reverse		Translation
597	LCA_LACTOBACILLUS_SAKEI_RS06930_|_50S_ribosomal_protein_L35_|_1362478:1362678_Reverse		Translation
598	LCA_LACTOBACILLUS_SAKEI_RS06935_|_translation_initiation_factor_IF-3_|_1362712:1363215_Reverse		Translation
599	LCA_LACTOBACILLUS_SAKEI_RS06950_|_primosomal_protein_DnaI_|_1367154:1368080_Reverse		Translation
600	LCA_LACTOBACILLUS_SAKEI_RS07000_|_1378741:1381095_Reverse		Translation
601	LCA_LACTOBACILLUS_SAKEI_RS07045_|_arginine--tRNA_ligase_|_1389483:1391174_Reverse		Translation
602	LCA_LACTOBACILLUS_SAKEI_RS07125_|_leucine--tRNA_ligase_|_1408408:1410831_Reverse		Translation
603	LCA_LACTOBACILLUS_SAKEI_RS07290_|_peptide_chain_release_factor_3_|_1445002:1446579_Reverse		Translation
604	LCA_LACTOBACILLUS_SAKEI_RS07340_|_23S_rRNA_(uracil-5-)-methyltransferase_RumA_|_1455300:1456691_Forward		Translation
605	LCA_LACTOBACILLUS_SAKEI_RS07670_|_23S_rRNA_(uracil-5-)-methyltransferase_RumA_|_1513347:1514711_Reverse		Translation
606	LCA_LACTOBACILLUS_SAKEI_RS07685_|_gatA_|_glutamyl-tRNA(Gln)_amidotransferase_subunit_A_|_1517273:1518739_Reverse		Translation
607	LCA_LACTOBACILLUS_SAKEI_RS07690_|_asparaginyl/glutamyl-tRNA_amidotransferase_subunit_C_|_1518742:1519038_Reverse		Translation
608	LCA_LACTOBACILLUS_SAKEI_RS07960_|_lysine--tRNA_ligase_|_1577615:1579132_Reverse		Translation
609	LCA_LACTOBACILLUS_SAKEI_RS07965_|_tRNA-dihydrouridine_synthase_|_1579208:1580194_Reverse		Translation
610	LCA_LACTOBACILLUS_SAKEI_RS08115_|_50S_ribosomal_protein_L31_type_B_|_1611937:1612200_Reverse		Translation
611	LCA_LACTOBACILLUS_SAKEI_RS08135_|_DNA-directed_RNA_polymerase_subunit_delta_|_1616829:1617437_Reverse		Translation
612	LCA_LACTOBACILLUS_SAKEI_RS08265_|_methionine--tRNA_ligase_|_1641460:1643505_Reverse		Translation

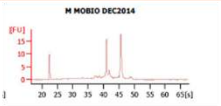
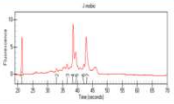
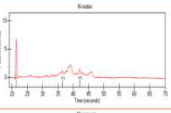
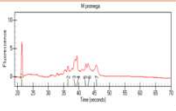
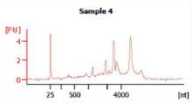
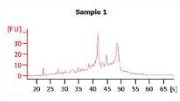
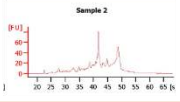
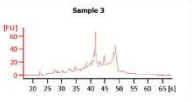
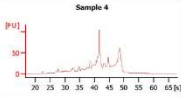
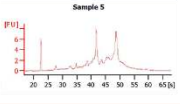
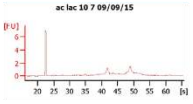
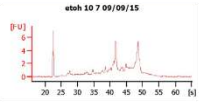
613	LCA_LACTOBACILLUS_SAKEI_RS08290_|_hypothetical_protein_|_1645708:1646670_Reverse		Translation
614	LCA_LACTOBACILLUS_SAKEI_RS08305_|_50S_ribosomal_protein_L7/L12_|_1648874:1649242_Reverse		Translation
615	LCA_LACTOBACILLUS_SAKEI_RS08310_|_50S_ribosomal_protein_L10_|_1649306:1649809_Reverse		Translation
616	LCA_LACTOBACILLUS_SAKEI_RS08335_|_50S_ribosomal_protein_L11_|_1655465:1655890_Reverse		Translation
617	LCA_LACTOBACILLUS_SAKEI_RS08355_|_50S_ribosomal_protein_L33_|_1658051:1658200_Reverse		Translation
618	LCA_LACTOBACILLUS_SAKEI_RS08375_|_1660375:1660782_Reverse		Translation
619	LCA_LACTOBACILLUS_SAKEI_RS08380_|_cysteine--tRNA_ligase_|_1660779:1662185_Reverse		Translation
620	LCA_LACTOBACILLUS_SAKEI_RS08575_|_30S_ribosomal_protein_S9_|_1705186:1705578_Reverse		Translation
621	LCA_LACTOBACILLUS_SAKEI_RS08580_|_50S_ribosomal_protein_L13_|_1705592:1706035_Reverse		Translation
622	LCA_LACTOBACILLUS_SAKEI_RS08665_|_30S_ribosomal_protein_S13_|_1725438:1725803_Reverse		Translation
623	LCA_LACTOBACILLUS_SAKEI_RS08670_|_50S_ribosomal_protein_L36_|_1725826:1725942_Reverse		Translation
624	LCA_LACTOBACILLUS_SAKEI_RS08675_|_infA_|_translation_initiation_factor_IF-1_|_1725975:1726193_Reverse		Translation
625	LCA_LACTOBACILLUS_SAKEI_RS08690_|_50S_ribosomal_protein_L15_|_1728391:1728825_Reverse		Translation
626	LCA_LACTOBACILLUS_SAKEI_RS08695_|_50S_ribosomal_protein_L30_|_1728860:1729045_Reverse		Translation
627	LCA_LACTOBACILLUS_SAKEI_RS08705_|_50S_ribosomal_protein_L18_|_1729579:1729938_Reverse		Translation
628	LCA_LACTOBACILLUS_SAKEI_RS08710_|_50S_ribosomal_protein_L6_|_1729979:1730512_Reverse		Translation
629	LCA_LACTOBACILLUS_SAKEI_RS08715_|_30S_ribosomal_protein_S8_|_1730543:1730941_Reverse		Translation
630	LCA_LACTOBACILLUS_SAKEI_RS08725_|_50S_ribosomal_protein_L24_|_1731747:1732058_Reverse		Translation
631	LCA_LACTOBACILLUS_SAKEI_RS08740_|_50S_ribosomal_protein_L29_|_1732793:1732987_Reverse		Translation
632	LCA_LACTOBACILLUS_SAKEI_RS08750_|_30S_ribosomal_protein_S3_|_1733414:1734073_Reverse		Translation
633	LCA_LACTOBACILLUS_SAKEI_RS08755_|_50S_ribosomal_protein_L22_|_1734087:1734440_Reverse		Translation
634	LCA_LACTOBACILLUS_SAKEI_RS08765_|_50S_ribosomal_protein_L2_|_1734825:1735658_Reverse		Translation
635	LCA_LACTOBACILLUS_SAKEI_RS08770_|_50S_ribosomal_protein_L23_|_1735697:1735981_Reverse		Translation
636	LCA_LACTOBACILLUS_SAKEI_RS08775_|_50S_ribosomal_protein_L4_|_1735981:1736604_Reverse		Translation
637	LCA_LACTOBACILLUS_SAKEI_RS08785_|_30S_ribosomal_protein_S10_|_1737304:1737612_Reverse		Translation
638	LCA_LACTOBACILLUS_SAKEI_RS08795_|_fusA_|_elongation_factor_G_|_1739070:1741157_Reverse		Translation
639	LCA_LACTOBACILLUS_SAKEI_RS08800_|_30S_ribosomal_protein_S7_|_1741253:1741723_Reverse		Translation
640	LCA_LACTOBACILLUS_SAKEI_RS08805_|_30S_ribosomal_protein_S12_|_1741813:1742226_Reverse		Translation
641	LCA_LACTOBACILLUS_SAKEI_RS09040_|_serine--tRNA_ligase_|_1792494:1793768_Reverse		Translation
642	LCA_LACTOBACILLUS_SAKEI_RS09425_|_tRNA_uridine(34)_5-carboxymethylaminomethyl_synthesis_GTPase_MnmE_|_1878167:1879555_Reverse		Translation
643	LCA_LACTOBACILLUS_SAKEI_RS09450_|_ribonuclease_P_protein_component_|_1883731:1884093_Reverse		Translation
644	LCA_LACTOBACILLUS_SAKEI_RS09455_|_50S_ribosomal_protein_L34_|_1884159:1884299_Reverse		Translation
645	LCA_LACTOBACILLUS_SAKEI_RS00095_|_GTP-binding_protein_YchF_|_20351:21451_Forward		Translation- GTPase interacting with 70S ribosome; ROS stress regulator
646	LCA_LACTOBACILLUS_SAKEI_RS05285_|_GTP-binding_protein_|_1053841:1054440_Reverse		Translation, GTPase involved in ribosome 50S subunit assembly (maturation of the central 50S protuberance)
647	LCA_LACTOBACILLUS_SAKEI_RS07320_|_hypothetical_protein_|_1452214:1452783_Reverse		Translation, putative tRNA binding factor
648	LCA_LACTOBACILLUS_SAKEI_RS05380_|_GTP-binding_protein_|_1072581:1074416_Reverse		Translation, ribosome-associated GTPase
649	LCA_LACTOBACILLUS_SAKEI_RS05020_|_CCA-adding_enzyme_|_995812:997005_Reverse	2.7.7.19	Translation, RNA modification
650	LCA_LACTOBACILLUS_SAKEI_RS06995_|_SsrA-binding_protein_|_1378262:1378732_Reverse		Translation, tmRNA-binding protein
651	LCA_LACTOBACILLUS_SAKEI_RS04620_|_912242:913393_Forward		Translation/RNA processing
652	LCA_LACTOBACILLUS_SAKEI_RS08685_|_preprotein_translocase_subunit_SecY_|_1727095:1728390_Reverse		Translation/secretion
653	LCA_LACTOBACILLUS_SAKEI_RS00145_|_hemolysin_|_28058:29401_Reverse		Transport protein
654	LCA_LACTOBACILLUS_SAKEI_RS06630_|_sodium:proton_antiporter_|_1306655:1308769_Reverse		Transport, Na(+)/H(+) antiporter
655	LCA_LACTOBACILLUS_SAKEI_RS01030_|_transporter_|_214032:215201_Forward		Transporter
656	LCA_LACTOBACILLUS_SAKEI_RS03985_|_glycerol_transporter_|_781764:782477_Forward		Transporter (facilitator) unknown substrate
657	LCA_LACTOBACILLUS_SAKEI_RS02450_|_AI-2E_family_transporter_|_489614:490753_Forward		Transporter unknown substrate

658	LCA_LACTOBACILLUS_SAKEI_RS08455_|_glutamate/gamma-aminobutyrate_family_transporter_YjeM_|_1678738:1680222_Reverse		Transporter, unknown substrate
659	~~LCA_LACTOBACILLUS_SAKEI_RS01005_|_integrase_|_210685:211605_Forward~~		~~Transposase~~
660	LCA_LACTOBACILLUS_SAKEI_RS04460_|_peptidase_T_|_874844:876085_Forward	3.4.11.4	Tripeptide aminopeptidase PepT
661	LCA_LACTOBACILLUS_SAKEI_RS01305_|_DNA-binding_response_regulator_|_272611:273297_Forward		Two component regulator (vanR?)
662	LCA_LACTOBACILLUS_SAKEI_RS01310_|_two-component_sensor_histidine_kinase_|_273297:274496_Forward		Two component regulator (vanR?)
663	LCA_LACTOBACILLUS_SAKEI_RS04925_|_ATP-dependent_protease_ATP-binding_subunit_HslU_|_977346:978764_Reverse		two-component ATP-dependent protease (ATPase and chaperone) ClpY
664	LCA_LACTOBACILLUS_SAKEI_RS04930_|_ATP-dependent_protease_subunit_HslV_|_978777:979322_Reverse		two-component ATP-dependent protease (N-terminal serine protease), ClpQ
665	LCA_LACTOBACILLUS_SAKEI_RS06795_|_DNA-binding_response_regulator_|_1337552:1338184_Reverse		two-component system response regulator, unknown target
666	LCA_LACTOBACILLUS_SAKEI_RS02355_|_glycosyl_transferase_|_472169:473371_Forward	2.4.1.-	UDP-Glycosyltransferase/glycogen phosphorylase family Cell wall?
667	LCA_LACTOBACILLUS_SAKEI_RS01280_|_multidrug_ABC_transporter_permease_|_265462:267255_Forward		Uncharacterized ABC transporter, 1 seule ssu surexprimée
668	LCA_LACTOBACILLUS_SAKEI_RS00855_|_amidase_|_175881:176387_Forward		Uncharacterized isochorismatase family protein
669	LCA_LACTOBACILLUS_SAKEI_RS00535_|_hypothetical_protein_|_105705:106592_Forward		Unknown
670	LCA_LACTOBACILLUS_SAKEI_RS01165_|_hypothetical_protein_|_244364:245428_Reverse		Unknown
671	LCA_LACTOBACILLUS_SAKEI_RS01170_|_hypothetical_protein_|_245582:246370_Forward		Unknown
672	~~LCA_LACTOBACILLUS_SAKEI_RS01430_|_hypothetical_protein_|_301745:302095_Forward~~		~~Unknown~~
673	~~LCA_LACTOBACILLUS_SAKEI_RS01495_|_hypothetical_protein_|_319208:319861_Forward~~		~~unknown~~
674	~~LCA_LACTOBACILLUS_SAKEI_RS01585_|_hypothetical_protein_|_338874:339218_Forward~~		Unknown
675	~~LCA_LACTOBACILLUS_SAKEI_RS01610_|_hypothetical_protein_|_342931:343185_Forward~~		~~Unknown~~
676	LCA_LACTOBACILLUS_SAKEI_RS01855_|_hypothetical_protein_|_397672:397932_Forward		Unknown
677	LCA_LACTOBACILLUS_SAKEI_RS01980_|_hypothetical_protein_|_419971:420408_Forward		Unknown
678	~~LCA_LACTOBACILLUS_SAKEI_RS02350_|_hypothetical_protein_|_471324:471755_Reverse~~		~~Unknown~~
679	~~LCA_LACTOBACILLUS_SAKEI_RS02490_|_hypothetical_protein_|_496720:497127_Forward~~		~~unknown~~
680	LCA_LACTOBACILLUS_SAKEI_RS02500_|_hypothetical_protein_|_499834:501105_Forward		Unknown
681	LCA_LACTOBACILLUS_SAKEI_RS02675_|_hypothetical_protein_|_535680:536081_Forward		Unknown
682	LCA_LACTOBACILLUS_SAKEI_RS03170_|_hypothetical_protein_|_626144:628855_Forward		unknown
683	LCA_LACTOBACILLUS_SAKEI_RS03185_|_hypothetical_protein_|_630948:631274_Reverse		unknown
684	~~LCA_LACTOBACILLUS_SAKEI_RS03240_|_membrane_protein_|_640800:641501_Forward~~		~~Unknown~~
685	LCA_LACTOBACILLUS_SAKEI_RS03395_|_hypothetical_protein_|_672406:672747_Forward		Unknown
686	LCA_LACTOBACILLUS_SAKEI_RS03530_|_hypothetical_protein_|_695401:697071_Forward		Unknown
687	LCA_LACTOBACILLUS_SAKEI_RS03705_|_hypothetical_protein_|_729437:729823_Forward		unknown
688	LCA_LACTOBACILLUS_SAKEI_RS03710_|_hypothetical_protein_|_729967:730491_Forward		unknown
689	~~LCA_LACTOBACILLUS_SAKEI_RS03965_|_hypothetical_protein_|_778359:779633_Forward~~		~~Unknown~~
690	~~LCA_LACTOBACILLUS_SAKEI_RS03975_|_hypothetical_protein_|_780424:781248_Forward~~		~~Unknown~~
691	~~LCA_LACTOBACILLUS_SAKEI_RS03980_|_hypothetical_protein_|_781230:781700_Forward~~		~~Unknown~~
692	~~LCA_LACTOBACILLUS_SAKEI_RS03990_|_hypothetical_protein_|_782498:782803_Forward~~		~~Unknown~~
693	~~LCA_LACTOBACILLUS_SAKEI_RS03995_|_TIGR00268_family_protein_|_782828:783664_Forward~~		~~Unknown~~
694	LCA_LACTOBACILLUS_SAKEI_RS04005_|_cysteine_desulfurase_|_785018:785365_Forward		Unknown
695	LCA_LACTOBACILLUS_SAKEI_RS04060_|_hypothetical_protein_|_796668:797327_Forward		Unknown
696	~~LCA_LACTOBACILLUS_SAKEI_RS04200_|_hypothetical_protein_|_820725:821699_Forward~~		~~Unknown~~
697	~~LCA_LACTOBACILLUS_SAKEI_RS04240_|_hypothetical_protein_|_828819:829424_Forward~~		~~Unknown~~
698	~~LCA_LACTOBACILLUS_SAKEI_RS04730_|_hypothetical_protein_|_932575:932991_Forward~~		~~Unknown~~
699	LCA_LACTOBACILLUS_SAKEI_RS04810_|_hypothetical_protein_|_950590:951459_Forward		Unknown
700	~~LCA_LACTOBACILLUS_SAKEI_RS04995_|_hypothetical_protein_|_990582:991427_Reverse~~		~~Unknown~~
701	~~LCA_LACTOBACILLUS_SAKEI_RS05065_|_hypothetical_protein_|_1004278:1005543_Reverse~~		~~Unknown~~
702	~~LCA_LACTOBACILLUS_SAKEI_RS05100_|_hypothetical_protein_|_1011907:1012935_Reverse~~		~~Unknown~~

703	~~LCA_LACTOBACILLUS_SAKEI_RS05130_|_hypothetical_protein_|_1016314:1016682_Reverse~~		~~Unknown~~
704	~~LCA_LACTOBACILLUS_SAKEI_RS05175_|_hypothetical_protein_|_1026404:1027279_Reverse~~		~~Unknown~~
705	~~LCA_LACTOBACILLUS_SAKEI_RS05415_|_hypothetical_protein_|_1081282:1081965_Reverse~~		~~Unknown~~
706	~~LCA_LACTOBACILLUS_SAKEI_RS05505_|_hypothetical_protein_|_1097506:1097793_Reverse~~		~~Unknown~~
707	LCA_LACTOBACILLUS_SAKEI_RS05600_|_membrane_protein_|_1113253:1113486_Reverse		Unknown
708	~~LCA_LACTOBACILLUS_SAKEI_RS05775_|_FMN_reductase_|_1145268:1146029_Reverse~~		~~Unknown~~
709	~~LCA_LACTOBACILLUS_SAKEI_RS06215_|_hypothetical_protein_|_1229043:1229342_Reverse~~		~~Unknown~~
710	~~LCA_LACTOBACILLUS_SAKEI_RS06325_|_hypothetical_protein_|_1256052:1256294_Reverse~~		~~Unknown~~
711	~~LCA_LACTOBACILLUS_SAKEI_RS06575_|_aluminum_resistance_protein_|_1295420:1296676_Reverse~~		~~Unknown~~
712	~~LCA_LACTOBACILLUS_SAKEI_RS06585_|_hypothetical_protein_|_1297727:1297906_Forward~~		~~Unknown~~
713	~~LCA_LACTOBACILLUS_SAKEI_RS06740_|_hypothetical_protein_|_1328015:1329136_Reverse~~		~~Unknown~~
714	~~LCA_LACTOBACILLUS_SAKEI_RS06875_|_hypothetical_protein_|_1354731:1355291_Reverse~~		~~Unknown~~
715	~~LCA_LACTOBACILLUS_SAKEI_RS06880_|_hypothetical_protein_|_1355285:1356460_Reverse~~		~~Unknown~~
716	~~LCA_LACTOBACILLUS_SAKEI_RS07170_|_CYTH_domain-containing_protein_|_1419015:1419596_Forward~~		~~Unknown~~
717	~~LCA_LACTOBACILLUS_SAKEI_RS07175_|_dithiol-disulfide_isomerase_|_1419674:1420318_Forward~~		~~Unknown~~
718	~~LCA_LACTOBACILLUS_SAKEI_RS07265_|_hypothetical_protein_|_1440056:1440247_Reverse~~		~~Unknown~~
719	~~LCA_LACTOBACILLUS_SAKEI_RS07285_|_hypothetical_protein_|_1443804:1444889_Forward~~		~~Unknown~~
720	~~LCA_LACTOBACILLUS_SAKEI_RS07310_|_membrane_protein_|_1449830:1450303_Forward~~		~~Unknown~~
721	~~LCA_LACTOBACILLUS_SAKEI_RS07635_|_hypothetical_protein_|_1507960:1508304_Forward~~		~~Unknown~~
722	~~LCA_LACTOBACILLUS_SAKEI_RS07790_|_hypothetical_protein_|_1539095:1539529_Reverse~~		~~Unknown~~
723	LCA_LACTOBACILLUS_SAKEI_RS07840_|_hypothetical_protein_|_1551386:1552945_Reverse		Unknown
724	LCA_LACTOBACILLUS_SAKEI_RS07855_|_hypothetical_protein_|_1555445:1556896_Reverse		Unknown
725	LCA_LACTOBACILLUS_SAKEI_RS08030_|_hypothetical_protein_|_1595161:1596540_Forward		Unknown
726	LCA_LACTOBACILLUS_SAKEI_RS08035_|_hypothetical_protein_|_1596722:1597402_Forward		Unknown
727	~~LCA_LACTOBACILLUS_SAKEI_RS08085_|_membrane_protein_|_1606162:1606728_Reverse~~		~~Unknown~~
728	~~LCA_LACTOBACILLUS_SAKEI_RS08140_|_hypothetical_protein_|_1617481:1617921_Reverse~~		~~Unknown~~
729	~~LCA_LACTOBACILLUS_SAKEI_RS08495_|_hypothetical_protein_|_1689164:1690165_Reverse~~		~~Unknown~~
730	~~LCA_LACTOBACILLUS_SAKEI_RS09045_|_hypothetical_protein_|_1794061:1795644_Reverse~~		~~Unknown~~
731	~~LCA_LACTOBACILLUS_SAKEI_RS09055_|_hypothetical_protein_|_1796583:1798175_Reverse~~		~~Unknown~~
732	~~LCA_LACTOBACILLUS_SAKEI_RS09140_|_hypothetical_protein_|_1815066:1815596_Reverse~~		~~Unknown~~
733	~~LCA_LACTOBACILLUS_SAKEI_RS09145_|_GNAT_family_acetyltransferase_|_1815693:1816220_Reverse~~		~~Unknown~~
734	~~LCA_LACTOBACILLUS_SAKEI_RS09500_|_hypothetical_protein_|_728958:729404_Forward~~		~~Unknown~~
735	~~LCA_LACTOBACILLUS_SAKEI_RS09545_|_hypothetical_protein_|_1471062:1471496_Reverse~~		~~Unknown~~
736	~~LCA_LACTOBACILLUS_SAKEI_RS09560_|_hypothetical_protein_|_1691588:1692406_Reverse~~		~~Unknown~~
737	~~LCA_LACTOBACILLUS_SAKEI_RS00050_|_hypothetical_protein_|_10683:12716_Forward~~		~~Unknown~~
738	~~LCA_LACTOBACILLUS_SAKEI_RS00100_|_hypothetical_protein_|_21469:22143_Forward~~		~~Unknown~~
739	~~LCA_LACTOBACILLUS_SAKEI_RS00195_|_hypothetical_protein_|_37871:38365_Forward~~		~~Unknown~~
740	~~LCA_LACTOBACILLUS_SAKEI_RS00785_|_hypothetical_protein_|_159980:160537_Forward~~		~~Unknown~~
741	LCA_LACTOBACILLUS_SAKEI_RS01130_|_CHAP_domain-containing_protein_|_236661:237815_Forward		Unknown cell suface protein
742	LCA_LACTOBACILLUS_SAKEI_RS03285_|_CHAP_domain-containing_protein_|_649323:650597_Forward		Unknown cell surface protein
743	~~LCA_LACTOBACILLUS_SAKEI_RS08220_|_cell_surface_protein_|_1634456:1635076_Forward~~		~~Unknown function~~
744	LCA_LACTOBACILLUS_SAKEI_RS03815_|_748650:749438_Forward		Unknown function, shape, cell division
745	LCA_LACTOBACILLUS_SAKEI_RS02690_|_hydrolase_|_539123:539755_Forward		unknown hydrolase
746	~~LCA_LACTOBACILLUS_SAKEI_RS05025_|_membrane_protein_|_997153:998025_Forward~~		~~Unknown membrane protein~~
747	LCA_LACTOBACILLUS_SAKEI_RS04390_|_hypothetical_protein_|_861331:861774_Forward		Unknown or translation

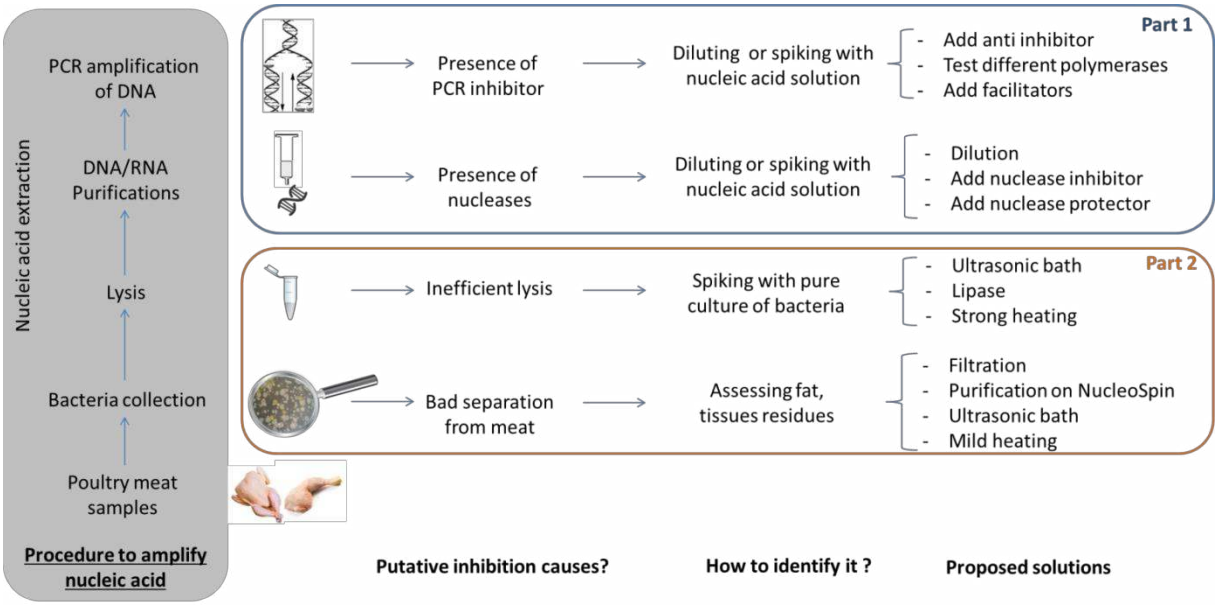
748	LCA_LACTOBACILLUS_SAKEI_RS02370_|_hypothetical_protein_|_475428:475661_Forward		unkwnown
749	LCA_LACTOBACILLUS_SAKEI_RS01985_|_dipeptidase_|_420820:421917_Reverse	3.4.13.9	Xaa Pro dipeptidase PepQ
750	LCA_LACTOBACILLUS_SAKEI_RS07735_|_xanthine_phosphoribosyltransferase_|_1530643:1531230_Reverse	2.4.2.22	Xanthine and xanthosine salvage, putine metabolism
751	LCA_LACTOBACILLUS_SAKEI_RS08080_|_zinc_metalloprotease_HtpX_|_1605249:1606148_Reverse		Zn-dependent protease with chaperone function, stress response
752	LCA_LACTOBACILLUS_SAKEI_RS00650_|_GMP_synthetase_|_132817:134370_Forward		
753	LCRIS_LACTOBACILLUS_CRISPATUS_RS02860_|_membrane_protein_|_544276:544524_Reverse		
754	LCRIS_LACTOBACILLUS_CRISPATUS_RS02865_|_integrase_|_544887:545807_Reverse		
755	RT94_PSEUDOMONAS_VIRIDIFLAVA_RS01750_|_ATP_synthase_subunit_alpha_|_164599:166143_Forward		

Annexe 2 Test d'extraction d'ARN

Date	Méthode de rinçage de la viande	Lot inoculation / Niveau de contamination (log CFU/g)	Charge bactérienne (log CFU/tube d'extraction)	Solution de protection ARN/Dilution	Culots bactérien en azote liquide	Kit d'extraction	Résultats
12/ 2014 marque 1	EtOH	M/3	7	-	+	Mobio	
12/ 2014 marque 1	EtOH	J/3	6				
12/ 2014 marque 1	EtOH	Non inoculé	5				
12/ 2014 marque 1	-	M/3	7	-	+	promega	
05/ 2015 marque 2	-	E ou U / 3	7	RNAlater – ½	-	Helsinki/Qiagen	
08/2015 marque 3	-	J/7	7	Azote liquide	-	Helsinki/Qiagen + 25 min chloroforme	
08/2015 marque 3	-	J/7	7	RNA later - ½	-	Helsinki/Qiagen + 25 min chloroforme	
08/2015 marque 3	-	J/7	7	-	+	Helsinki/Qiagen + 25 min chloroforme	
08/2015 marque 3	-	J/7	7	RNA later - ½	+	Helsinki/Qiagen + 25 min chloroforme	
08/2015 marque 3	-	J/7	7	Centrifugation différentielle	-	Helsinki/Qiagen + 25 min chloroforme	
08/2015 marque 3	-	J/7	7	Centrifugation différentielle	-	Helsinki/Qiagen + 25 min chloroforme	
08/2015 marque 3	EtOH	J/7	6	Centrifugation différentielle	-	Helsinki/Qiagen + 25 min chloroforme	

Date	Méthode de rinçage de la viande	Lot inoculation / Niveau de contamination (log CFU/g)	Charge bactérienne (log CFU/tube d'extraction)	Solution de protection ARN/Dilution	Culots bactérien en azote liquide	Kit d'extraction	Résultats
08/2015 marque 3	-	J/3	3	Centrifugation différentielle	-	Helsinki/Qiagen + 25 min chloroforme	
08/2015 marque 3	-	J/5	4	Centrifugation différentielle	-	Helsinki/Qiagen + 25 min chloroforme	
08/2015 marque 3	-	J/7	7	Centrifugation différentielle	-	Helsinki/Qiagen + 25 min chloroforme	
08/2015 marque 3	-	J/8	8	Centrifugation différentielle	-	Helsinki/Qiagen + 25 min chloroforme	
09/2015 marque 1	EtOH	M/3	7	-	+	MOBIO	
09/2015 marque 1	EtOH	M/3	7	-	+	Helsinki/Qiagen + 25 min chloroforme	
09/2015 marque 1	EtOH	Ajout culture pure/7	7	-	+	Helsinki/Qiagen + 25 min chloroforme	
10/2015 marque 4	EtOH	E ou U / 5	5	Centrifugation différentielle	+	Helsinki/Qiagen + 25 min chloroforme	
01/2016 marque 1	EtOH	E/5	5	RNAprotect pur + Centrifugation différentielle	-	Helsinki/Qiagen + 25 min chloroforme	
01/ 2016 marque 1	EtOH	E/5	5	RNA later pur + Centrifugation différentielle	-	Helsinki/Qiagen + 25 min chloroforme	
01/2016 marque 1	EtOH	E/5	5	10µl RNAse inhibitor Qiagen + Centrifugation différentielle	-	Helsinki/Qiagen + 25 min chloroforme	

Annexe 3 Schéma du projet eNABLE



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UNIVERSITE BRETAGNE LOIRE

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Thèse de Doctorat

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Description et comportement des communautés bactériennes de la viande de poulet conservée sous atmosphère protectrice

Description and behavior of bacterial communities of chicken meat samples stored under modified atmosphere packaging

Résumé

Contrôler les bactéries altérantes des aliments, notamment les produits carnés crus, est un enjeu majeur pour les industries agroalimentaires. Les conditions de stockage de la viande sous différentes atmosphères exercent une pression de sélection et modifient le comportement et le développement des communautés bactériennes initialement présentes. Des méthodes de séquençage à haut débit, utilisées pour caractériser différents écosystèmes microbiens, ont été appliquées pour étudier la dynamique des communautés bactériennes de la viande de poulet au cours du stockage.
Nous avons développé une méthode pour constituer des écosystèmes microbiens standards dont la composition a été déterminée par pyroséquençage du gène de l'ARNr 16S. La présence *de Brochothrix thermosphacta* et de *Pseudomonas* parmi les espèces dominantes a été confirmée et nous avons mis en évidence que *Shewanella* et *Carnobacterium* étaient sous dominantes. Nous avons sélectionné deux écosystèmes pour effectuer des challenges tests reproductibles sur de la viande de poulet conservée sous 3 atmosphères couramment utilisées. Une analyse métatranscriptomique et métagénomique a été réalisée afin de savoir "Quelles bactéries étaient présentes ?", "Qu'étaient-elles capables de faire?" et "Qu'exprimaient-elles?" suivant les conditions.
Nous avons ainsi pu évaluer l'impact des mélanges gazeux sur la dynamique bactérienne et les fonctions exprimées par les bactéries suivant les contaminants initiaux. Cela nous donne des pistes pour fournir des indications afin d'optimiser la conservation de la viande en contrôlant les écosystèmes microbiens.

Mots clés
Viande de poulet; Ecologie microbienne; Séquençage à haut débit; Pyroséquençage; Métatranscriptomique; Métagénomique; Atmosphère protectrice modifiée; Altération.

Abstract

Controlling spoilage microorganisms, especially in raw meat products, is challenging for the food industry. Storage conditions such as modified atmosphere packaging (MAP) have selective effects on the microbiota dynamics. Thanks to the recent development of next generation sequencing methods widely used for characterizing microbes in different ecosystems, we studied bacterial community dynamics during chicken meat storage.

We developed a method to constitute a standard meat microbial ecosystem hosting known bacterial species previously described by 16S rRNA sequencing. Our results confirmed the presence of *Brochothrix thermosphacta* and *Pseudomonas* and we also showed the presence of subdominant species as *Shewanella* and *Carnobacterium*. We selected 2 bacterial communities enabling reproducible challenge tests on meat during 9 days of storage at 4°C under 3 different atmospheres currently used in the industry. Metatranscriptomic and metagenomic analyses were performed to know "Who is there?", "What can they do?" and "What are they expressing?" depending on the gaseous mixtures and on the initial microbiota.

Consequently, we could evaluate the impact of storage atmosphere on the microbiotas dynamics and on the functions the bacteria expressed, depending on the storage condition and on the nature of the bacterial communities present. This led to indications of optimized storage conditions of poultry meat by managing their ecosystems.

Key Words
Chicken meat; Microbial ecology; Next generation sequencing; Pyrosequencing; Metatranscriptomic; Metagenomic; Modified atmosphere packaging; Spoilage.