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Isolation and identification of lactic acid bacteria from Swedish foods

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Abstract

Food fermentation is a method widely used in the past to extend the storage life of food. Numerous studies on fermented food have revealed that they not only have biopreservative properties but also health benefits. Lactic acid bacteria are the major group of microorganisms involved in food fermentation and the properties that influence food are primarily due to the compounds released from microorganisms such as organic acids and bacteriocins. Their health benefits are exerted through several mechanisms including inhibiting the growth of pathogenic bacteria and modifying the host immune response. A number of strains that have been investigated show different properties even between the same species thus emphasizing the importance of strain identification. To determine if some traditional fermented Swedish foods contain lactic acid bacteria, bacteria from four fermented Swedish foods (two surströmming and two sausages) were isolated using MRS broth. Bacterial isolates were examined for their colony and cell morphology and Gram staining and were found to be predominantly Gram-positive cocci or rods. 16S rRNA PCR amplifications of selected isolates was performed using universal prokaryotic primers and sequenced. The sequencing results showed that the bacterial isolates from Oskars surströmming Filéer and Gognacs medvurst were *Lactobacillus sakei* and the isolate from Mannerströms surströmming was *Enterococcus* sp. This study showed that the traditional Swedish fermented food evaluated did contain lactic acid bacteria.

Contents

1. Introduction

Today, the main purpose of eating food is not only to satisfy hunger but also to improve health. Through consuming certain foods, people believe they can prevent disease and live longer (Siró, et al., 2008). Thus, people are interested in added function of food products. Functional food products are a new type of processed foods, which not only have a role in supplying nutrition but also in modulating body function, and are related to health benefits (Kaur, et al., 2011; Siró, et al., 2008). People may improve their physical and mental capabilities by consuming functional foods (Urala, et al., 2007). The global functional food market was estimated to comprise about US$ 63 billion and is continuously growing (Kaur, et al., 2011).

Not every food featuring health benefits is a functional food. Thus, natural products that have positive effects on health cannot be considered as functional foods. For example, cranberries contain two compounds, fructose and proanthocyanidin that have been demonstrated to affect urinary tract infections (Raz, et al., 2004). However, cranberries are not a functional food. In order to be considered as a functional food, some additional process should be entailed (Kaur, et al., 2011). These processes divide functional foods into groups such as fortified, enriched, altered and enhanced products (Spence, 2006). A fortified product is a food fortified with certain nutrients naturally existing in the food; an enriched product is a food containing added new nutrients or components; an altered product is a food where an existing substance is replaced with another beneficial component (Siró, et al., 2008); and an enhanced product is a food supplemented with certain components through a change in growing conditions such as, new feed composition and genetic modification (Spence, 2006).

Certain functional foods can be classified into probiotic and prebiotic. Probiotic foods are defined as “live microorganisms that are proven to provide a health benefit on the host when they are consumed in sufficient amounts” by the Food and Agriculture Organization and World Health Organization expert consultation (Reid, et al., 2003). The most widely known probiotic bacteria are Lactic acid bacteria (LAB) and many studies on these bacteria have been conducted. In 1995, Gibson and Roberfroid (Gibson, et al., 1995) defined

prebiotics as non-digestible food ingredients such as starches, dietary fibers, other non-absorbable sugars, sugar alcohols, and oligosaccharides that have a positive influence on the host. Stimulating the growth and/or activity of existing bacteria is the main mechanism of this benefit (Kaur, et al., 2011). Investigating the existence of microorganisms in certain foods and clear identification of the strains are prerequisites for being employed in the potential industrial application as a biopreservative and probiotic product.

2. Lactic acid bacteria

A large number of microorganisms such as bacteria, fungi, parasites and viruses exist on skin and mucosal surfaces of spinal animals. About 10^{14} microorganisms colonize the mammalian gut to form the so called microbiota, and these microorganisms contribute to their health (Kamada, et al., 2013). Among these microorganisms, LAB have been shown to interrelate with body and food. Soil and plants are the hypothetical first niche of the ancestral LAB, however over time, the niche of LAB had moved to the gut of grass eating animals (Quinto, et al., 2014). Transiting niche from soil and plants to the animal intestine requires three areas of genomic adaptation: resistance to host barriers, sticking to intestinal cells, and fermentation of some substrates in the gut (Quinto, et al., 2014). This adaptation leads LAB to develop defenses against stress which allow them to survive harsh conditions and sudden environmental changes (van de Guchte, et al., 2002).

Lactic acid fermentation is the transformation of sugar into lactic acid. Fermentation as a means of food preservation has been used for centuries. The fermentation process also improves nutritional value of perishable foods such as dairy products, vegetables, meat and fish (Sarao, et al., 2015) and LAB are the primary organisms that producing this fermentation process. Lactic acid is the main end product that LAB produce during carbohydrate metabolism. Generally, bacteria that belong to this group are Gram positive, immobile, catalase negative, non-sporulating, anaerobic or facultative aerobic cocci or rods (Fhoula, et al., 2013). *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* are referred to typical LAB genera (Quinto, et al., 2014).

LAB are considered as the most general and significant starter cultures used in fermented dairy products. The microflora of raw milk often contains LAB; however, inoculating LAB during manufacturing process is more common since they influence dairy products in numerous ways. Controlling pH during milk ripening is the initial function of LAB via conversion of naturally occurring lactose in milk to lactic acid (glycolysis). Through this conversion, pH is reduced quickly in fermented dairy product. Since only acid-tolerant bacteria can survive in such an environment, it leads to the control of non-starter microflora originating from the surrounding environment (Hickey, et al., 2015). Secondly, LAB are involved in developing dairy products' flavor during fermentation. Developing cheese flavor is concomitant of the microbial breakdown and subsequent transformation of the degradation products of the carbohydrate, fat, and protein components existing initially in the curd (Williams, et al., 1997). Three main biochemical pathways including glycolysis (lactose), lipolysis (fat) and proteolysis (caseins) are involved in this flavor development (Smit, et al., 2005). Glycolysis is the pathway which converts glucose into pyruvate, lactic acid or ethanol. Breakdown of lipids is called lipolysis. In this process, the triglycerides are converted into glycerol and free fatty acids through hydrolysis. Proteolysis is the breakdown of protein into polypeptides or amino acids (Hickey, et al., 2015). Several enzymes are needed in these pathways and their primary source is the starter cultures used in fermentation (Smit, et al., 2005). Examples of typical LAB used in the dairy industry are: *Lactococcus lactis* spp. *lactis, Lactococcus lactis* spp. *cremoris* (Cheddar), *Lactobacillus helveticus*, *Lactobacillus delbrueckii* spp. *bulgaricus* and *Lactobacillus casei* (Swiss-/Italian-type cheese), *Streptococcus thermophilus* (Swiss-type cheese/yogurts) and *Lactobacillus acidophilus* (yogurts, soured creams) (Leroy, et al., 2004). Non-starter LAB can be derived from the manufacturing environment or from raw milk, where they are present as contaminants (Hickey, et al., 2015). Some non-starter LAB - illustrated as adventitious species - can potentially contribute to the overall maturation process and deeper flavor in fermented dairy product by acting as a source of enzymes involved in glycolysis, lipolysis and proteolysis (Williams, et al., 1997). For example, cheddar cheese made from aseptic cheese vats and specific starter strains is considered to lack the full mature flavor (Crow, et al., 2001). Non-starter LAB develop the flavor in dairy foods as well as be competitive against other bacteria (Hickey, et al., 2015). Competitive inhibition makes it possible to

achieve further control of bacteria. Their competitive inhibition allows non-starter bacteria to remain and this enhances flavor (Crow, et al., 2001). The most frequent non-starter LAB species found in the dairy industry include *Lactobacillus casei, L. paracasei, L. plantarum, L. curvatus, L. brevis, L. fermentum*, *Enterococcus durans, Pediococcus acidilactici, P. pentosaceus, E. faecalis*, and *E. faecium* (Settanni, et al., 2010).

Bifidobacteria are among the widespread group of culturable anaerobic bacteria within the human and animal gastrointestinal tract (Pokusaeva, et al., 2011). They are Gram-positive, heterofermentative, non-motile, non-spore forming, high GC and catalase-negative anaerobic microorganisms. Since they also produce lactic acid as one of their main fermentation products, they belong to LAB group (Martinez, et al., 2013). Bifidobacteria also have health-promoting properties such as, protection against pathogens by releasing antimicrobial agents (e.g. bacteriocins) and/or blocking pathogen adhesion, and modulation of the immune response. Bifidobacteria and lactobacilli do not include pathogenic species and protection against infection might be assumed to be imparted through their dominance in the faeces of breast-fed babies (Pokusaeva, et al., 2011). These properties allow both genera to be regarded as the main potentially health-enhancing bacteria. Some dairy products include bifidobacteria, especially fermented milk, and they usually have a significantly lower growth-rate than the starter culture. Their proliferation, however, would contribute to increased levels of lactate and acetate in the end products (Fernández, et al., 2015). Bifidobacteria have saccharolytic abilities like most intestinal bacteria, and are believed to be important to the fermentation of carbohydrates in the colon. They are able to ferment many kinds of complex carbon sources such as gastric mucin, xylo-oligosaccharides, (trans)-galactooligosaccharides, soy bean oligosaccharides, malto-oligosaccharides, fructo-oligosaccharides, pectin and other plant derived-oligosaccharides, and their ability to metabolize certain carbohydrates is species- and strain-dependent (Pokusaeva, et al., 2011).

2.1Lactic acid bacterial bacteriocins

LAB contribute to food preservation and safety due to the production of one or more active metabolites with antimicrobial properties. One of these properties is the ability to reduce

the pH of the media with organic acids (Sobrino-López, et al., 2008). Antimicrobial ability by organic acid such as lactic acid, acetic acid and propionic acid is also highly related to bacterial cytoplasmic membrane. The acids show antimicrobial ability by interfering with the membrane potential and inhibiting active transport (Caplice, et al, 1999). They also produce certain low-molecular mass protein or peptides, called bacteriocins that are involved in inhibiting the growth of spoilage bacteria and pathogens in foods (Hickey, et al., 2015). Bacteriocin also allows the producing strain a competitive advantage in the food environment. Its antimicrobial activity has less effect against Gram-negative bacteria compared to Gram-positive bacteria due to outer membrane which protective barrier provided by lipopolysaccharide (Caplice, et al, 1999).

2.1.1 Classification of bacteriocins

Bacteriocins are antimicrobial peptides first characterized in Gram-negative bacteria. Colicin, a bacteriocin released by *E. coli*, has been the most studied and it kills bacteria by various mechanisms such as inhibiting cell wall synthesis or permeabilizing the target cell membrane (Kim, et al., 2014). Among the Gram-positive bacteria, bacteriocins produced by LAB have been the most studied and utilized in food (Cleveland, et al., 2001). Bacteriocins originated from LAB can be organized into several classes by molecular weight and chemistry. Class I LAB bacteriocins known as lantibiotics, contain lanthionine and β-lanthionine (Abee, et al., 1995). Class II LAB bacteriocins are small heat stable peptides (<10 kDa) excluding lanthionine residues (Abee, et al., 1995; Drider, et al., 2006). Class III LAB bacteriocins are large heat unstable proteins (>30 kDa) and class IV LAB bacteriocins are complex bacteriocins associated with carbohydrate or lipid moieties (Abee, et al., 1995; Holzapfel, et al., 1995).

2.1.1.1 Class I bacteriocins

Class I LAB bacteriocins are the best studied bacteriocin group. They are small (<5 kDa) heat stable peptides that are widely modified after translation, forming particular thioether amino acids, lanthionine and methyl-lanthionine (Deegan, et al., 2006). Lantibiotics are derived from an abbreviation of lanthionine-containing antibiotic peptides. They are produced by large numbers of Gram-positive bacteria and have their lanthionines imbedded

within cyclic peptides. They usually contain a methyl-substituted lanthionine derivative, (2S,3S,6R)-3-methyllanthionine. They also typically – but not always - have the unsaturated amino acids 2,3-didehydroalanine and (Z)-2,3-didehydrobutyrine that are derived from serine and threonine residues (Chatterjee, et al., 2005). Based on structural similarities, the LAB lantibiotics can be classified into two subclasses. Subclass Ia Includes the prototype lantibiotic nisin which has been used for food preservation in more than 80 countries (Caplice, et al, 1999). This subclass contains flexible, elongated and positively charged peptides, which usually act on cytoplasmic membranes by creating pores (Martinez, et al., 2013). Subclass Ib peptides are either negatively charged or have no net charge. They interfere with the important enzymatic reactions of susceptible bacteria, bind to the lipid II, peptidoglycan precursor molecule, and inhibit cell wall biosynthesis (Deegan, et al., 2006).

The most well-known Class I bacteriocin is nisin which has been generally recognized as safe for use as a direct human food ingredient (Caplice, et al, 1999). Thus, it has been widely used in a large number of fresh and processed foods as a biopreservative. It is approved by the European Commission, the Food and Drug Administration in the USA and the World Health Organization through extensive studies, and commercialized as a dried concentrated powder (Sobrino-López, et al., 2008). It is a peptide that consists of 34 amino acid residues and its name is derived from Lancefield Group N inhibitory substance, the initial classification of the compound (Chatterjee, et al., 2005). It is produced by several strains of *Lactococcus lactis*. Each bacteriocin has a range of inhibitory activity against microorganisms and nisin is active against a broad variety of bacteria including *Lactococcus, Streptococcus, Staphylococcus, Listeria* and *Mycobacterium*, as well as the vegetative cells and outgrowing spores of *Bacillus* and *Clostridium* species (McAuliffe, et al., 2001). *Bacillus* and *Clostridium* spp. are Gram-positive spores that are influenced by nisin, with spores being more susceptible than vegetative cells. When the spores become more sensitive to nisin, they are more easily damaged by heat. This is an important factor when nisin is used as a food preservative in heat processed foods (de Arauz, et al., 2009). However, many Gram-positive bacteria have been demonstrated to have resistance to nisin because of their ability to synthesize a nisin inactivating enzyme, nisinase (Abee, et al., 1995). Generally Gram-negative bacteria, fungi and virus are rarely affected by the bacteriocin nisin (de Arauz, et al.,

2009). Nisin A and nisin Z are well-known forms of nisin and they are sorted by a single amino acid at position 27, which is His in nisin A and Asn in nisin Z. Another natural form of nisin, nisin Q, was also isolated from *L. lactis* and differs at four amino acid positions (Val 15, Leu 21, Asn 27, and Val 30) from nisin A (Ala 15, Met 21, His 27, and Ile 30) (Chatterjee, et al., 2005).

The major target for nisin is the cytoplasmic membrane of the bacterial cell and the action of nisin is highly related to permeabilization of bacterial cell membranes (Abee, et al., 1995). Nisin causes cell death by forming pores, which disrupt the proton motive force and the pH equilibrium. It causes ions to leak and stimulates hydrolysis of ATP (de Arauz, et al., 2009). Furthermore, nisin inhibits cell wall biosynthesis by binding with lipid II, a peptidoglycan precursor. This interference toward cell wall biosynthesis is also an inherent property of nisin forming pores (Deegan, et al., 2006). Treatment with nisin causes membrane depolarization in a voltage dependent manner (Chatterjee, et al., 2005). Nisin A and Z showed increased activity at acidic pH values and caused permeabilization of the membranes at membrane potentials that were very low and even completely missing. When negative potential is applied, transient pores can be formed by nisin in black lipid membranes (planar lipid bilayers) and their diameters range from 0.2 to 1.2 nm. They allow hydrophilic solutes to pass if the solutes are below 0.5 kDa. Therefore, nisin A and Z can induce leakage of ions and ATP from target cells and this is called the barrel-stave mechanism (Abee, et al., 1995). The phospholipid composition of the membrane strongly influences the efficiency of pore formation and this is an important feature in Gram-positive bacteria which have a high content of anionic lipids in their membrane (Chatterjee, et al., 2005). There are other mechanism models, barrel-stave model and wedge model, used to describe the mode of action. Insertion and pore formation is enhanced (barrel-stave model) or mediated (wedge model) by proton motive force components (Bauer, et al., 2005). During the barrel-stave mechanism, the cationic lantibiotic monomer (the stave) binds to the membrane surface through electrostatic attraction, and pores (barrel) are formed at a certain membrane potential in which the nisin estimates a position perpendicular to the membrane. In contrast, surface bound nisin molecules bind parallel to the membrane in the wedge mechanism. Biding of the nisin molecules produces local strain which can cause

bending of the membrane (Chatterjee, et al., 2005). The local perturbation of the lipid bilayer causes pore formation (Bauer, et al., 2005).

2.1.1.2 Class II bacteriocins

Class II LAB bacteriocins are small, hydrophobic membrane-active peptides and include unmodified bacteriocins that can be divided into three subclasses - class IIa, IIb and IIc. Class IIa is a pediocin-like bacteriocin including pediocin PA-1/AcH, class IIb is a two peptide bacteriocin, and class IIc includes non-pediocin-like bacteriocins and one-peptide bacteriocins (Drider, et al., 2006). Class II LAB bacteriocins are considered to form amphiphatic helices with changeable amounts of hydrophobicity and β-sheet structure (Abee, et al., 1995). Pediocin PA-1 is the best studied bacteriocin among class II LAB bacteriocins and originated from *Pediococcus acidilactici*. Pediocin AcH is also a well-known class II bacteriocin belonging to class II (Deegan, et al., 2006). Both bacteriocins share sequence similar to other important anti-listerial bacteriocins such as Sakacin A and P, Leucocin A, and Carnobacteriocin BM1 and B2 produced by LAB associated with meat (Abee, et al., 1995). These two bacteriocins induce leakage of K^+, amino acids, and other molecules that have low molecular weight in sensitive cells and rapid exhaustion of intracellular ATP in these bacteria. If carboxyfluorescein-loaded vesicles derived from membranes bind with pediocin PA-1/AcH, the vesicles become leaky. Furthermore, class IIa bacteriocins promote permeabilization of the target cell membrane by forming ion-selective hydrophilic pores which disrupt the proton motive force and exhaust intracellular ATP (Drider, et al., 2006). Class IIb includes lactacin F and lactococcin G. This subgroup refers to two-component bacteriocins that require two peptides to work synergistically (Deegan, et al., 2006).

2.1.1.3 Class III and class IV bacteriocins

Class III LAB bacteriocins are large (>30 kDa) and heat unstable proteins. Helveticin J belongs to class III bacteriocin produced by *Lactobacillus helveticus* 481. This antimicrobial agent active against closely related species such as *L. helveticus* 1846 and 1244, *L. bulgaricus* 1373 and 1489, and *L. lactis* 970 (Joerger, et al., 1986). However, it is hard to use this bacteriocin as a food preservative because of its narrow activity range and heat unstable feature (Rodríguez, et al., 2003). Class IV LAB bacteriocins are large and form complexes with other

macromolecules such as carbohydrate or lipid moieties (Cleveland, et al., 2001). Sublancin 168 and glycocin F belong to this group. Glycocin F has heat and acid resistant (stable between pH 2-10 and 100 °C for 2 hours) (Stepper, et al., 2011). Both classes have not been as extensively studied as class I and class II bacteriocins.

2.1.2 Bacteriocins for food preservation and safety

It is very important to protect food from spoilage and pathogenic bacteria and help to preserve the nutritive quality of the raw material. Bacteriocin is a typical example of investigated compounds as a preservative (de Arauz, et al., 2009). Bacteriocins can be used in the preservation of different food products and the source of bacteriocins can be either as purified compounds, crude bacterial fermentates, or the bacteriocin-producing organisms (Drider, et al., 2006). They have several properties that allow them to be suitable for use as a food preservative: being recognized as safe substances in general, inactivated by digestive proteases, having little influence on the intestinal microbiota, having a relatively wide antimicrobial ability (against many food-borne pathogenic and spoilage bacteria), facilitating genetic manipulation (genetic determinants are usually plasmid-encoded), not active and nontoxic on eukaryotic cells, usually pH and heat stable and no cross resistance with antibiotics (Gálvez, et al., 2007). Although several kinds of LAB bacteriocins have been studied, usage of bacteriocins is still limited. For example, pediocin, class II LAB bacteriocins, has potential to be used as a food preservative; however, it has not been approved as antimicrobial food additives (de Arauz, et al., 2009). Nisin, class I LAB bacteriocins, have been commercialized for decades to prevent spore germination and growth of pathogenic bacteria contaminating food products. They have been widely used to make cheese (Gharsallaoui, et al., 2015). During the making of cheese, the outgrowth of butyric acid bacteria such as *Clostridium tyrobutyricum* is a common problem. Nitrate is commonly used in cheese milk as a chemical preservative in order to prevent clostridia spores and it can be replaced by nisin A. Nisin A acts as an inhibitor of *L. monocytogenes* - one of the most virulent food-borne pathogens - and inhibits the growth of pathogens in camembert cheese (Abee, et al., 1995; Richard, 1995). Furthermore, bacteriocins can be used in meat and fish products. Fermented sausages are a typical example of fermented meat products produced as a result of the lactic fermentation and formulated with nitrite (Caplice, et al, 1999).

Nitrite curing system has been widely used to extend food storage life during manufacturing of meat and fish products. However, its danger has been proposed, thus replacing the usage of nitrite is needed. In fish products, several bacteriocins such as nisin A, nisin Z and bavaricin A have been demonstrated to have the ability to serve as preservatives (Abee, et al., 1995). Many class IIa LAB bacteriocins have been investigated for a long time, however, a purified form of class IIa bacteriocins has not yet been commercialized. Most of the studies about the application of class IIa bacteriocins have focused on bacteriocin-producing cultures in foods to modulate the growth of spoilage organisms or food-borne pathogens, such as *L. monocytogenes* (Drider, et al., 2006). Therefore, several benefits can be expected when using bacteriocins as preservatives in foods. Food has longer storage life, heat-tolerance and loses less nutrients and vitamins due to less severe heat treatments. It can also decrease the risk of spreading food-borne pathogens and the use of chemical preservatives (Gálvez, et al., 2007).

2.1.3 Bacteriocins for food quality and flavor

Bacteriocins can also influence the quality and flavor of certain foods. During the cheese making process, non-starter LAB that are not added deliberately contribute to cheese in different ways. These bacteria affect the quality of cheese in a positive way; however, sometimes they can have negative effects on its quality such as off-flavors and calcium-lactate crystal formation (Guinane, et al., 2005). Lacticin 3147, bacteriocin from *Lactococcus lactis*, has been demonstrated to show significant reduction in the levels of non-starter LAB. Therefore, using bacteriocins allows making a more predictable product after cheese ripening (Ryan, et al., 1996). Bacteriocins produced by LAB also induce cell-lysis, therefore, cause and increased rate of proteolysis in cheese. This allows the cheese to take on extra-creamy texture and enhance taste and flavor (Deegan, et al., 2006). Some bacteriocins such as lactococcin ABM, class II bacteriocins, result in releasing of intracellular enzymes such as lactase dehydrogenase and post-proline dipeptidyl aminopeptidase, and accelerate cheese ripening. These enzymes act in cheese to break down the casein into small peptides and amino acids. Since amino acids play an important role as precursor compounds which are in charge of flavor, lysis is known to be advantageous for improved flavor development (Guinane, et al., 2005).

2.2Probiotic bacteria

Strains of LAB are the most common microorganisms employed as probiotics. *Lactobacillus* and *Bifidobacterium* are the most commonly investigated and widely used genera in the food industry (Sarao, et al., 2015). Likewise, *Propionibacterium, Streptococcus, Bacillus, Enterococcus, Escherichia coli*, and yeasts are also used as commercially available probiotics (Fernández, et al., 2015). Probiotic bacteria show several common properties. Their beneficial effects on health are based on the presence of selected viable bacteria. Therefore, probiotic strains must be able to survive the manufacturing process (Sarao, et al., 2015). Good manufacturing technologies must be undergirded in order to prevent losing viability and functionality of bacteria and creating unpleasant flavor or textures (Saarela, et al., 2000). Maintaining stability of the strains and the claimed abilities in the food products is essential not only during the process but also during storage (Sarao, et al., 2015). Surely probiotic bacteria should withstand gastric juices and bile. They must survive when they pass through the gastrointestinal tract and arrive alive at their site of action (Saarela, et al., 2000). Thus, they should be able to survive both acidic conditions of the stomach and alkaline conditions of the duodenum (Gupta, et al., 2009). If a probiotic adhere to intestinal mucosa, it can enhance its beneficial effects due to close contact and prolonged colonization (Sarao, et al., 2015). A dose of 5×10^9 colony forming units per day (cfu/day) is recommended for at least five days for a sufficient amount of health benefits (Gupta, et al., 2009). At least, 10^5 cfu/g should be contained in probiotic food and consumed (Sarao, et al., 2015). Probiotics must be generally recognized as safe, thus being nonpathogenic and nontoxic (Gupta, et al., 2009). Through several mechanisms, probiotics should have an ability to protect the host against pathogenic microorganisms (Saarela, et al., 2000). Probiotics have been proved to have a number of effects on the host and different bacteria strains of the same genus and species may show different effects, i.e. species- and strain-dependent. Additionally, probiotics should be deficient of transferable antibiotic resistance genes (Fernández, et al., 2015). They should also be of human origin and attach to the gut epithelium to resist the flushing effect of peristalsis. Even if the probiotic strain cannot colonize the gut, they should have the ability to persist in the intestine. Moreover, they should be able to interact or send signals

to the immune cells associated with the gut and must have the ability to influence local metabolic activity (Gupta, et al., 2009).

2.3The beneficial effects and mechanism of probiotics

The human gastrointestinal track consists of up to 10^{13}-10^{14} cells and is a complex ecosystem containing the gastrointestinal epithelium, immune cells and resident microbiota (Quinto, et al., 2014). The resident gastrointestinal microbiota provides a protective barrier against pathogenic microorganisms (Liévin-Le Moal, et al., 2006). Human gastrointestinal tract can be divided into three major sections that have its own distinct microbiota; the stomach, the small intestine, and the large intestine (Quinto, et al., 2014). The stomach is primarily populated by aerobic Gram-positive microorganisms (<10^3 cfu/g) (Biedermann, et al., 2015). The genera *Lactobacillus, Bifidobacterium, Bacteroides* and *Streptococcus* (10^3-10^4 cfu/g) are the main inhabitants in the small intestine and the large intestine is inhabited by genera *Bacteroides, Fusobacterium, Lactobacillus, Bifidobacterium* and *Eubacterium* in large numbers (10^{11}-10^{12} cfu/g) (Biedermann, et al., 2015 ;Quinto, et al., 2014). There is scientific evidence supporting the concept that the maintenance of health gut microflora may provide protection against gastrointestinal disorders including gastrointestinal infections, inflammatory bowel diseases, and cancer (Saarela, et al., 2000). Therefore, consuming probiotics may allow people to have health promoting benefits through the maintenance of health gut microflora.

Probiotics provide certain health-enhancing properties through the release of bioactive compounds as metabolites of LAB, Propionibacteria, yeast, and moulds (Fernández, et al., 2015). *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB-12 have been reported to have a beneficial effect of probiotics especially for prevention of acute diarrhea and *L. reuteri* SD2222 has been established to have a health benefit for treatment of acute diarrhea (Reid, et al., 2003). The promising health benefits of probiotics include amelioration of acute diarrhea in children, reduction of the risk of respiratory tract infections, relief of children's milk allergy/atopic dermatitis, and alleviation of irritable bowel syndrome (Fernández, et al., 2015). Probiotics are considered as exerting health-promoting properties by normalization of the host's microbiota, inhibiting the intestinal

colonization with pathogenic microorganisms, interaction with the gut associated lymphoid tissue (GALT) to modulate the immune system, and through their own metabolic activity (Quinto, et al., 2014). Although several mechanisms have been postulated, the exact molecular mechanisms of action of probiotics behind the effects are largely unknown.

2.3.1 Inhibition of pathogens

Several studies have demonstrated that specific probiotics have health beneficial properties, such as the treatment of acute diarrhea associated with rotavirus, ulcerative colitis, *Clostridium difficile*-associated diarrhea, and *Helicobacter pylori* infection (Quinto, et al., 2014). These health beneficial properties can vary considerably among different strains from the same species, i.e. often disparate and strain-specific (Ebel, et al., 2014). Several mechanisms have been proposed regarding action of probiotics: disturbing the adsorption, cell internalization of the pathogen (Fernández, et al., 2015), producing the metabolites and substances directly influencing the pathogens (Power, et al., 2014), enhancing the epithelial barrier and inhibiting pathogen adhesion to intestinal mucosa (Bermudez-Brito, et al., 2012).

Mucosal epithelial surfaces cover the gastrointestinal and respiratory tracts (Fernández, et al., 2015). The intestinal barrier is a major defense mechanism used to maintain epithelial integrity protecting the organism from the environment and is composed of the mucous layer, antimicrobial peptides, secretory Immunoglobulin A (IgA) and epithelial junction adhesion complex (Bermudez-Brito, et al., 2012). The gastrointestinal tract is unceasingly exposed to abundant microorganisms and considered as primary parts of entry for most infectious viruses (Fernández, et al., 2015). Once this barrier malfunctions, pathogenic bacteria can reach the submucosa and induce inflammatory responses, which may result in intestinal disorders (Bermudez-Brito, et al., 2012). When the probiotic bacteria adhere to a host cell they hindering the attachment of pathogens and thus, may help the host fight against infection (Fernández, et al., 2015). Consuming non-pathogenic probiotic bacteria may contribute to the prevention of intestinal disorders by enhancing the intestinal barrier and inhibiting pathogen adhesion (Bermudez-Brito, et al., 2012). Moreover, the several compounds produced by LAB contribute to inhibit pathogens. Organic acids such as lactic acid, acetic acid and propionic acid make the intestinal pH decrease, thus suppressing the

growth of pathogenic bacteria (Williams, 2010). Furthermore, they produce metabolites that can help protect the gut, and regulate intestinal motility and mucus production (Gupta, et al., 2009). Other substances released by LAB include hydrogen peroxide, bacteriocins and biosurfactants which are toxic to pathogenic bacteria (Williams, 2010).

2.3.2 Interaction with the immune system

The immune system is one of the most important parts in the body; maintaining physiological integrity and health is its main function. The immune system protects against infectious diseases and provides defense against infections caused by pathogenic microorganisms. It also modulates our body in diverse ways sometimes by up- or down-regulating the defense system (Fernández, et al., 2015). The immune system can be divided into the innate immune response and adaptive immunity (Bermudez-Brito, et al., 2012). The innate immune response is immediate and activated within minutes or hours of encountering the antigen. In contrast, the specific adaptive immunity takes days or weeks to develop and to come into effect (Rijkers, et al., 2011). The innate immune system responds to certain molecules associated with a group of pathogens so called pathogen-associated molecular patterns. They are recognized by specific receptors such as toll-like receptors. These stimulate the production of cytokines (TNF- α IFN- γ, IL-10), chemokines, and other innate effectors by modulating cytokine expression patterns through epithelial cells and/or macrophages and dendritic cells (Rijkers, et al., 2011; Bermudez-Brito, et al., 2012). The adaptive immunity depends on B and T lymphocytes, which are specific for particular antigens (Bermudez-Brito, et al., 2012). Innate immunity arranges adaptive immunity that consists of Band T lymphocytes responsible for antibody production and generation of cytotoxic T lymphocytes. The adaptive immune system's function is to create and develop an immunological memory which is able to be used as vaccination to prevent infectious diseases (Fernández, et al., 2015; Rijkers, et al., 2011). Probiotics are able to stimulate a variety of components involved in the immune system and cause innate and adaptive immune response in the host.

The human body features a multitude of natural barriers aimed at preventing infection and disease. These barriers can be divided into external and internal barriers. The external

barrier includes the largest organ in the body, skin. External microorganisms can cross the intestine wall by translocation via the epithelial layer or Peyer's patches. Native intestinal bacteria including lactobacilli can penetrate the intestinal mucous layer and are able to survive in the spleen or in other organs for many days where they stimulate phagocytic activity (Quinto, et al., 2014). Therefore, the thickness and physical state of the intestinal mucus layer and its response to orally consumed probiotics are important contributors to the immune response (Strugala, et al., 2001). Several studies have established that some probiotic strains have the ability to stimulate the immune response by promoting the phagocytic activity of lymphocytes and macrophages, i.e. probiotic bacteria have been shown to involve in immunomodulation (Williams, 2010). Lymphocytes and macrophages are representative type of white blood cells that highly contribute to the immune system. IgA plays a significant role in mucosal immunity and the secretion of IgA is stimulated by probiotics. Especially *Lactobacillus rhamnosus* strain GG has been shown to increase IgA, immunoglobulin G, and immunoglobulin M response (Gupta, et al., 2009; Williams, 2010). Many *in vitro* studies have shown that different strains and species of probiotics modulate the immune response in different ways by stimulating different effectors. Furthermore, certain strains of probiotics can provide defense against infections by inducing antiviral, cytokine and chemokine responses in the gastrointestinal and respiratory epithelial cells or immune cells (Fernández, et al., 2015). However, numerous probiotic studies *in vivo* with many strains of *Lactobacillus* have been performed and yielded ambiguous results. Positive results have been shown in treating acute infectious diarrhea and certain allergic diseases such as the prevention of atopic eczema or dermatitis (Quinto, et al., 2014).

3 Isolation LAB from Swedish foods

3.1Introduction

Fermentation has been an extensively used method to preserve food. Large numbers of studies on fermented food have revealed that they do not only possess biopreservative properties but can also yield health benefits. Surströmming, canned fermented herring, is a traditional food from northern area of Sweden. It is well-known for its unique smell, which can be described as a pungent smell of rotting fish. In order to make surströmming, salted herring is fermented for several months in barrels and thereafter canned. It does not go

through a sterilization process during manufacturing; therefore, herring are preserved by being fermented. The fish continues to ferment after canning, giving the can a swollen appearance (Kobayashi, et al., 2000b). Medvurst and isterband are traditional Swedish fermented sausages. One of the most common fermented sausages is salami and the manufacturing of fermented sausage is complex with many parameters to be considered, producing a tasteful, visually attractive and safe sausage. It takes weeks or even month to produce salami. In contrast, medvurst is a fast fermented sausage, hence, it can be produced and sold within a few days. Furthermore, it can be eaten without further cooking or heating (Nguyen, et al., 2010). The starter cultures are added during the process and usually LAB, especially *Lactobacillus* genus are the most significant microorganisms in the starter culture (Stolzenbach, et al., 2009).

LAB are a major group of microorganisms involved in food fermentation. They have been demonstrated to have positive effects on food and consumers. Swedish people are considered healthy and this may in part be due to the presence of LAB in their foods. To evaluate this hypothesis, I investigated several selecting Swedish foods and the bacteria was isolated on selective media and identified by sequencing 16S rRNA after polymerase chain reaction (PCR).

3.2Materials and methods

3.2.1 Food samples

Two brands of surströmming (Oskars surströmming Filéer and Mannerströms surströmming) were obtained from the local supermarket in Örebro, Sweden. The fish was manufactured in 2014 and the cans were opened on April 8, 2015. Moreover, two kinds of Swedish fermented sausages (Gammaldags isterband and Gognacs medvurst) were purchased April 9, 2015. Mannerströms surströmming contained the whole fish including the intestinal tract while Oskars surströmming filéer only consisted of fillets. Gammaldags isterband was purchased at the local supermarket in Örebro, Sweden, and Gognacs medvurst was purchased at the local traditional market in Örebro, Sweden.

3.2.2 Sampling and LAB isolation

The surströmming can was placed in a plastic bag and opened inside a fume hood. Microbial samples were collected using a sterile cotton swab. The cotton swab was spread on a labeled 1.5% Man, Rogosa, Sarpe (MRS) agar plate (BD Difco, USA). Each surströmming sample was spread onto two MRS agar plates and incubated at 37°C under anaerobic conditions by using a BD GasPak EZ Anaerobe Container System (BD, USA) and an anaerobic jar (Mitsubishi, Japan). Gammaldags isterband and Gognacs medvurst samples were taken by cotton swabs soaked in phosphate buffered saline solution (PBS). The cotton swab was rubbed inside the sausage and skin. Thereafter, the cotton swab was spread on each MRS agar plate and incubated at 37°C under anaerobic conditions by using the BD GasPak EZ Anaerobe Container System (BD, USA) and an anaerobic jar (Mitsubishi, Japan).

After one to two overnight cultures, 4 colonies from Gammaldags isterband and 8 colonies from each surströmming and Gognacs medvurst were selected and re-streaked onto MRS plates by sterile loop and incubated at 37°C under anaerobic conditions using the BD GasPak EZ Anaerobe Container System (BD, USA) and an anaerobic jar (Mitsubishi, Japan). The colonies were stored as bacterial glycerol stocks created by adding 4-5 colonies per each re-streaked colonies into 15% glycerol (Duchefa Biochemie, The Netherlands) in Brain Heart Infusion media (BHI) (BD, USA). The tubes were stored in a freezer at -80°C.

3.2.3 Characterization of isolates

The cultures were examined for their colony morphology, cell morphology and Gram staining. Colony morphology was examined on the plate and recorded. Cell morphology was examined by wet-mount, Gram staining and microscopy. Wet-mount was prepared by suspending bacteria in PBS on a glass slide of examined usage a light microscopy under 100x objective (ZEISS, Germany). To perform Gram staining, few colonies (2-3) were taken by sterile loop, spread on slide glass with PBS, allowed to air dry and fix using flame. The slides were placed into a jar with crystal violet for 1 min, thereafter placed into iodine solution for 1 min, and washed with alcohol for 20 sec and finally placed into safranin stain for 1 min. Between each process, the slide was washed with water.

3.2.4 Genomic DNA extraction

DNA was isolated from bacteria by taking 3-5 colonies (depending on its size) from a MRS agar plate with an overnight culture prepared from the freezer stocks. Bacteria were diluted with 1 ml of MQ water and centrifuged (Pico 17, Thermo Scientific, USA) for 1 min at 12000 rpm. The supernatant was removed and 200 ul of InstaGene matrix (Bio-Rad, USA) was added to the pellets, incubated at 56 °C for 30 min, voltexed for 10 sec, boiled in a waterbath for 8 min and voltexed again for 10 sec. The bacterial cell debris was removed by centrifugation (Pico 17, Thermo Scientific, USA) for 3 min at 12000 rpm and the genomic DNA (gDNA) was in the supernatant. Afterwards, the concentration of DNA was measured by adding 2 ul of gDNA on NanoVue Plus Spectrophotometer (GE Healthcare, UK).

3.2.5 PCR amplification of 16S rRNA

A 16S rRNA PCR amplification of extracted gDNA (20ul) was conducted with universal prokaryotic primers. A 2 ul of each primer pA (5' – AGAGTTTGATCCTGGCTCAG; nucleotide 8 to 27 of the 16S rRNA gene of *E. coli*) and pH (5' – AAGGAGGTGATCCAGCCGCA; nucleotide 1541 to 1522 of the 16S rRNA gene of *E. coli*) (Falsen, et al., 1999) were used to amplify the gDNA. Amplification mixtures were subjected to denaturation at 94°C for 5 min; 30 cycles of 95°C for 30 sec (denaturation), 55°C for 45 sec, and 72°C for 1 min (extension); followed by a final extension period of 10 min at 72°C. The PCR products were analyzed on agarose gel electrophoresis. A 1% agarose gel (Sigma-Aldrich, USA) and 4.5 ul of 10% Ethidium Bromide (Sigma-Aldrich, USA) were prepared and the agarose gel was placed in a buffer-filled case (filled with 1x TAE buffer). A volume of 5 ul of GeneRuler™ 1 kb Plus DNA Ladder (Thermo Fisher Scientific, USA) and mixture of 2 ul of 6x DNA loading dye and 8 ul of PCR reaction were loaded on each well. Electrical filed was applied via the power supply at 90 V for 40 min. The DNA bands on the gel were visualized with UV light, using the BioDoc-It Imaging System (UVP, USA).

3.2.6 DNA sequence analysis

The PCR products were purified using NucleoSpin® Extract II kit (MACHEREY-NAGEL, Germany). Quantification of DNA was performed via a NanoVue Plus Spectrophotometer (GE Healthcare, UK) with 2 ul of purified DNA on it. Before sequencing preparation, all of the

purified DNA samples were analyzed on agarose gel electrophoresis. A 1% agarose gel (Sigma-Aldrich, USA) and 4.5 ul of 10% Ethidium Bromide (Sigma-Aldrich, USA) were prepared and the agarose gel was placed in a buffer-filled case (filled with 1x TAE buffer). Thereafter, a volume of 5 ul of GeneRuler™ 1 kb Plus DNA Ladder (Thermo Fisher Scientific, USA) and mixture of 2 ul of 6x DNA loading dye and 5 ul of purified DNA sample were loaded in each well. Electrical filed was applied via the power supply at 90 V for 40 min. The DNA bands on the gel were visualized with UV light using the BioDoc-It Imaging System (UVP, USA).

Sequencing preparation was performed by adding two primers and MQ water into PCR products depending on concentration that was measured through NanoVue Plus Spectrophotometer (GE Healthcare, UK). A concentration of Oskars surströmming Filéer (C8) was 60 ng/ul, a concentration of Gognacs medvurst (C2) was 45 ng/ul and a concentration of Mannerströms surströmming (C5) was 37 ng/ul. A certain volume of each PCR product (2.5 ul, 3.3 ul, 4.1, ul), calculated by the concentration of the PCR products, 1.5 ul of primer and certain volume of MQ water (11 ul, 10.2 ul, 9.4 ul), calculated by the volume of the PCR products, were added into a sequencing tube. The reactions for sequencing were sent to Eurofins MWG Opreon, Ebersberg, Germany. Using the Basic Local Alignment Search Tool (BLAST) program from the National Center for Biotechnology Information (NCBI) database facilitates sequence similarity searches (Nguyen, et al., 2010). The sequences were aligned with the ClustalW program in SIB ExPASy Bioinformation Resource Portal (Swiss Institute of Bioinformatics, Switzerland) and then gene sequences obtained from both primers were aligned and combined.

3.3Results

A total of twenty-eight colonies were isolated and cultured from Oskars surströmming Filéer, Mannerstöms surströmming, Gammaldags isterband and Gognacs medvurst. The cultures were first characterized for their bacterial shape and Gram stain as shown in Table 1. There were small differences between colonies regarding size; however, colonies from the same food had similar shape and size. Most of the bacteria were rod-shaped and Gram-positive except for Oskars surströmming Filéer (C7) and Gognacs medvurst (C2). These two colonies

showed ambiguous color (light purple). The bacteria isolates from Mannerstöms surströmming showed the most similarity in cell morphology to *L. rhamnosus* strain GG.

Table 1. Characteristics of isolates from the samples

ID	Colony number	Cell structure	Size (x width)	Gram reaction*
L. rhamnosus strain GG	control	rod	3-4	+
Oskars surströmming Filéer	1	cocci	1-1.5	+
	2	cocci	1-1.5	+
	3	rod	2.5	+
	4	cocci	1-1.5	+
	5	cocci	1-1.5	+
	6	cocci	1-1.5	+
	7	cocci	1.5	+/-
	8	cocci	1.5	+
Mannerströms surströmming	1	rod	3-4	+
	2	rod	3-4	+
	3	rod	3-4	+
	4	rod	3-4	+
	5	rod	3-4	+
	6	rod	3-4	+
	7	rod	3-4	+
	8	rod	3-4	+
Gognacs medvurst	1	rod	2.5	+
	2	rod	2.5	+/-
	3	rod	2.5	+
	4	rod	3-4	+
	5	rod	3-4	+
	6	rod	3-4	+
	7	rod	3-4	+
	8	rod	3-4	+
Gammaldags isterband	1	rod	5	+
	2	rod	5	+
	3	rod	5	+
	4	rod	5	+

*+, positive; -, negative; +/-, undetermined

Bacteria could not be recovered from the freezing stock for Gammaldags isterband therefore PCR was only done on *L. rhamnosus* (control), Oskars surströmming Filéer (C3), Oskars surströmming Filéer (C7), Oskars surströmming Filéer (C8), Mannerströms surströmming (C1), Mannerströms surströmming (C5), Mannerströms surströmming (C7), Gognacs medvurst

(C1), Gognacs medvurst (C2) and Gognacs medvurst (C5). The PCR products were purified and Figure 1 shows the single bands of the purified PCR from all isolates. The PCR products were of the expected size, 1500 bp.

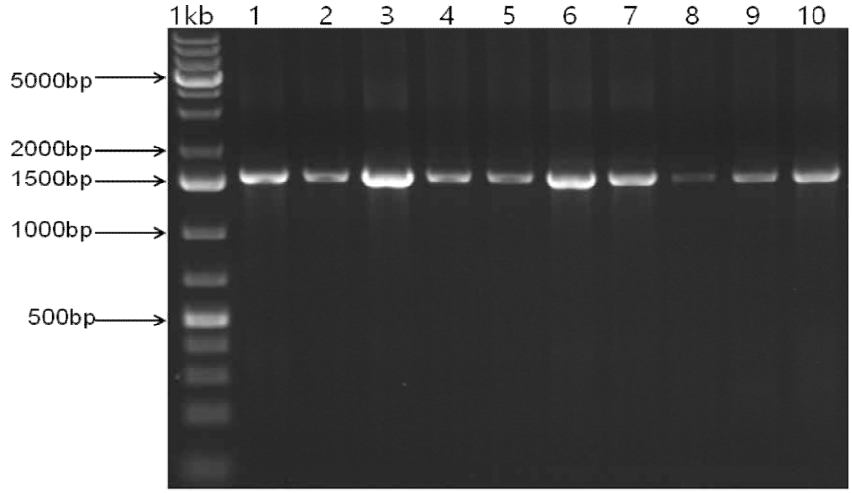

Figure 1. An agarose gel presenting purified PRC products from the isolates (1kb, 1kb Plus DNA Ladder; lane 1, *L. rhamnosus* control; lane 2, Oskars surströmming Filéer (C3); lane 3, Oskars surströmming Filéer (C8); lane 4, Gognacs medvurst (C1); lane 5, Gognacs medvurst (C5); lane 6, Oskars surströmming Filéer (C7); lane 7, Gognacs medvurst (C2); lane 8, Mannerströms surströmming (C1); lane 9, Mannerströms surströmming (C5); lane 10, Mannerströms surströmming (C7)).

One culture from each Oskars surströmming Filéer (C8), Mannerströms surströmming (C5) and Gognacs medvurst (C2) was selected for sequencing and the sequences were examined by the BLAST program from the NCBI database for sequence similarity. The sequence of the 16S rRNA region are shown in Appendix 1. Their sequence similarities were examined via BLAST and the sequence similarity of LAB isolates are shown in Table 2.

Each sequence was aligned with the ClustalW program in SIB ExPASy Bioinformation Resource Portal (Swiss Institute of Bioinformatics, Switzerland). Sequence alignments from bacterial isolates are shown in Appendix 2. Both sequences from forward and reverse primers were combined together and combined sequences are displayed in Appendix 3. Combined sequences were examined with respect to their similarity by BLAST and the sequence similarities are shown in Table 3.

Table 2. The sequence similarity of LAB isolates

ID	Primer	Size (bp)	Description	Identity
Oskars surströmming Filéer (C8)	Forward	940	*E. pseudoavium* *E. viikkiensis* *E. devriesei*	100%
Oskars surströmming Filéer (C8)	Reverse	980	*E. pseudoavium* *E. avium* *E. malodoratus* *E. gilvus* *E. pallens* *E. raffinosus*	100%
Mannerströms surströmming (C5)	Forward	747	*L. sakei*	99%
Mannerströms surströmming (C5)	Reverse	985	*L. sakei*	99%
Gognacs medvurst (C2)	Forward	928	*L. sakei*	99%
Gognacs medvurst (C2)	Reverse	989	*L. sakei*	99%

Table 3. The sequence similarity of combined gene

ID	Size (bp)	Description	Identity
Oskars surströmming Filéer (C8)	1447	*E. pseudoavium* *E. viikkiensis* *E. devriesei*	99%
Mannerströms surströmming (C5)	1473	*L. sakei*	99%
Gognacs medvurst (C2)	1292	*L. sakei*	99%

3.4Discussion

In the present study, *Lactobacillus sakei* was identified as the Gognacs medvurst isolate and Mannerströms surströmming isolate. *Lactobacillus sakei,* originally found in rice wine, belongs to LAB, however, its properties show striking differences among LAB group. *Lactobacillus sakei* shows high tolerance to a variety of environmental conditions such as low temperature, high salt concentration and diverse oxygen levels (McLeod, et al., 2008). This species has been found in several raw fermented food products including fermented plant, fish and meat, however, its primary habitat is meat. The main flora of fresh meat contains this species and it is dominant during meat fermentation (Champomier-Vergès, et al., 2001). This species plays an important role in the production process of fermented sausage and has been widely used as a biopreservative in meat and fish products. Its metabolism, which produces lactic acid and inhibition compounds against pathogenic bacteria such as *Escherichia coli* O157:H7 or spoilage bacteria, mainly contributes to act as a biopreservative (Najjari, et al., 2008; Champomier-Vergès, et al., 2001; McLeod, et al., 2008).

However, there are some different features between strains. For example, there are studies that reported some *L. sakei* strains have been shown to cause meat spoilage through releasing ropy slime and spoilage of fish products such as cold-smoked salmon and rainbow trout (Lyhs, et al., 2002; Joffraud, et al., 2001; Najjari, et al., 2008).

Another LAB species was isolated from Oskars surströmming Filéer isolate. Based on 16S rRNA sequence, the species was shown to have a 99% match with *Enterococcus pseudoavium, Enterococcus viikkiensis* and *Enterococcus devriesei*. LAB consist of a heterogeneous group of microorganisms having similar metabolic features which produces lactic acid as a major metabolite. The genus *Enterococcus* is a group which falls under the category of LAB (Yerlikaya, et al., 2011). Said genus is Gram-positive and cocci, therefore, it features the same characteristics as shown in Table 1. It can be easily detected in human and animal intestinal tracts and is also commonly found in vegetables, dairy products, animal products, water and soils, which could be a result of diffusion of fecal sources (Ogier, et al., 2008). Most of *Enterococcus* species have been shown to be tolerant in harsh environment and have the highest heat resistant species among non-sporulating bacteria, being resistant to even pasteurization temperatures. Furthermore, it has been shown to grow on different substrates, a wide-temperature range, extreme pH and salinity (Javed, et al., 2011). It is likely to exist everywhere in the environment; therefore, it is not surprising that it is found to enter food products during processing via direct or indirect contamination (Javed, et al., 2011; Ogier, et al., 2008).

When surströmming is manufactured, the Baltic Sea herring is put into strong brine and fermented. Several kinds of compounds are produced in sufficient amounts during fermentation. Especially, two organic acids, propionic acid and butyric acid, mainly contribute to its unique flavor and odor (Kobayashi, et al., 2000b). In Kobayashi's study (Kobayashi, et al., 2000b), all the isolates from the fermented herrings were identified as *Haloanaerobium praevalens* and this species is mainly in charge of producing the two organic acids. In this case, there is a species difference between the present study and Kobayashi's study (Kobayashi, et al., 2000b). This might be due to the use of different media. In the present study, the MRS broth media were used, which have been widely used as

selective media for *lactobacillus* but *Enterococcus* can also grow in MRS broth media (Khay, et al., 2012). In contrast, the anaerobic bacterial culture medium (ABCM) was used for growing *Haloanaerobium praevalens*. ABCM features a high concentration of sodium because of supplementation with 10% (w/v) NaCl while this was not the case in MRS media used in our study. *Haloanaerobium praevalens* has high intracellular salt concentration and has been isolated from various hypersaline environments (Zeikus, et al, 1983). It cannot grow in the presence of less than 5% (w/v) NaCl and the optimal salt concentration for growth of *Haloanaerobium praevalens* is 10% (w/v) (Kobayashi, et al., 2000a). Furthermore, sodium acetate, an ingredient of MRS broth, has been reported to cause a significant reduction in the competing bacteria (Ibrahim Sallam, 2007). Therefore, it would not be able to grow in the MRS broth media even if present in surströmming.

4. *General conclusion*

A large numbers of microorganisms exist in the mammalian gut and LAB have been shown to have positive effect on health and food preservation among these microorganisms (Kamada, et al., 2013). Several metabolites produced by LAB such as organic acids and bacteriocins have been demonstrated to contribute to food preservation and safety (Sobrino-López, et al., 2008). There are different effects between bacteria strains of the same genus and species (Fernández, et al., 2015). From the results presented in this study, the presence of LAB in selected Swedish foods was confirmed. Some strains from genus, *Lactobacillus* and *Enterococcus*, isolated from the three Swedish foods, have been well-known to contribute to food preservation and flavor, however, a large phenotypic diversity has been studied among strains. Therefore, identifying the strains is very important. Further studies for developing more efficient methods to identify the strains should be performed. The food preservative and health promoting property of the identified strains should be examined through several processes such as acid and bile tolerance determination, antibiotic susceptibility test, antimicrobial spectrum analysis and potential virulence factor detection (Sahoo, et al., 2015). This may help identify the best strains for the food industry.

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