ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA

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Abstract

Lactic acid bacteria (LAB) have long history of application in fermented foods as a starter cultures to produce dairy, meat, bakery and vegetable fermentation because of their beneficial influence on nutritional, organoleptic, and shelf-life characteristics. LAB are also known to produce and excrete compounds with antimicrobial activity, such as hydrogen peroxide, carbon dioxide, diacetyl, bacteriocins and bacteriocin like substances. All antimicrobial compounds can antagonize the growth of some spoilage and pathogenic bacteria in foods and have been explored in the control of most unwanted organisms. Looking for antimicrobial compounds is crucial to provide an alternative to chemical additives, offering to the market new and appealing food products. The present work aims to give an overview of the recent advances in antimicrobial activity of LAB for the production of industrially important compounds.

Key words: *lactic acid bacteria, antimicrobial activity, antimicrobial compounds, biyopreservation*

1. INTRODUCTION

Food safety is one of the major concerns in public health due to outbreaks of food-borne diseases. Consumers have become concerned about the safety of synthetic preservatives used in food. As a result, there is increasing demand for natural products that can serve as alternative food preservatives (Gyawali & Ibrahim 2014). In fermented foods, LAB displaying antimicrobial activities could be used as natural biopreservatives to prevent or inhibit the growth of pathogenic and spoilage bacteria and fungi. LAB also preserves the nutritive qualities of various foods (Ammor et. al. 2006, Ahmadova et. al. 2013). This century has been a major effect in describing, cataloging, and characterizing the wide variety of antagonistic compounds produced by LAB (Liu 2003). The preservative effect of LAB is due to the production of one or more active metabolites, such as organic acids (lactic, acetic, formic, propionic acids), that intensify their action by reducing the pH of the media, and other substances, like fatty acids, acetoin, hydrogen peroxide, diacetyl, antifungal compounds (propionate, phenyl-lactate, hydroxyphenyl-lactate, cyclic dipeptides and 3-hydroxy fatty acids), bacteriocins (nisin, reuterin, reutericyclin, pediocin, lacticin, enterocin and others) and bacteriocin-like inhibitory substances-BLIS (Favaro et. al. 2015). This paper reviews antimicrobial activity of LAB.

2. LACTIC ACID BACTERIA

LAB are gram-positive, non-sporulating, catalase-negative, aero tolerant, acid tolerant, nutritionally fastidious, strictly fermentative microorganisms which ferment sugar and produce lactic acid (El-Ghaish et al. 2010, Pringsulaka et al. 2012). Their long history of safe use commonly referred to as the GRAS (Generally Regarded As Safe) status or QPS (Qualified Presumption of Safety) status (Noreen et al. 2011). LAB were first isolated from milk (Carr et. al. 2002) and have since been found in such foods and fermented products as meat, milk products, vegetables, beverages and bakery products (Liu 2003)(Table 1). LAB consist of a number of bacterial genera within the phylum Firmicutes. The genera *Carnobacterium, Enterococcus, Lactobacillus, Tetragenococcus, Lactococcus, Weissella, Lactosphaera, Leuconostoc, Melissococcus, Oenococcus, Pediococcus, Streptococcus* and *Vagococcus* are recognized as LAB. *Lactococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc, Lactobacillus* and *Carnobacterium* are the genera most commonly used as starter cultures in the fermentation processes of milk, meat and vegetable products. The classification of lactic acid bacteria into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance (Rattanachaikunsopon & Phumkhachorn 2010). The important characteristics used in differentiation of the lactic acid bacteria genera is the mode of glucose fermentation under standard conditions (non limited supply with glucose, growth factors such as vitamins, amino acids and nucleic acids precursors, and limited oxygen availability). Under these conditions, LAB can be divided into two groups: (I) homofermentative and (II) heterofermentative bacteria. Homofermentative LAB such as *Pediococcus*, *Streptococcus*, *Lactococcus* convert sugars almost quantitatively to lactic acid. The second group, the heterofermentative bacteria such as *Weissella* and *Leuconostoc* produce not only lactic acid but ethanol/acetic acid and carbon dioxide (Rattanachaikunsopon & Phumkhachorn 2010, Papagianni 2012).

Table 1. Lactic acid bacteria used in fermented foods (taken from Rattanachaikunsopon & Phumkhachorn 2010)

Fermented Products	Lactic Acid Bacteria*
Dairy product	
Hard cheeses without eyes	*L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris*
Cheeses with small eyes	*L. lactis* subsp. *lactis, L. lactis* subsp. *lactis* var. *diacetylactis, Leuc. menesteroides* subsp. *cremoris*
Butter and buttermilk	*L. lactis* subsp. *lactis, L. lactis* subsp. *lactis* var. *diacetylactis, L. lactis* subsp. *cremoris, Leuc. menesteroides* subsp. *cremoris*
Yoghurt	*Lb. delbrueckii* subsp. *bulgaricus, S. thermophilus*
Fermented, probiotic milk	*Lb. casei, Lb. acidophilus, Lb. rhamnosus, Lb. johnsonii, B. lactis, B. bifidum, B. breve*
Kefir	*Lb. kefir, Lb. kefiranofacies, Lb. brevis*
Fermented meats	
Fermented sausage (Europe)	*Lb. sakei, Lb. curvatus*
Fermented vegetables	
Sauerkraut	*Leuc. mesenteroides, Lb. plantarum, P. acidilactici, Leuc. mesenteroides, P. cerevisiae, Lb. brevis*
Pickles	*Lb. plantarum, Leuc. mesenteroides, Lb. pentosus, Lb. plantarum*
Fermented olives	*P. acidilactici, P. pentosaceus, Lb. plantarum*
Fermented vegetables	*Lb. fermentum*
Fermented cereals	
Sourdough	*Lb. sanfransiscensis, Lb. farciminis, Lb. fermentum, Lb. brevis, Lb. plantarum, Lb. amylovorus, Lb. reuteri, Lb. pontis, Lb. panis, Lb. alimentarius, W. cibaria*

*B. *Bifidobacterium*, L. *Lactococcus*, Lb. *Lactobacillus*, Leuc. *Leuconostoc*, P. *Pediococcus*, S. *Streptococcus*, W. *Weissella*

3. ANTIMICROBAL COMPOUNDS PRODUCED BY LACTIC ACID BACTERIA

LAB produce various antimicrobial compounds, which can be classified as low molecular mass compounds such as hydrogen peroxide, carbon dioxide, diacetyl, uncharacterized compounds, and high molecular mass compounds like bacteriocins (Ammor et. al. 2006, Mobolaji & Wuraola 2011). Antimicrobial compounds can be identified by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), the latter being often used for the determination of molecular mass. The agar and broth dilution methods are available for evaluation of the antimicrobial activity (Yang 2000). This review will focus on some of antimicrobial compounds produced by LAB such as lactic acids, hydrogen peroxide, diacetly, reutein and bacteriocins.

3.1. Lactic Acids

The primary antimicrobial effect exerted by LAB is the production of lactic acid (Mobolaji & Wuraola 2011). Lactic acid is the major organic acid of LAB fermentation where it is in equilibrium with its undissociated and dissociated forms, and the extent of the dissociation depends on pH (Ammor et. al. 2006). Lactic acid can be produced by LAB in its L- or D-isomer form. L-lactic acid is preferred for food and pharmaceutical applications and as starting material in the production of biopolymers, while D-lactic acid is toxic for humans. Therefore, metabolic engineering studies have focused on the production of pure L-lactic acid by homofermentative LAB. In addition, the stereoisomers of lactic acid also differ in antimicrobial activity, L-lactic acid being more inhibitory than the D-isomer (Papagianni 2012). Lactic acids are generally thought to exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic functions (Rattanachaikunsopon & Phumkhachorn 2010). The production of lactic acid and reduction of pH are depended on species or strain, culture composition and growth conditions (Olaoye & Onilude 2011). They have a very broad mode of action and inhibit both gram-positive and gram-negative bacteria as well as yeast and moulds (Rattanachaikunsopon & Phumkhachorn 2010). At low pH, a large amount of lactic acid is in the undissociated form, and it is toxic to many bacteria, fungi and yeasts. However, different microorganisms vary considerably in their sensitivity to lactic acid. At pH 5.0 lactic acid was inhibitory toward spore-forming bacteria but was ineffective against yeasts and moulds (Yang 2000).

3.2. Diacetyl

One of the end products that can be generated from private is diacetly, which is an important flavor compound in dairy products. Strains of *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, and some species belonging to *Leuconostoc* and *Weissella* genera are used as diacetly producers. It is produced from citrate in co-metabolic fermentation with lactose (Kleerebezem et. al. 2000). Diacetyl may be synthesized by either homolactic or heterolactic pathways of sugar metabolism. Citrate-utilizing LAB produce diacetly during milk fermentation in the production of butter, buttermilk and several cheeses while diacetly generates the typical butter aroma in these products. Because of its value as aroma compound, efficient production of diacetly from lactose rather than citrate has been the aim of several metabolic engineering strategies (Papagianni 2012). It inhibits the growth of gram negative by reacting with the arginine-binding protein, thus affecting the arginine utilization. Gram negative bacteria are more sensitive to diacetly than gram positive bacteria. Diacetly at 344 µg/ml inhibits strains of *Listeria, Salmonella*, *Yersinia, Esherichia coli*, and *Aeromonas* (Yang 2000, Ammor et. al 2006).

3.3. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is widely used in the fields of foods, pharmaceuticals, dental products, textiles, environmental protection, and it is also involved in advanced oxidation processes and various biochemical processes (Abbas et. al. 2010). H_2O_2 is also produced by LAB in the presence of oxygen.

The antimicrobial effect of H_2O_2 may result from the oxidation of sulfhydryl groups causing denaturing of a number of enzymes, and from the peroxidation of membrane lipids thus the increased membrane permeability and also be as a precursor for the production of bactericidal free radicals such as superoxide (O^{-2}) and hydroxyl (OH^-) radicals which can damage DNA (Yang 2000, Ammor et al. 2006, Sunil & Narayana 2008). H_2O_2 can have a strong oxidizing effect on membrane lipids and cellular proteins and is produced using such enzymes as the flavo protein oxidoreductases, NADH peroxidase, NADH oxidase and α-glycerophosphate oxidase (Rattanachaikunsopon & Phumkhachorn 2010). The synthesized H_2O_2 can inhibit the growth of psychotropic and pathogenic microorganisms (Yang 2000, Zalan et. al. 2005). Psychotropic species and strains among the following genera can cause food spoilage at cold temperature: *Alcaligenes, Shewanella, Brochotrix, Enterococcus, Lactobacillus, Pseudomonas*, and others. Some food borne pathogens, such as *Aeromonas hydrophila, Listeria monocytogenes, Yersinia enterocolitica,* and *Clostridium botulinum* type E, can grow 5 °C (Ito et. al. 2003, Abbas et. al. 2010).

3.4. Reuterin

The glycerol derived antimicrobial compound reuterin has been shown to be produced by a number of lactobacilli. Reuterin is produced under anaerobic conditions, and production is enhanced by the presence of glycerol. Reuterin is able to inhibit the growth of *Aspergillus* and *Fusarium*, and it may therefore play a role in preventing mycotoxin formation in fermented foods. It is also active against gram positive and gram negative bacteria, including enteropathogens, yeast, fungi, protozoa, and viruses (Nes et. al. 2012). Spoilage organisms sensitive to reuterin include species of *Salmonella, Shigella, Clostridium, Staphylococcus, Listeria, Candida,* and *Trypanosoma* (Yang 2000).

3.5. Bacteriocin

Bacteriocins are ribosomally synthesized antimicrobial peptides that are active against other bacteria, either of the same species (norrow spectrum) or across genera (broad spectrum) (Soomro et. al. 2002, Yang et. al. 2012). Some members of LAB produce bacteriocins and bacteriocins-like substances (Mobolaji & Wuraola 2011) (Table 2). Most of LAB bacteriocins are small thermostable or large thermolabile proteins or protein complexes that display antimicrobial properties against other bacteria often closely related gram positive bacteria, whereas producer cells are immune to their own bacteriocin(s) (Zacharof & Lovitt 2012). Bacteriocin production is strongly dependent on pH, nutrient sources and incubation temperature. Activity levels of bacteriocin production are often obtained at conditions lower than required for optimal growth (Todorov & Dicks 2005, de Vuyst & Leroy 2007). Four distinct classes of LAB bacteriocins have been identified on the basis of biochemical and genetic characterization: lantibiotics (class 1), small, heat-stable nonlanthionine peptides (class 2), large heat-labile proteins (class 3) and complex bacteriocins containing chemical moieties such as lipid and carbohydrate (class 4) (Hernandez et. al. 2005). The LAB bacteriocins have many attractive characteristics that make them suitable candidates for use as food preservatives, such as: 1) Protein nature, inactivation by proteolytic enzymes of gastrointestinal tract, 2) Non-toxic to laboratory animals tested and generally non-immunogenic, 3) Inactive against eukaryotic cells, 4) Generally thermo resistant (can maintain antimicrobial activity after pasteurization and sterilization), 5) Broad bactericidal activity affecting most of the gram-positive bacteria and some, damaged, gram-negative bacteria including various pathogens but usually only when the integrity of the outer membrane has been compromised, for example after osmotic shock or low pH treatment, in the presence of a detergent or chelating agent, 6) Genetic determinants generally located in plasmid, which facilitates genetic manipulation to increase the variety of natural peptide analogues with desirable characteristics (Juodeikiene et. al. 2012). Nisin, produced by *Lactococcus lactis,* is the most thoroughly studied bacteriocin to date and has been applied as an additive to certain foods worldwide including cheese, canned food and cured meat. Nisin is active against a wide range of gram-positive pathogenic organisms and also gram negative bacteria, but only when used at high concentration or when the target cells have been pre-treated with EDTA (Todorov & Dicks 2005). Nisin solubility and stability increase substantially with increasing acidity. Nisin is stable at pH 2 and can be autoclaved at 121ºC. Under alkaline pH there is an increasing loss of activity, with complete inactivation after 30 min at 63ºC at pH 11 (Jozala et. al.2005). Another commercially produced bacteriocin is pediocin PA-1 produced by *Pediococcus acidilactici* and marketed as ATTA^{TM} 2431 (Yang et. al. 2012).

Table 2. Some well characterized bacteriocins produced by lactic acid bacteria (taken from Soomro et. al. 2002)

Bacteriocin	Producer organism	Properties
Nisin	*Lactococcus lactis* subsp. *lactis* ATCC 11454	Lantibiotic, broad spectrum, chromosome/plasmid mediated, bactericidal, produced late in the growth cycle
Pediocin A	*Pediococcus pentosaceus* FBB61 and L-7230	Broad spectrum, plasmid mediated
Pediocin AcH	*Pediococcus acidilactici* H	Broad spectrum, plasmid mediated
Leucocin	*Leuconostoc gelidum* UAL 187	Broad spectrum, plasmid mediated, bacteriostatic, produced early in the growth cycle
Helveticin J	*L.helveticus* 481	Narrow spectrum, chromosomally mediated, bactericidal
Carnobacteriocin	*Carnobacterium piscicola* LV17	Narrow spectrum, plasmid mediated, produced early in the growth cycle

4. CONCLUSION

Lactic acid bacteria display numerous antimicrobial activities in fermented foods. This is mainly due to the production of organic acids, but also of other compounds, such as ethanol, H_2O_2 , diacetyl, reuterin and bacteriocins. Bacteriocins ,that inhibit not only closely related species but also effective against food-borne pathogens and many other gram-positive spoilage microorganisms, have attracted considerable interest for use as natural food preservatives in recent years. Each antimicrobial compound produced during fermentation provides an additional hurdle for pathogens and spoilage bacteria to overcome before they can survive and/or proliferate in a food or beverage, from time of manufacture to time of consumption. The antimicrobial-producing LAB may be used as protective cultures to improve the microbial safety of foods and they also play an important role in the preservation of fermented foods.

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