Bacteriocins and lactic acid bacteria - a minireview

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Fermentation of various foods by lactic acid bacteria (LAB) is one of the oldest forms of biopreservation practised by mankind. Bacterial antagonism has been recognized for over a century but in recent years this phenomenon has received more scientific attention, particularly in the use of various strains of lactic acid bacteria. One important attribute of LAB is their ability to produce antimicrobial compounds called bacteriocin. In recent years, interest in the compounds has grown substantially due to their potential usefulness as natural substitute for chemical food preservatives in the production of foods with enhanced shelf life and/or safety. This balance is achieved by its inhibitory effect upon the harmful pathogenic microorganisms. This paper presents some background on the scientific research about lactic acid bacteria as probiotics and their bacteriocins for healthy nutrition of fermented food. Probiotics had been of interest in the promotion of good health in animals and man. Some of the positive effects of probiotics are: growth promotion of farm animals, protection of host from intestinal infections, alleviation of lactose intolerance, relief of constipation, anticarcinogenic effect, anticholesterolaemic effects, nutrient synthesis and bioavailability, prevention of genital and urinary tract infections and immunostimulatory effects.

Key words: Bacteriocins, lactic acid bacteria, fermented food, probiotics

INTRODUCTION

Lactic acid bacteria (LAB) occur naturally in several raw materials like milk, meat and flour used to produce foods (Rodriguez et al., 2000). LAB are used as natural or selected starters in food fermentations in which they perform acidification due to production of lactic and acetic acids flavour. Protection of food from spoilage and pathogenic microorganisms by LAB is through producing organic acids, hydrogen peroxide, diacetyl (Messens and De Vugst, 2002), antifungal compounds such as fatty acids (Corsetti et al., 1998) or phenullactic acid (Lavermicocca et al., 2000) and/or bacteriocins (De Vugst and Vandamme, 1994). LAB play an important role in food fermentation as the products obtain with their aid are characterized by hygienic safety, storage stability and attractive sensory properties.

Many bacteria of different taxonomic branches and residing in various habitats produce antimicrobial substances that are active against other bacteria. Both Gram negative and Gram positive bacteria produce bacteriocins. Bacteriocins are proteinaceous antibacterial compounds, which constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides (De Vugst and Vandamme, 1994). In general these substances are cationic peptides that display hydrophobic or amphiphilic properties and the bacterial membrane is in most cases the target for their activity. Depending on the producer organism and classification criteria, bacteriocins can be classified into several groups (Ennahar et al., 2000; Jack and Jung, 2000; Cleveland et al., 2001; McAuliffe et al., 2001) in which classes I and II are the most thoroughly studied. Class I, termed lantibiotics, constitute a group of small peptides that are characterized by their content of several unusual amino acids (Gruder et al., 2000). The class II bacteriocins are...
small, nonmodified, heat stable peptides (Nes and Holo, 2000). Many bacteriocins are active against food borne pathogens (Vignolo et al., 1996; De Martins and Franco, 1998; Bredhott et al., 1999).

A large number of bacteriocins have been isolated and characterized from lactic acid bacteria and some have acquired a status as potential antimicrobial agents because of their potential as food preservatives and antagonistic affect against important pathogens. The important ones are nisin, diplococcin, acidophilin, bulgarican, helveticins, lactacins and plantaricins (Nettles and Barefoot, 1993). The lantibiotic nisin which is produced by different Lactococcus lactis spp. is the most thoroughly studied bacteriocin to date and the only bacteriocin that is applied as an additive in food worldwide (Delves Broughton et al., 1996). One of the reasons for increased consumption of fermented milk products is that fermented dairy products containing probiotics which have many proposed health benefits are available on the market. In this paper the diversity of bacteriocins their application and lactic acid bacteria used as probiotics are reviewed.

**Taxonomy of lactic acid bacteria**

The classification of LAB was initiated in 1919 by Orla-Jensen (Table 1) and was until recently primary based on morphological, metabolic and physiological criteria. Lactic acid bacteria comprise a diverse group of Gram-positive, non spore forming, non motile rod and coccus shaped, catalase-lacking organisms. They are chemoorganotrophic and only grow in complex media. Fermentable carbohydrates and higher alcohols are used as the energy source to form chiefly lactic acid. LAB degrades hexoses to lactate (homofermentatives) or lactate and additional products such as acetate, ethanol, CO$_2$, formate or succinate (heterofermentatives). They are widely distributed in different ecosystems and are commonly found in foods (dairy products, fermented meats and vegetables, sourdough, silage, beverages), sewage, on plants but also in the genital, intestinal and respiratory tracts of man and animals.

Current methodolgies used for classification of LAB mainly rely on 16S ribosomal ribonucleic acid (rRNA) analysis and sequencing (Olsen et al., 1994). Based on these techniques, Gram-positive bacteria are divided into two groups depending on their G + C content. The Actinomycetes have a G + C content above 50 mol% and contain genera such as Atopobium, Bifidobacterium, Corynobacterium and Propionibacterium. In contrast, the Clostridium branch has a G + C content below 50 mol% and include the typical LAB genera Carnobacterium, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and Streptococcus.

**Lactic acid bacteria as probiotics**

Lactic acid bacteria were referred to as probiotics in scientific literature by Lilley and Stillwell (1965). However probiotic took on a different terminology when Sperti (1971) used the term « probiotic » to describe tissue extracts that stimulated microbial growth. Parker (1974) redefined it as organisms and substances that contribute to the intestinal microbial balance. The most recent and accurate description of probiotics was undertaken by Fuller (1989) who redefined it as « a live microbial feed supplement beneficial to the host (man or animal) by improving the microbial balance within its body ». Another recent definition was by Schrezenmeir and De Vrese (2001) who defined probiotics as viable microbial food supplements which beneficially influence the health of the host.

The gastrointestinal tract contains food in different stages of digestion, digestive ferments, liquids and solid waste. Within the gut are also wide ranges of microbes that may be either harmful or beneficial. The beneficial ones assist in the breakdown of food while they also manufacturers vitamins essential to the body, breaking down and destroying some toxic chemicals that may

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**Table 1.** Orla-Jensen (1919) key to differentiation of the lactic acid bacteria and current taxonomic classification.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Shape</th>
<th>Catalase</th>
<th>Nitrite reduction</th>
<th>Fermentation</th>
<th>Current genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betapectinum</td>
<td>Rod</td>
<td>-</td>
<td>-</td>
<td>Hetero</td>
<td>Lactobacillus Weissella</td>
</tr>
<tr>
<td>Thermobacterium</td>
<td>Rod</td>
<td>-</td>
<td>-</td>
<td>Homo</td>
<td>Lactobacillus</td>
</tr>
<tr>
<td>Streptobacterium</td>
<td>Rod</td>
<td>-</td>
<td>-</td>
<td>Homo</td>
<td>Lactobacillus Carnobacterium</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Coccus</td>
<td>-</td>
<td>-</td>
<td>Homo</td>
<td>Streptococcus Enterococcus</td>
</tr>
<tr>
<td>Betacoccus</td>
<td>Coccus</td>
<td>-</td>
<td>-</td>
<td>Homo</td>
<td>Lactococcus Vagococcus</td>
</tr>
<tr>
<td>Microbacterium</td>
<td>Rod</td>
<td>+</td>
<td>+</td>
<td>Homo</td>
<td>Leuconostoc Oenococcus Weissella</td>
</tr>
<tr>
<td>Tetracoccus</td>
<td>Coccus</td>
<td>+</td>
<td>+</td>
<td>Homo</td>
<td>Brochothrix</td>
</tr>
</tbody>
</table>

*According to Orla Jensen (1919).  
In genera Pediococci are catalase negative but some strains produce a pseudocatalase that results in false positive reactions.
have been ingested with the food. Under both healthy and sick conditions, several different types of bacteria compete or fight with each other to establish dominance in the warm and moist environment of the alimentary canal that serves as an ecosystem for their survival and propagation. The average human large intestine harbors over 400 different species of bacteria with a total population far outnumbering even the number of human cells in the body. Under ideal conditions of health and diet, the different strains of bacteria on microflora compete and check the excessive number of any one strain. Healthy condition can be achieved if a balance is maintained between the « good » and « bad » bacteria in the ratio of 85 percent to 15 percent. Oral supplement of diet with viable Lactobacillus acidophilus of human origin, which is bile resistant, led to a significant decline of three different fecal bacterial enzymes (Goldin and Gorbach, 1977). This decrease in the fecal bacterial enzyme activity observed in both humans and rats included beta glucuronidase, azoreductase and nitroreductase. All these enzymescatalyse the conversion of procarcinogens to proximal carcinogens in the large bowel leading to colon cancer.

Lactic acid bacteria including Lactobacillus, leuconostoc, lactococcus, pediococcus and Bifidobacterium spp. are prominent members of the commensal intestinal flora and are the commonly studied probiotics bacteria. They cause reduced lactose intolerance alleviation of some diarrhoeas, lowered blood cholesterol, increased immune response and prevention of cancer (Marteau and Ramband, 1993, 1996; Gilliland, 1996; Salminen et al., 1998a). The selection criteria for probiotic LAB include: human origin, safety, viability/activity in delivery vehicles, resistance to acid and bile, adherence to gut epithelial tissue ability to colonise the gastro intestinal tract, production of antimicrobial substances, ability to stimulate a host immune response and the ability to influence metabolic activities such as vitamin production, cholesterol assimilation and lactose activity (Salminen et al., 1996).

Fuller (1989) and Conway (1996) listed the following organisms as species used in probiotic preparation: Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus casei subsp. rhamnosus, Lactobacillus fermentum, Lactobacillus reuteri Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactobacillus bulgaricus, Lactococcus plantarum, Streptococcus thermophilus, Enterococcus faecium, Enterococcus faecalis, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium adolescentis, Bifidobacterium longum, Bifidobacterium breve.

**Probiotics benefit in the gastro intestinal tract and immune system**

Certain LAB species are found not only as components of the human intestinal microflora but also of the man made ecosystem present in fermented food. That is why fermented milks containing viable LAB are known to be beneficial to health acting as prophylaxis against intestinal infections. Thus many investigators have evaluated the effect of yoghurt on the immune response of animals and humans.

Many studies have been conducted on their effect on the incidence and duration of various types of diarrhoea (Isolauri, 2001; Bhatnagar et al., 1998). LAB can be effective in preventing gastrointestinal disorders and in the recovery from diarrhoea of miscellaneous causes (Marteau et al., 2001). A decrease in the severity and duration of persistent diarrhoea has been reported with LAB (Bhatnagar et al., 1998). Guandalini et al. (2000) also reported that the administration of Lactobacillus rhamnosus GG to 287 children aged 1-36 months with acute diarrhoea significantly reduced the duration in infected children by rotavirus compared with those receiving placebo. Administration of Lb rhamnosus GG also shortened the duration of the hospital stay.

**CLASSIFICATION OF BACTERIOCINS**

The bacteriocins produced by Gram-positive bacteria like LAB are small peptides, 3-6 kDa, in size (Nes et al., 1996), although there are exceptions (Jorger and Klaenhammer, 1990). On a sound scientific basis three classes of bacteriocins have been established: Class I, the lanthionines; class II, the small heat stable non lanthionines; and class III, large heat labile bacteriocins (Table 2). A fourth class of bacteriocins is composed of an undefined mixture proteins, lipids and carbohydrates. The existence of the fourth class was supported mainly by the observation that some bacteriocin activities obtained in cell free supernatant, exemplified by the activity of Lb plantarum LPCO 10 were abolished not only by protease treatments, but also by glycolytic and lipolytic enzymes (Jimenez-Diaz et al., 1993).

Most of the Gram positive bacteriocins are membrane active compounds that increase the permeability of the cytoplasmic membrane (Jack et al., 1995). They often show a much broader spectrum of bactericidal activity than the colicins (Gram negative bacteriocins which are produced by Esherichia coli). They fall with in two broad classes, viz the lantibiotics (Jack et al., 1995) and the non lantibiotic bacteriocins (Nes et al., 1996). Nisin (Table 3) prevents clostral spoilage spoilage of processed and natural cheeses, inhibits the growth of some psychrotropic bacteria in cottage cheese, extends the shelf life of milk in warm countries, prevents the growth of spoilage lactobacilli in beer and wine fermentations and provides additional protection.
Table 2. Antimicrobial peptides (peptide-bacteriocins) produced by lactic acid bacteria (Nissen-Meyer et al., 1997).

<table>
<thead>
<tr>
<th>Group I: Modified bacteriocins (the lantibiotics)</th>
<th>Group II: Unmodified bacteriocins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type A</strong></td>
<td><strong>Type B</strong></td>
</tr>
<tr>
<td>Nisin</td>
<td>NK a</td>
</tr>
<tr>
<td>Lactocin S</td>
<td></td>
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<tr>
<td>Lacticin 481</td>
<td></td>
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<tr>
<td>Carnocin UI 49</td>
<td></td>
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<tr>
<td>Cytolysin</td>
<td></td>
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<td></td>
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<tr>
<td>Nonpediocin-like bacteriocins:</td>
<td></td>
</tr>
</tbody>
</table>

a Not known: lantibiotics of type B produced by lactic acid bacteria are presently not known
b References for the pediocin-like bacteriocins are: Pediocin PA1 (Henderson et al., 1992; Marug et al., 1992), leucocin A (Hastings et al., 1991), sakacin P (Tichaczek et al., 1992), curvacin A (Tichaczek et al., 1992; Holck et al., 1992), mesentericin Y105 (Hechard et al., 1992), carnobacteriocin BM1 and B2 (Quadri et al., 1994), enterocin A (Aymerich et al., 1996), piscocin 126 (Jack et al., 1996), bavaricin MN (Kaiser, Montville, 1996), piscicocin V1a (20).
c Reference for plantaricin S: (Tichaczek et al., 1993).
d Originally published as a modified one peptide bacteriocin (Cintas et al., 1995), but recent results indicate that it is an unmodified two-peptide bacteriocin (Cintas et al., unpublished results).
e As-48 is a cyclic antimicrobial peptide produced by Enterococcus faecalis (Martinez-Bueno et al., 1994).

Table 3. Properties of some well characterized bacteriocins (Soomro et al., 2002).

<table>
<thead>
<tr>
<th>Bacteriocin</th>
<th>Producer organism</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin</td>
<td>Lactococcus lactis subsp. lactis ATCC 11454</td>
<td>Lantibiotic, broad spectrum, chromosome / plasmid mediated, bactericidal, produced late in the growth cycle. Broad spectrum, plasmid mediated.</td>
</tr>
<tr>
<td>Pediocin A</td>
<td>Pediococcus Pentosaceus FBB61 and L-7230</td>
<td>Broad spectrum, plasmid mediated.</td>
</tr>
<tr>
<td>Pediocin AcH</td>
<td>Pediococcus Acidilactici H Leuconostoc gelidum UAL 187</td>
<td>Broad spectrum, plasmid Mediated, bacteriostatic, produced early in the growth cycle.</td>
</tr>
<tr>
<td>Leucocin J</td>
<td>L. helveticus 481 Carnobacterium piscicola LV17</td>
<td>Narrow spectrum, chromosomally mediated, bactericidal. Narrow spectrum, plasmid mediated, produced early in the growth cycle.</td>
</tr>
</tbody>
</table>

against Bacillus and clostridial spores in canned foods. Nisin is a permitted food additive in more than 50 countries including the US and Europe under the trade name Nisaplin (Vandenberg, 1993; Delves-broughton et al., 1996). Nisin is active against many gram positive bacteria including Listeria spp.

BACTERIOCIN BIOSYNTHESIS

Bacteriocins are synthesized as pre-propeptide which are processed and externalised by dedicated transport machinery (Nes et al., 1996). Bacteriocin production in LAB is growth associated: it usually occurs throughout the growth phase and ceases at the end of the exponential phase (or sometimes before the end of growth (Parente et al., 1997; Lejeune et al., 1998). Bacteriocin production is affected by type and level of the carbon, nitrogen and phosphate sources, cations surfactants and inhibitors. Bacteriocins can be produced from media containing different carbohydrate sources. Nisin Z can be produced from glucose, sucrose and xylose by Lactococcus lactis IO-1 (Matsuaki et al., 1996; Chinachoti et al., 1997a,b) but better results were obtained with glucose compared to xylose. Glucose followed by sucrose, xylose and galactose were the best carbon sources for the production of Pediocin AcH in an unbuffered medium.
All bacteriocins are synthesized with an N terminal leader sequence and until recently only the double glycine type of leader was found in class II bacteriocins (Holo et al., 1991; Muriana and Klaenhammer, 1991; Klaenhammer, 1993; Havarstein et al., 1994). However, it has now been disclosed that some small, heat stable and non modified bacteriocins are translated with sec dependent leaders (Leer et al., 1995; Worobo et al., 1995). The structural bacteriocin gene encodes a preform of the bacteriocin containing an N-terminal leader sequence (termed double glycine leader) whose function seems to prevent the bacteriocin from being biologically active while still inside the producer and provide the recognition signal for the transporter system. A number of genes, often found in close proximity to each other are required for production of lantibiotics. These genes include:

(a) The structural gene, lan A,
(b) immunity genes (Lan I and in some cases Lan E, Lan F and Lan G) encoding proteins that protect the producer from the producer lantibiotic,
(c) a gene Lan T encoding what appears to be a membrane associated ABC transporter that transfers the lantibiotic across the membrane,
(d) a gene, lan P, encoding a serine proteinase which removes the leader sequence of the lantibiotic prepeptide,
(e) two genes, lan B and Lan C (or in some cases only one gene, Lan M), with no sequence similarity to other known genes thought to encode enzymes involved in the formation of lanthionine and methyl lanthionine, and
(f) two genes lan k and lan R encoding two component regulatory proteins that transmit an extracellular signal and thereby inducing lantibiotic production.

CONCLUSION

The potential application of bacteriocins as consumer friendly biopreservatives either in the form of protective cultures are as additives is significant. LABS are typically involved in a large number of spontaneous fermentations but they are also closely associated with the human environment. Food fermentations have a great economic value and it has been accepted that these products contribute in improving human health. LABS have contributed in the increased volume of fermented foods world wide especially in foods containing probiotics or health promoting bacteria. Bacteriocins produced by LAB are the subject of intense research because of their antibacterial activity against foodborne bacteria.

Further studies should be focused on the mechanisms of action of LAB within the gastro intestinal tract and in the immune system which stimulate the in vivo immunity effects. Furthermore, genetic engineering of already identified probiotics and those newly discovered to make them more efficacious should be pursued.

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Goldin B, Gorbach SL (1977). Alterations in fecal microflora enzymes related to diet, age, lactobacillus supplements, and


