Production of anti-microbial substances by probiotics

Churamati Mishra, MSc and John Lambert, MBBS, FRACP, PhD

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Bacterial antagonism has been recognised 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 (LAB). Anti-microbial compounds produced by LAB have provided these organisms with a competitive advantage over other microorganisms. Lactic acid bacteria have a natural ecological niche in many foods as well as in the intestinal tract. The efficacy and spectrum of anti-microbial products of lactic acid bacteria are broad and include lactic and acetic acid, hydrogen peroxide, carbon dioxide, diacetyl as well as bacteriocins or bacteriocin-like substances. Further screening for agents with a broad spectrum of activity is required. This will involve genetic or protein engineering of such compounds to commercialise these agents.

Introduction

The gastrointestinal tract is a site for proliferation of microorganisms with the domination of different strains being influenced by various factors such as changes in tissue surface chemistry (by hormonal fluctuation), administration of antibiotics, age and diet. The impact of antibiotic treatment includes reduction in colonisation resistance, implantation of new and pathogenic microorganisms and development of resistant strains in the normal microbial flora. Many probiotics, including bifidobacteria, resist invasion by pathogens has been referred to as colonisation resistance. Selective colonisation of the intestinal flora by using dietary supplements has been attempted since the work of Metchnikoff who showed that cholera could be prevented by the presence of ‘desirable’ microflora such as those found in cultured milk products

Interest in biocontrol that involves the incorporation of lactic acid bacteria (LAB or lactic) in food supplements (or ‘probiotics’), is presently experiencing a resurgence in the food and pharmaceutical industries. A probiotic is defined as a cultured product or live microbial feed supplement which has been shown to benefit the host animal (by improving its intestinal balance). The most commonly used lactics in probiotic preparation, either singly or in a mixture of different organisms, include Lactobacillus bulgaricus, Lactococcus acidophilus, Streptococcus thermophilus and Bifidobacterium. The use of other organisms for example Enterobacter faecalis, Escherichia coli and Bacillus subtilis is questionable because of public health concern.

Fermentation with LAB is one of the oldest known methods of food preservation. However, it was not until the 1970s that the mechanisms of their action was were investigated. The inhibition of many pathogenic bacteria in fermented foods is believed to be the result of anti-microbial substances produced by LAB resulting in natural preservation through bacterial antagonism. The principle of bacterial antagonism applies to the preparation of new probiotics as well.

Desirable characteristics of organisms used as probiotics

There are several desirable characteristics for organisms to be used as dietary adjuncts. Organisms should be a normal inhabitant of the human intestinal tract, non-pathogenic, non-toxic and be capable of surviving passage through the gastrointestinal tract. Within the gut it must produce the desired effects. Furthermore, it must maintain viability and activity in the carrier food before consumption. Finally, the organism should be sensitive to antibiotics used to treat infection and should not harbour plasmids resulting in antibiotic resistance. It is also important to know the number of organisms needed to colonise human subjects to estimate the effective therapeutic dose.

Anti-microbial compounds produced by LAB have provided these organisms with a competitive advantage over other microorganisms. Exploitation of antibiosis of LAB is the best choice for not only improving the microbial safety of the food products but as a probiotic preparation because of their natural adaptation to the gut environment. Lactics need to be acid tolerant bacteria and exhibit resistance to lysozyme present in the saliva and other enzymes, gastric juice and duodenal fluids. Many lactics are resistant to the bile salt present in the gut and survive the intestinal motility and adhere well to gastric mucosa.

Possible modes of action of probiotics are: suppression of viable count by production of antibacterial compounds; competition for nutrients and adherence sites; alteration of microbial metabolites and stimulation of immunity. Lactics are known to produce a wide range of anti-microbial substances which will be discussed in the following section.

Inhibitory compounds produced by lactic acid bacteria

Anti-microbial activity of LAB isolated from food has been the subject of intensive research due to the potential application of these bacteria as protective cultures in several ways such as by rendering the environment more anaerobic, by inhibiting enzymatic decarboxylation by disrupting the cell membrane with the accumulation of the gaseous phase in the lipid bilayer.

Diacetyl (2,3 butanediol) is synthesised by certain species of LAB from pyruvate. It inhibits the growth of Gram-negative bacteria and Gram-positive bacteria other than Listeria monocyctogenes, Staphylococcus aureus, Bacillus cereus, Clostridium botulinum and several others. Bacitracins have considerable promise for application as natural food preservatives. Bacitracin produced mainly by LAB may be very important in competing with other organisms in the intestine. They consist of a biologically active protein moiety, have a bactericidal mode of action and attach to specific cell receptors. Wide variation exists in their chemical composition but a specific mode of action. The effects of bacteriocins have been elucidated in the food systems, however, a prophylactic role in the intestine has yet to be proven conclusively. In the following section, our knowledge of bacteriocins produced by LAB is reviewed.

General characteristics of bacteriocins

Although not exactly defined, bacteriocins differ from classical antibiotics. They are a heterogeneous group of bacterial antagonists that vary considerably in molecular weight, biochemical properties, range of sensitive hosts and mode of action. Klassenhammer redefined them as follows: "Bacteriocins are proteins with bactericidal activity directed against species that are usually closely related to the producer bacterium".

In the past few years many bacteriocins or bacteriocin-like compounds have been characterised. Many of these substances do not comply with the definition proposed above. Bacteriocins are generally active against closely related species and thus the inhibitory spectrum is very narrow (especially bacteriocins from LAB). However, nisin from Lactococcus lactis and pediocin A from Pediococcus pentaeus B6 and L-7320 are active against a broader range of foodborne pathogens. Pediococcus damnosus and P. pentaeus inhibit the growth of Gram-negative organisms such as Yersinia enterocolitica, Pseudomonas fragi and Pseudomonas aeruginosa.

Exceptions exist with regards to Klassenhammer’s definition of bacteriocins, including their bactericidal mode of action, the pure protein nature and the narrow spectrum of antibacterial activity. Bacteriocins from P.
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Introduction

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Interest in biocontrol that involves the incorporation of lactic acid bacteria (LAB or lactic) in food supplements (or ‘probiotics’), is presently experiencing a resurgence in the food and pharmaceutical industries. A probiotic is defined as a cultured product or live microbial feed supplement which beneficially affects the host animal (by improving its intestinal balance). The most commonly used lactic acid bacteria for probiotic preparation, either singly or in a mixture of different organisms, include Lactobacillus bulgaricus, Lactobacillus acidophilus, Streptococcus thermophilus and Bifidobacterium. The use of other organisms for example Enterobacter faecalis, Escherichia coli and Bacillus subtilis is questionable because of public health concern.

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Inhibitory compounds produced by lactic acid bacteria

Antimicrobial activity of LAB isolated from food has been the subject of intensive research due to the potential application of these bacteria as protective cultures in several ways such as by rendering the environment more biologically preservation. The major groups of inhibitory compounds produced by LAB are:

1. lactic and other volatile acids with a resulting decrease in pH
2. other primary metabolites such as hydrogen peroxide, carbon dioxide and diacetyl
3. bacteriocins – special antimicrobial compounds.

Each of these groups of compounds, especially a combination of them, can be used to extend the shelf life and safety of food products. Antibiosis of LAB has been extensively reviewed.

The mechanism of antibiosis produced by LAB are summarised in Table 1.

Table 1. Inhibitory compounds produced by lactic acid bacteria and their mechanism of action.

<table>
<thead>
<tr>
<th>Inhibitory compound</th>
<th>Mechanism of action</th>
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<tbody>
<tr>
<td>Lactic and other volatile acids</td>
<td>Disruption of cellular metabolism</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Inactivation of essential biomolecules by superoxide anion chain reaction, activation of lactoperoxidase system</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>Anaerobic environment and/or inhibition of enzyme desacetylolation and/or disruption of the cell membrane</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>Interference with arginine utilization</td>
</tr>
<tr>
<td>Bacteriocin (secondary metabolites)</td>
<td>Little is known, disruption of cytoplastic membrane (in the case of nisin)</td>
</tr>
</tbody>
</table>
| Lactic acid | is the major metabolite produced by LAB and depends on the substrate and microorganisms, lactic acid has been reported as having good, active, poor antimicrobial properties. Acetic acid is another organic acid produced by LAB. Both lactic and acetic acids and their salts are generally regarded as safe by the United States Food and Drug Administration. Acetic acid and its salts assert their antimicrobial activity up to pH 4.5 and the effect is due to undissociated molecules. The inhibitory effect of lactic acid produced by LAB has been most extensively investigated. Low pH affects every aspect of cellular metabolism, especially the growth of unwanted microbes in culture media. Undissociated lactic and acetic acids penetrate the cell membrane and disturb the transmembrane potential, resulting in inhibition of substrate transport and membrane-bound FoFATPase activity. The minimum inhibitory concentration of undissociated lactic acid shows strain specificity.

Hydrogen peroxide produced by LAB is inhibitory to both Gram-negative (Pseudomonas sp.) and Gram-positive Staphylococcus aureus. Because LAB do not possess catalase, H2O2 accumulates in the surrounding medium, resulting in anaerobic conditions. The lethal effect of H2O2 may be due to the inactivation of essential biomolecules by the superoxide anion chain reaction. It may also function via the hexose monophosphate shunt system, which oxidises the thiocyanate to release toxic oxidation products that are detrimental to foodborne pathogens. The H2O2 is more effective as a sporicide than as a bactericide. Carbon dioxide may exert its antimicrobial effect in several ways. Diacetyl, (2,2 butanedione) is synthesised by certain species of LAB from pyruvate. It inhibits the growth of Gram-negative bacteria and Gram-positive bacteria other than LAB and yeasts. Diacetyl interferes with arginine utilisation by reacting with arginine-binding proteins of Gram-negative organisms.

Bacteriocins produced by LAB are the subject of intense research because of their antimicrobial activity against other bacteria. Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Clostridium botulinum and several others. Bacteriocins have considerable promise for application as natural food preservatives. Lactic acid producing strains of LAB may be very important in competing with other organisms in the intestine. They consist of a biologically active protein moiety, have a bactericidal mode of action and attack specific cell receptors. Wide variation exists in their chemical composition but specific mode of action. The effects of bacteriocins have been elucidated in the food systems, however, a prophylactic role in the intestine has yet to be proven conclusively. In the following section, our knowledge of bacteriocins produced by LAB is reviewed.

General characteristics of bacteriocins

Although not exactly defined, bacteriocins differ from classical antibiotics. They are a heterogeneous group of bacterial antagonists that vary considerably in molecular weight, biochemical properties, range of sensitive hosts and mode of action. Klaenhammer redefined them as follows: "Bacteriocins are proteolytic enzymes with bactericidal activity directed against species that are usually closely related to the producer bacterium."

In the past few years many bacteriocins or bacteriocin-like compounds have been characterized. Many of these substances do not comply with the definition proposed above. Bacteriocins are generally active against closely related species and thus the inhibitory spectrum is very narrow (especially bacteriocins from LAB). However, nisin from Lactococcus lactis and pediocin A from Pediococcus pentosaceus B6 and L-7230 are able to act as a broader range of foodborne pathogens. Pediococcus damnosus and P. pentaceus inhibited the growth of Gram-negative organisms such as Yersinia enterocolitica, Pseudomonas fragi and Pseudomonas fluorescens.

Exceptions exist with regards to Klaenhammer’s definition of bacteriocins, including their bactericidal mode of action, the pure protein nature and the narrow spectrum of antibacterial activity. Bacteriocins from P.
produced by LAB are bactericidal. However, lactococcus 27 from Lb. helveticus, lactococcus from Lb. sake 148, and lactococci from Lactococcus gelidum have a bacteriostatic effect. The term ‘bacteriocin-like substance’ was suggested for those antagonistic substances that do not fit the traditional definition of a bacteriocin.

There is no clear cut boundary between antibiotics, bacteriocins or microcins. Like antibiotics, bacteriocins therefore may be bacteriostatic or bactericidal with narrow or broad ranges of action, they may also be included in a family of peptide antibiotics. Antibiotics are synthesised biosynthetically by multi-step enzyme pathways. There are many ribosomally synthesised bacteriocins. While many antimicrobials promote the development of resistant strains, development of resistance to bacteriocins is rare. However, the possibility of resistance to bacteriocins may have been overlooked. Many antibiotics can be chemically synthesised. There is no report to date in which bacteriocins are chemically synthesised. However, it is appropriate to expect that, with genetic engineering, analogues of bacteriocins may be designed and constructed.

There is considerable overlap in the definition of antimicrobial substances. It is thus generally accepted that bacteriocins are a heterogeneous group of proteinaceous compounds that may vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties. Their ability to inhibit growth and the fact that all are proteinaceous may be the only common features of this mixed group of substances.

Lipid and/or carbohydrate moieties can be associated with these proteinaceous compounds and part of the bacteriocin complex. Bacteriocins can be either cell bound or released extracellularly and may be produced early or late in the growth cycle. They are susceptible to proteases and have variable sensitivity to pH and temperature.

The first detailed characterisation of bacteriocinogenic activity of lactobacilli was reported in 1961. Since then, considerable research has been continuing on bacteriocins from various groups of LAB. However important properties of some well characterised bacteriocins are shown in Table 2.

Nisin is produced by Lactococcus lactis subsp. lactis of dairy origin. Initially, nisin was accepted as a food preservative. It has a broad spectrum of activity against Gram-positive organisms including spoilforming bacteria. Nisin acts as a subunit, A, B, C, D or E, that differ in amino acid composition and activity. It is a pentacyclic catened polypeptide, referred to as a lantibiotic. Nisin contains 34 amino acids and is synthesised by posttranslational processing of ribosomally synthesised prepeptides. Nisin is a bacteriocin of A type, but it also mediates dehydrogenase activity on serine and threonine to dehydro forms, some of which react with cysteine residues to form thioether cross linkages. In addition, a leader peptide is cleaved and the mature bacteriocin is exported from the cell. The modified peptide bacteriocin is characterised by the occurrence of the sulphur-containing amino acids lanthionine and β-methylallantoin. The unusual amino acids of nisin, dehydroalanine (DHA) and dehydrobutyrine (DHB), are thought to inactive sulphhydryl groups in germinated spores. The biochemistry, genetics and mode of action of nisin A has been extensively reviewed.

Table 2. Properties of some well characterised bacteriocins

<table>
<thead>
<tr>
<th>Bacteriocin</th>
<th>Organism</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin</td>
<td>Lactococcus lactis subsp. lactis ATCC 11454</td>
<td>Chromosomalplasmid mediated, bactericidal, produced in the growth cycle</td>
</tr>
<tr>
<td>Pediocins A</td>
<td>Lactobacillus sake</td>
<td>Broad spectrum, plasmid mediated</td>
</tr>
<tr>
<td>Pediocin A1I</td>
<td>Lactobacillus sake</td>
<td>Broad spectrum, plasmid mediated</td>
</tr>
<tr>
<td>Leuconostoc gelidum</td>
<td>Broad spectrum, plasmid mediated</td>
<td></td>
</tr>
<tr>
<td>Helveticin</td>
<td>Lb. helveticus 481</td>
<td>Narrow spectrum, chromosomally mediated, bactericidal</td>
</tr>
<tr>
<td>Carnobacterium Carnobacterium piscicola</td>
<td>Narrow spectrum, plasmid mediated, produced early in the growth cycle</td>
<td></td>
</tr>
</tbody>
</table>

Mechanisms of action of bacteriocins

Little is known about the mechanism of action of bacteriocins. For instance, nisin is not bactericidal for the cells in which it is produced. The membrane is the target organ but this property is not found in Gram-negative bacteria. The cell wall is disrupted with phospholipid composition of the membrane may be influenced in the effectiveness of nisin. The combined results obtained in cells, vesicles and liposomes, suggest that the specificity of lactococci A is not unique to Lactococcus lactis and that the nisin receptor protein associated with the cytoplasmic membrane.

The treatment of the cell walls to remove lipopolysaccharide prevented the binding of pediocin A1 from P. acidilactici. It has also been suggested that lipopolysaccharide molecules only in Gram-negative bacteria may be one of the binding sites for pediocin A1. This may be the reason why LAB bacteriocins are not active. Gram-positive bacteria, and not to Gram-negative bacteria. The cell wall is disrupted with phospholipid composition of the membrane may be influenced in the effectiveness of nisin. The combined results obtained in cells, vesicles and liposomes, suggest that the specificity of lactococci A is not unique to Lactococcus lactis and that the nisin receptor protein associated with the cytoplasmic membrane.

Genetic determinants for bacteriocins

The gene or genes encoding bacteriocin production may be located on the chromosome or on plasmids. The production of bacteriocins include plasmid-mediated; while production of helveticins J and lactacin B from Lb. acidophilus N2 are chromosomally-mediated. In the case of nisin, it is not known whether the gene is located on a plasmid or on the chromosome, because no physical evidence linking this phenotype to a distinct plasmid has been obtained. The conjugal transfer of plasmid encoded genes could not be detected in lysates of transconjugants of L. lactis 11454.

Little attention has previously been directed towards the chromosome of these industrially important organisms. The genetic analysis of transposable elements in genetic analysis has received attention in the genus Lactococcus. A broad distribution of the insertion elements has been observed in many lactococcal plasmids and chromosomal DNA, encoding lactose fermenting ability, proteinase activity, and bacteriophage resistance. Many researchers observed the involvement of insertion elements (IS) in the nisin gene. Multiple copies of IS904 within the lactococcal chromosomal backbone downregulate the expression of nisin gene. The nuclear acid sequence of the nisin precursor gives rise to a prepropeptide of 57 amino acids, including a 23 amino acid leader region and a 34 amino acid structural region (spa). No promoter or ribosome binding sites were found. The conclusion of the spaV gene is transcribed from polyribonucleic mRNA.

The genes for nisin production in L. lactis ATCC 11454 are thus located on the chromosome. The restriction patterns indicate that the size of L. lactis genome is about 2.500 kb. The fact that nisin genes lie on a DNA restriction fragment that is one-half the size of the genome rules out the possibility of it being plasmid encoded, unless it is a plasmid that rivals the size of the chromosome which is unprecedented.

To date, antibiotic resistance vectors have been used to study the genetics of LAB because of the ease with which the desired clones can be selected, but these markers are unsuitable for food-grade bacteria. Ideally, a food-grade cloning vector should be constructed from DNA derived from foodborne sources that are approved for food use and contain a selectable marker that does not compromise human drug therapy. A major objective at present is the development of homologous vector systems based upon LAB DNA that are suitable for metabolic or otherwise accessible selection phenotypes for food use, such as genes associated with carbohydrate metabolism, bacteriocin production and resistance. Recently, a food-grade cloning vector, pFM011, employing the Nis phenotype as a selectable marker, has been described that lead to a better understanding of LAB bacteriocins.

Nisin which is produced by lactococci of dairy origin, has been approved for use as a food preservative in over 45 countries. In 1988, it was approved by the US Food and Drug Administration for use in pasteurised cheese to inhibit outgrowth of spores of C. botulinum. Nisin is of particular interest because of its effectiveness against a wide range of Gram-positive bacteria, including the human pathogen C. perfringens, C. botulinum and S. aureus. Although normally resistant to nisin, Gram-negative organisms can be sensitised when the outer membrane is weakened in the presence of cationic agents. Nisin is being exploited as a food preservative mainly in the dairy foods. It is nontoxic and digested by intestinal enzymes. It is heat stable and does not contribute to off-flavours. It has no value in medical therapy because it is typically insoluble in blood at physiological pH. The large size of the molecule precludes absorption if intramuscular injection is used. However, nisin can be used for topical applications.

The role of bacteriocins in the suppression of pathogens is therefore mainly because of their susceptibility to proteolytic enzymes within the gut. However, the search for strains which produce bacteriocins and bacteriocin-like substances resistant to degradation continues because of the potential importance of these agents.

Conclusion

To derive the optimum human benefit from this bacterial antagonist requires the selection of strains with desirable properties. The LAB are not only crucial to the manufacture of fermented products, but they have been shown to be effective in the control of food-borne pathogens, in controlling the growth of unwanted microorganisms, in development of probiotics, in modulation of intestinal flora, in modulation of intestinal flora, and in prevention of tumours.

Although claims for health benefits have been made for LAB in fermented dairy products for nearly a century, the nutritional and therapeutic value of these organisms remains controversial. Clarification of these claims will require further scientific evaluation of antimicrobial products of LAB with stringent strain selection criteria and conduct of well controlled clinical trials. Further research is also required in the area of host specificity, methods of assessing bacterial adherence and bacterial labelling so that quantification, localisation and identification of microbial species can be performed as they pass through the gastrointestinal tract. The future of beneficial interference will depend on a greater understanding of the genetic mechanisms involved.

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Charunmiti Mishra and John Lambert


原始菌（Probiotics）的抗菌素類物質的產生

摘要

細菌的抗菌作用已確認為公元1世紀，但最近這方面所受到科學界的注意，特別是在不同乳酸菌種類的應用上。乳酸菌種類的原始菌類物質使這些細菌具備超過其他細菌的天然殺菌能力。這些微小的天然殺菌劑被稱為乳酸菌。乳酸菌可被用作一種天然殺菌劑，廣泛於食品和飲料業。它們包括乳酸菌、乳酸菌、乳酸菌、乳酸菌、乳酸菌和乳酸菌。乳酸菌的天然殺菌成分將進一步著重於活物質的微生物學研究。
P. aeruginosa 43200 and 43201 are sensitive to proteases and are not protein in nature. Most bacteriocins produced by LAB are bactericidal. However, lactococcin 27 from Lb. helveticus, 2 bacteriocins from Lb. sake 148P, and bacteriocin from Leuconostoc gelidum 3 have a bacteriostatic effect. The term ‘bacteriocin-like substance’ was suggested for those antagonistic substances that do not fit the traditional definition of bacteriocins.

There is no clear boundary between antibiotics, bacteriocins or microcins. Like antibiotics, bacteriocins therefore may be bactericidal or bacteriocal with narrow or broad ranges of action, that might be included in a family of peptide antibiotics. Antibiotics are synthesised nonribosomally by multi-step enzyme pathways. There are many ribosomally synthesised bacteriocins. While many antibiotics are produced by the development of resistant strains, development of resistance to bacteriocin or microcins is rare. However, the possibility of resistance to bacteriocins may have been overlooked. Many antibiotics can be chemically synthesised. There is no report to date in which bacteriocins are chemically synthesised. However, it is appropriate to expect that, with genetic engineering, analogues of bacteriocins may be designed and constructed.

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Lipid and/or carbohydrate moieties can be associated with these proteinaceous components and part of the bacteriocin complex. Bacteriocins can therefore be cell bound or released extracellularly and may be produced early or late in the growth cycle. They are susceptible to proteases and have variable stability to pH and temperature.

The first detailed characterization of bacteriocinogenic activity of lactococcal was reported in 1961. Since then, considerable research has been continuing on bacteriocins from many bacterial species, and the more important properties of some well characterised bacteriocins are shown in Table 2.

Nisin is produced by Lactococcus lactis subsp. lactis of dairy origin. Nisin is the first bacteriocin to be accepted as a food preservative. It has a broad spectrum of activity against Gram-positive organisms and is not destroyed by heat or pasteurization. Nisin is sold in a variety of forms and is used in beverages, ice cream, cheese, and other dairy products.

Mechanisms of action of bacteriocins

Bacteriocins work by binding to the cell wall and membrane of the target bacterium. This binding activates the bacteriocin, which then enters the cell and disrupts the cell membrane, leading to cell death. The bacteriocin may also activate enzymes that destroy the cell wall, leading to cell lysis. Some bacteriocins are able to inhibit the growth of many different bacterial species, including some that are resistant to antibiotics.

Table 2. Properties of some well-characterised bacteriocins

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</tr>
<tr>
<td>Pediocin A</td>
<td>Pediococcus pentosaceus</td>
<td>Produces bacteriocin activity, high broad spectrum, chromosome-mediated, bacterial, produced in the growth cycle</td>
</tr>
<tr>
<td>Pediocin A11</td>
<td>Pediococcus acidilactici</td>
<td>Produces bacteriocin activity, high broad spectrum, chromosome-mediated, bacterial, produced early in the growth cycle</td>
</tr>
<tr>
<td>Leuconocin</td>
<td>Leuconostoc gelidum UAL-187</td>
<td>Produces bacteriocin activity, high broad spectrum, chromosome-mediated, bacterial, produced in the growth cycle</td>
</tr>
<tr>
<td>Helveticin J</td>
<td>Lb. helveticus 48T</td>
<td>Produces bacteriocin activity, high broad spectrum, chromosome-mediated, bacterial, produced in the growth cycle</td>
</tr>
<tr>
<td>Carnobacterin</td>
<td>Carnobacterium piscicola LV17</td>
<td>Produces bacteriocin activity, high broad spectrum, chromosome-mediated, bacterial, produced in the growth cycle</td>
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The restriction patterns indicate that the size of L. lactis genome is about 2.500 kb. The fact that nisin gene lies on a DNA restriction fragment that is one-half the size of the genome rules out the possibility of it being plasmid-encoded, unless it is a plasmid that rivals the size of the chromosome which is unprecedented.

To date, antibiotic resistance vectors have been used to study the effects of LAB because of the ease with which the desired clones can be selected, but these markers are unsuitable for food-grade bacteria. Ideally, a food-grade cloning vector should be constructed from DNA derived from cells that are approved for food use and contain a selectable marker that does not compromise human drug therapy. A major objective at present is the development of homologous vector systems based only upon LAB DNA that are either metabolically or otherwise acceptable for selection phenotypes for food use, such as genes associated with carbohydrate metabolism, bacteriocin production and resistance. Recently, a food-grade cloning vector, pM011, employing the Nis prototype as a selectable marker for LAB, has been lead to a better understanding of LAB bacteriocins.

Nisin which is produced by lacticocci of dairy origin, has been approved for use as a food preservative in over 45 countries. In 1988, it was approved by the US Food and Drug Administration for use in pasteurised cheese and inhibited growth of certain species of bacteria. Nisin is of particular interest because of its effectiveness against a broad range of Gram-positive bacteria such as Lactococcus, Staphylococcus, and Bacillus. Nisin is a potent competitor in the dairy industry, as it is able to inhibit the growth of Gram-positive bacteria and has a low toxicity to humans.

Little attention has previously been directed towards the chromosome of these industrially important organisms. The study of the genetic and metabolic properties of the bacteriocin, in this case nisin, has revealed new insights into the mechanisms of action of these important substances. The properties of nisin have been extensively reviewed in recent years.

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Introduction

Probiotic bacteria are commonly defined as viable bacteria, in single or mixed culture, that have a beneficial effect on the health of the host.

In the dairy industry the most widely used probiotic bacteria belong to the group of lactic acid bacteria, though some bifidobacteria and yeasts are also used. The term ‘probiotic bacteria’ (LBA) currently includes the genera Lactobacillus, Leuconostoc, Pedococcus and Lactococcus. Although some strains of Streptococcus and Enterococcus share the properties of LAB, Streptococcus thermophilus is the only strain currently used in fermented dairy products. The use of LAB in foods has a long history and most strains are considered commensal microorganisms with no pathogenic potential. Their ubiquitous presence in the intestinal microflora and the human gastrointestinal tract, and their traditional use in fermented foods and dairy products without significant problems attest to their safety. Members of the genus Lactobacillus are most commonly given safe or generally recognized as safe (GRAS), whilst members of the genera Streptococcus and Enterococcus contain many opportunist pathogenic factors (Table 1).

The safety of probiotics has been questioned in recent reviews and clinical reports which have drawn attention to cases of human bacteremia associated with the presence of LAB.25

The need for strays of probiotic organisms have been used in the clinical treatment of gastrointestinal disorders in both children and adults. These include conditions where mucosal integrity is impaired by antibiotics or radiotherapy, acute diarrhea of bacterial or viral origin, and in antibiotic-related diarrhoea.3 No evidence of opportunistic infection by probiotics was seen in these studies.

In addition to these clinical studies and animal studies showing an absence of infectivity, toxicity studies have also been carried out confirming the absence of acute toxicity of the studied strains of probiotic bacteria. Although acute toxicity tests were originally designed for chemicals they also give an indication of any harmful effects associated with extremely high doses of freeze-dried bacteria.

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Safety of probiotic bacteria

In recent years interest has been renewed in health promotion and disease prevention by the incorporation of probiotic bacteria into foods to constitute healthful bacteria in the intestinal tract. There is considerable interest in extending the range of foods containing probiotic organisms from dairy foods to infant foods, meat, fish-based products, cereal-based products and pharmaceuticals. New and more specific strains of probiotic bacteria are being sought. Traditional probiotic dairy strains of lactic acid bacteria have a long history of safe use and most strains are considered commensal microorganisms with no pathogenic potential. No evidence of opportunistic infection by probiotics was seen in these studies.

Recent analyses by Saxelin et al26 of clinical isolates of lactobacilli from bacterial infections, particularly those from both strains and strains used in pharmaceutical preparations have confirmed that these LAB are not involved in human infections.

Table 1. Classification of probiotic organisms and their safety status

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pathogenic potential</th>
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<tbody>
<tr>
<td>Lactobacillus</td>
<td>Mainly non-pathogens, some opportunistic infections (usually in immunocompromised patients)</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>Mainly non-pathogens</td>
</tr>
<tr>
<td>Pedococcus</td>
<td>Mainly non-pathogens, some isolated cases of infection</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Oral streptococci mainly non-pathogens (including Streptococcus thermophilus); some may cause opportunistic infections</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>Some strains are opportunistic pathogens with haemolytic activity and antibiotic resistance</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>Mainly non-pathogens, some isolated cases of human infection</td>
</tr>
<tr>
<td>Saccharomyces</td>
<td>Mainly non-pathogens, some isolated cases of human infection</td>
</tr>
</tbody>
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