Antimicrobial Peptides of Probiotic Lactobacillus strains

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In recent years, numerous food poisoning outbreaks, involving various pathogens and food products, and the increasing concern over the preservation of minimally processed foods have spurred growing awareness of the importance of food safety. This has prompted new approaches to inhibit foodborne pathogens. There has been a rapid worldwide increase in pathogenic bacteria that are resistant to multiple antibiotics. There is, therefore, a pressing need to develop new antibiotics and novel antimicrobial agents. Under such conditions potential probiotic cultures could play an important role if taken daily as food adjuncts. In particular, there has been a renewed interest in the antimicrobial activity of lactic acid bacteria (LAB), which has been important for centuries in the preservation of food. Probiotic Lactobacillus rhamnosus strain shows broad spectrum of activity against GIT pathogens and food spoilage organisms.

Keywords: Probiotics; Lactic acid bacteria (LAB); Antimicrobial peptides; bacteriocins

1. Introduction

In the last two decades, antimicrobial peptides have been gaining attention as antimicrobial alternatives to chemical food preservatives and commonly used antibiotics. Due to emergence of multi-drug resistant bacteria as serious problem over the past decades, major research efforts are aimed at finding effective drug(s). Under such conditions Lactic acid bacteria and their metabolites are good alternatives as a source of antimicrobial agents. Primarily Lactobacillus and Bifidobacterium species are found in many dairy foods, normal inhabitants of the human gut and supplements and are currently attracting keen interest as health supplements from both consumers and researchers because of heightened awareness of the beneficial links between health, nutrition and diet [1]. A probiotic is a ‘live microbial food supplements that beneficially affects the host animal by improving the intestinal microbial balance’ [2]. Nowadays, the treatment of gastrointestinal disorders with probiotics is a widely used remedy for intestinal complications in humans. There is a concern that industry will no longer be able to develop effective antibiotics at a rate sufficient to compete with the development of microbial resistance to old antibiotics. These factors have renewed interest in the possibility of deliberately feeding beneficial microorganisms to humans as an alternative to antibiotic therapy in gastrointestinal disorders. The principle of using harmless bacteria for conquering pathogens has been recognized for many years. In fact, probiotics have been used for as long as people have eaten fermented foods. However, it was Metchnikoff at the turn of the 20th century who first suggested that ingested bacteria could have a positive influence on the normal microbial flora of the intestinal tract [3]. He hypothesized that lactobacilli were important for human health and longevity and promoted yogurt and other fermented foods as healthy. Probiotics are usually targeted for use in intestinal disorders in which specific factors (such as antibiotics, medication, diet or surgery) disrupt the normal flora of the gastrointestinal tract, making the host susceptible to disease(s). Examples of such diseases include antibiotic associated diarrhoea, inflammatory bowel diseases. The goal of probiotic therapy is to increase the numbers and activities of those microorganisms suggested to possess health-promoting properties until such time that the normal flora can be re-established.

2. Lactic acid bacteria as Probiotics

During the last decades, it became clear that the human body lives in close harmony with a complex ecosystem that is composed of more than 1000 different bacterial species inhabiting the oral cavity, upper respiratory tract, gastrointestinal tract (GIT), vagina and skin. This collection known as the ‘microbiota’ is acquired soon after birth and persists throughout life. Together, these microbes play an important role in the physiology of their host, including the digestion and assimilation of nutrients, protection against pathogen colonization, modulation of immune responses, regulation of the fat storage, and stimulation of intestinal angiogenesis [4]. However, understanding how these different species contribute to human health remains a major challenge. One main difficulty is correlating the health status of the host with the presence or absence of certain bacterial species, bearing in mind that the microbiota varies extensively among individuals. Within this complex research area on the microbiota, the deliberate administration of probiotic bacteria can contribute substantially to gain better knowledge of beneficial microbe-host interaction whereby fundamental, medical, nutritional, and commercial aspects are taken into account. Lactobacillus and Bifidobacteria are the normal inhabitants of the gastrointestinal tract of humans. The lactobacilli belong to the lactic acid bacteria (LAB), since their main end product of carbohydrate metabolism is lactic acid. The genus Lactobacillus comprises a large heterogeneous group of low G+C content, Gram-positive, non-sporulating and microaerophilic bacteria [5].
Taxonomically, the genus Lactobacillus belongs to the phylum Firmicutes, class Bacilli, order Lactobacillales, family Lactobacillaceae. They are nutritionally fastidious, requiring rich media to grow (carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives, and vitamins) [6].

2.1 Selection of Probiotics
To understand how probiotics work, it is important to understand a little about the physiology, microbiology of GIT and the digestive process. The digestive process begins as soon as food enters the mouth and to stomach, the microbes present in the GIT have the potential to act in a favourable, a deleterious or a neutral manner. Microbes in small intestine and in the large intestine complete the digestion process. Certain intestinal microbes are known to produce vitamins and they are non-pathogenic, their metabolism is non-putrefactive, and their presence is correlated with a healthy intestinal flora. The metabolic end products of their growth are organic acids (lactic and acetic acids) that tend to lower the pH of the intestinal contents, creating conditions less desirable for harmful bacteria. Probiotics may also influence other protective functions of the intestinal mucosa including synthesis and secretion of antibacterial peptides, mucus. The GIT also serves as large mucosal surfaces that bridge the gap between ‘inside the body’ and ‘outside the body’. Along this mucosal interface, microbes and foreign antigens colonizing or passing through the GIT interact with important components of the immune system. This interaction serves to prime or stimulate the immune system for optimal functioning. Normal microbial inhabitants of the GIT also reinforce the barrier function of the intestinal lining, decreasing ‘translocation’ or passage of bacteria or antigens from the intestine into the blood stream [7]. This function has been suggested to decrease infections and possibly allergic reactions to food antigens. When selecting a probiotic strain, a number of aspects should be considered, and the theoretical basis for selection should involve safety, functional as well as technological aspects [8-10].

3. Probiotic properties
Probiotic bacteria must be of human origin, non-pathogenic and genetically stable. Furthermore it is important that they are able to survive passage through the GIT (i.e. low gastric pH and bile acids) and should preferably adhere to the intestinal mucosa so that they are able to colonize the host. The beneficial effects of probiotics are suggested to be due to modulation of the intestinal bacterial flora, adherence to the mucosa thereby preventing pathogens from adhering, changes of total enzyme activities in the colon contents, influences on the immune system of the host and changes in the availability of nutrients and competition for nutrients [11].

3.1 Potential health benefits of probiotic cultures
Probiotic confers the health benefits to the consumers but the efficacy demonstrated for one given bacterial strain cannot be necessarily transferred to other probiotic organisms.

3.2 Antimicrobial action of probiotics
Antimicrobial action of probiotic Lactobacilli may be manifested by one or combination of the following actions including competition for nutrients, adhesion and production of different antimicrobial metabolites such as organic acids, H\textsubscript{2}O\textsubscript{2}, bacteriocins, etc.
LAB produce lactic acid and other organic acids thus lower the pH of the environment and consequently inhibit the growth of the bacterial pathogens. The cell free L. casei subsp. rhamnosus Lcr35 supernatant inhibited the growth of human pathogenic bacteria: Enterotoxigenic Escherichia coli (ETEC), enteropathogenic Escherichia coli, Klebsiella pneumoniae, Shigella flexneri, Salmonella typhimurium, Pseudomonas aeruginosa, Enterococcus faecalis, and Clostridium difficile [12]. In vitro antimicrobial activity of L. acidophilus against clinical isolates of Helicobacter pylori is attributed to lactic acid [13]. In another report, in vitro anti-H. pylori by different Lactobacilli strain are associated with production of lactic acid and other organic acids [14]. Alokomi et al., observed that the lactic acid produced by Lactobacillus acts as a permeabilizer of the Gram-negative bacterial outer membrane, allowing other antimicrobial substances produced by the host to penetrate and thereby increasing the susceptibility of pathogens to these antimicrobial molecules [15].
Production of H\textsubscript{2}O\textsubscript{2} by Lactobacillus spp. may be a non-specific antimicrobial defense mechanism of the normal vaginal ecosystem [16-17]. Hydrogen peroxide inhibits both Gram-positive and Gram-negative organisms. Production of bacteriocins, recent reports have revealed that some intestinal lactobacilli and bifidobacteria produce antimicrobial substances that are active against these enteropathogens. Bacteriocins are ribosomally synthesized antimicrobial peptides and bactericidal proteinaceous molecule produced by bacteria. The term “bacteriocins” was originally coined in 1953 by Jacob, specifically to define protein antibiotics of the colicin type, but it is now accepted to include peptide inhibitors from any bacteria [18]. Tagg in 1991 proposed the term “bacteriocins-like inhibitory substance” for designating the antimicrobial protein from Gram-positive microorganisms, to tell them apart from...
colicins which is produced by *E. coli*. Today, however, most antimicrobial peptides are named “bacteriocin”, irrespective of Gram-positive or Gram-negative origin [19]. The bacteriocin family includes a wide variety of peptides and proteins in terms of their size, microbial targets, and mechanism of action and immunity [20]. Bacteriocins are divided into four main categories as described in table 1 [21-22]. Although bacteriocin may enhance survival of LAB in complex ecological system, interest has focused on prevention of growth of harmful bacteria in the fermentation, preservation of dairy products and as anti-infective drug. It is therefore more interesting with respect to probiotics that individual strains may inhibit growth or adhesion of pathogenic microorganism by secreted products like bacteriocin and not merely an effect of acidic pH. There are many evidences reporting secretory antibacterial components produced by LAB having broad range of activity against Gram-positive and Gram-negative organisms [23], which are independent of lactic acid and hydrogen peroxide. However the overall antimicrobial activity of LAB may be due to a synergistic action of lactic acid and proteinaceous substances. Many lactic acid bacteria produce antibacterial peptides, bacteriocins; including lactacin B from *Lactobacillus acidophilus*, plantaricin from *Lactobacillus plantarum* and nisin from *Lactococcus lactis*, have a narrow spectrum of activity acting only against closely related bacteria [24]. Antimicrobial peptides produced by *L. rhamnosus* are distinct from bacteriocins produced by other *Lactobacillus* spp., as they exhibit a broad spectrum of activity against Gram-positive and Gram-negative organisms and may belong to least characterized fourth class of complex bacteriocins [25-26].

### Table 1. Classification of bacteriocins from Lactic acid bacteria

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
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<tbody>
<tr>
<td>I. Lantibiotics</td>
<td>Ribosomally produced peptides that undergo extensive post-translational modification</td>
</tr>
<tr>
<td></td>
<td>Small (&lt;5 kDa) peptides containing lanthionine and methyl lanthionine</td>
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<td></td>
<td>Ia. Flexible molecules compared to Ib</td>
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<td></td>
<td>Ib. Globular peptides with no net charge or net negative charge</td>
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<tr>
<td>II. Nonlantibiotics</td>
<td>Low-molecular-weight (&lt;10 kDa), Heat stable peptides</td>
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<tr>
<td></td>
<td>Formed exclusively by unmodified amino acids</td>
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<td></td>
<td>Ribosomally synthesized as inactive peptides that get activated by post-translational cleavage</td>
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<tr>
<td></td>
<td>of the N-terminal leader peptide</td>
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<tr>
<td></td>
<td>Iia. Anti-listerial single peptides that contain YGNGGVXC amino acid motif near their N termini</td>
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<td></td>
<td>Iib. Two peptide bacteriocins</td>
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<tr>
<td></td>
<td>Iic. Bacteriocin produced by the cell’s general see-pathway</td>
</tr>
<tr>
<td>III. Nonlantibiotics</td>
<td>High-molecular-weight (&gt;30 kDa), heat labile proteins</td>
</tr>
<tr>
<td>IV Complex bacteriocins</td>
<td>Carrying lipid or carbohydrate moieties, which appear to be required for activity</td>
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<tr>
<td></td>
<td>Such bacteriocins are relatively hydrophobic and heat stable</td>
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</table>

### 3.3 Mode of action of bacteriocins

Bacteriocins may possess a bactericidal or bacteriostatic mode of action on sensitive cells, this distinction being greatly influenced by several factors such as bacteriocins dose and degree of purification, physiological state of the indicator cells (growth phase) and experimental conditions (eg., temperature, pH, other antimicrobial compounds). Most bacteriocins exert bactericidal mode of action against the sensitive microorganisms, although some have been shown to act in a bacteriostatic manner. The majority of bacteriocins kill susceptible bacteria by membrane permeabilization or by interference with essential enzymes; Nisin forms a complex with ultimate cell wall precursor lipid II, thereby inhibiting the cell wall biosynthesis. Subsequently the complex aggregates and incorporates further peptides to form a pore in the bacterial membrane. Several theories have been proposed to explain the exact mechanism by which antimicrobial peptides kill bacteria. The ‘barrel-stave’ mechanism describes the formation of transmembrane channels/pores by bundles of peptides. Progressive recruitment of additional peptide monomers leads to steadily increasing pore size. Leakage of intracellular components through these pores subsequently causes cell death. The ‘carpet-like’ peptide first bind on to the surface of the target microbial cell membrane and subsequently the membrane is covered by ‘carpet-like’ clusters of peptides and cause membrane permeation, leads to lysis of the microbial cell [27].
4. Antimicrobial peptides of probiotic Lactobacillus rhamnosus

Antimicrobial activity of probiotic L. rhamnosus is associated with cell free culture filtrate (CFC) and extracellular low molecular weight proteins (EPC) present in CFC filtrate. L. rhamnosus produces antimicrobial peptides which inhibit Escherichia coli, Enterobacter aerogenes, Salmonella typhi, Shigella sp., Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Bacillus cereus, Bacillus megaterium, Bacillus subtilis and Staphylococcus aureus and also reported against Klebsiella pneumoniae, Helicobacter pylori, Campylobacter jejuni, Micrococcus luteus and Listeria monocytogenes [25-26]. The antimicrobial peptides are produced during exponential and stationary phases. These antimicrobial peptides are active over broad pH range (2-9) except against few test organisms like P. vulgaris, Bacillus spp., S. aureus and also sensitive to proteolytic enzymes like proteinase K, trypsin and pepsin indicates proteinaceous nature of inhibitory compound. Heat stability of low-molecular-weight (4 kDa) peptides, which would be advantageous for transport and usage as a drug has been suggested to arise from complex patterns of disulphide intramolecular bonds that stabilise secondary structures by reducing the number of possible unfolded structures [27-29]. Interestingly, the novel antimicrobial agent was not active against several related bacteria, including L. casei, L. rhamnosus. Antimicrobial peptides from Lactobacillus rhamnosus GG has been characterised seven small peptides from, two of which were NPSRQERR and PDENK, that retained the antimicrobial activity detected with LGG conditional media. The antimicrobial activity of these peptides was observed against both Gram-negative E. coli EAEC 042 and S. typhimurium and with less potency against Gram-positive Staph. aureus [30]. The most well-known natural antimicrobial peptides in commercial use is nisin. This natural peptide was derived from Lactococcus lactis and was approved for use in food by the Joint FAO/WHO Expert Committee on Food Additives in 1969. Nisin is now used in over 50 countries as a food preservative in a broad range of foods ranging from dairy products and meat to beer [31]. A number of other bacterially-derived antimicrobial peptides are also used as food preservatives [32]. Use of small peptides as anti-infective drugs is a novel approach and would offer several advantages over current treatments [33]. Here we have demonstrated that novel antimicrobial peptides from L. rhamnosus have a broad spectrum of activity against a wide range of pathogens, including major GIT pathogens and food spoilage organisms. It is accepted that the use of antimicrobial peptides would be safe for the treatment of infectious diseases owing to the fact that they are produced by probiotic bacteria [30]. However, more studies are necessary on purification of these peptides, characterization, mode of action and the possibility of using this novel antimicrobial agent as an anti-infective agent and to progress it through pre-clinical studies in animal model.

5. Other health benefits of Probiotics

A number health benefits are claimed in favour of probiotic products including antimicrobial activity and gastrointestinal infections, acute diarrhoea, antibiotic-associated diarrhoea, rotaviral diarrhoea, improvement in inflammatory bowel diseases, anti-Helicobacter pylori activity, lactose intolerance, antimutagenic properties [34], anticarcinogenic properties, reduction in serum cholesterol, immune system modulation, decreasing the activity of some colonic enzymes such as azoreductase and nitroreductase. Health benefits imparted by probiotic bacteria are strain specific, and not species- or genus-specific. It is important to note that no strain will provide all proposed benefits, not even strains of the same species, and not all strains of the same species will be effective against defined health conditions [35].

6. Conclusions

In the past decades there has been considerable interest in probiotics. Lactic acid bacteria have been used in fermentation since antiquity and recently have attracted keen interest due to their additional health benefits. Moreover, Lactobacillus rhamnosus strain has shown probiotic properties like acid-bile, phenol tolerance and antimicrobial activity against food spoilage organisms and GIT pathogens. Antimicrobial peptides of L. rhamnosus have gained attention due to their broad antimicrobial spectrum and novel antimicrobial agents due to rapid increase in pathogenic microbes which are resistant to conventional antibiotics. Probiotics Lactobacillus can be used as an adjuvant or alternative therapy in gastrointestinal disorders as they are considered safe, non-pathogenic and normal inhabitants of human GIT. Therefore, it is possible to conceptualize the use of probiotics or probiotics-derived peptides as anti-infective agents, due to their broad antimicrobial spectrum providing good alternative over the antibiotics. Probiotic L. rhamnosus strain has potential to be used as an antimicrobial microbe, anti-infective agent, bio-control, food additive and as biopreservatives due to its broad antimicrobial spectrum.

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References


