

**BACTERIOCIN PEPTIDES OF PROBIOTICS****Rita Narayanan\* and B. Suresh Subramonian**

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**ABSTRACT**

Human body and health encompass a close harmony with a complex ecosystem that is composed of more than 1000 different bacterial species inhabiting the gastrointestinal tract, vagina and skin. This ecosystem of microbiota' is acquired soon after birth and persists throughout life. 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. Consumers are now aware of the importance of food safety, as many of the chemical additives used in food may raise issues of health risks. Commercially available preservatives may cause certain health discomforts. Moreover the use of antibiotics or residues in food is illegal. Antimicrobial peptides have been gaining attention as antimicrobial alternatives to chemical food preservatives. Unlike chemical preservatives, bacteriocins are "generally recognized as safe" (GRAS) and promises safe use as food preservatives in vegetables, dairy, processed cheese, meats, and other food products, as they inhibit microorganisms contamination (Deegan *et al.*, 2006; Settanni and Corsetti, 2008). Bacteriocins are a kind of ribosomal synthesized antimicrobial peptides produced by bacteria, which can kill or inhibit other bacterial strains closely-related or non-related.

One of the major concerns is the emergence of multi-drug resistant bacteria over the past decades. 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 and normal inhabitants of the human gut. Their



use as health supplements are currently attracting keen interest from both consumers and researchers due to the awareness of the beneficial links between health, nutrition and diet (Stanton *et al.*, 2001).

Probiotic which include *Lactobacillus* and *Bifidobacterium* 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 to colonize the host. The beneficial effects of probiotics are manifested by modulation of the intestinal bacterial flora, adherence to the mucosa thus preventing pathogens from adhering, production antimicrobial metabolites such as organic acids, H<sub>2</sub>O<sub>2</sub>, bacteriocins, changes of total enzyme activities in the colon contents, influence on the immune system of the host and competing for nutrients (Goossens *et al.*, 2003).

LAB produces lactic acid and other organic acids thus lowering the pH of the environment and consequently inhibit the growth of 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* (Forestier, *et al.*, 2001) *In vitro* antimicrobial activity of *L. acidophilus* against clinical isolates of *Helicobacter pylori* is attributed to lactic acid (Bhati *et al.*, 1989). In another report, *in vitro* anti-*H. pylori* by different Lactobacilli strain are associated with production of lactic acid and other organic acids (Midolo *et al.*, 1995). Alokomi *et al.*, (2000) 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.

Production of H<sub>2</sub>O<sub>2</sub> by *Lactobacillus spp.* may be a non-specific antimicrobial defense mechanism of the normal vaginal ecosystem (Reid and Burton, 2002).

Hydrogen peroxide inhibits both Gram-positive and Gram-negative organisms. Production of bacteriocins, in recent reports have revealed that some of the intestinal lactobacilli and bifidobacteria produce antimicrobial substances that are active against 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. Many microorganisms, such as bacteriocin producing LAB, are used to start cultures or co-cultures in food production processes for increasing flavor and prolonging shelf-life. 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. The bacteriocin family includes a wide variety of peptides and proteins in terms of their size, microbial targets, and mechanism of action and immunity.

## CLASSIFICATION OF BACTERIOCINS ARE AS FOLLOWS

### Bacteriocins from Gram-Negative Bacteria

#### Colicins

Colicins are antibacterial proteins produced by bacteria, which can kill bacterial strains closely related to a produced species, in order to reduce environmental competitors for acquiring nutrients and living space. Colicins are organized in three specific domains, an amino-terminal translocation (T) domain, which is implicated in the transfer across the outer membrane via the translocator protein; a central receptor-binding (R) domain, which is bound with a bacterial outer membrane receptor; and a carboxy-terminal cytotoxic (C) domain, which has antibacterial activity (Cascales *et al.*, 2007; Kleanthous, 2010). When a bacterial outer membrane surface has the colicins recognition receptors protein and the translocators protein system, the colicins are transported into the bacteria, which kills it, and are known as sensitive strains. For a particular colicin, non-receptor protein bacteria are classified as resistant strains. Bacteria with a deficiency of translocator protein system are classified as tolerant strains, which produce immunity proteins are classified as immune strains. Resistant, tolerant, and immune strains of bacteria would not be killed by corresponding colicins. When colicins enter the target cell, they can be divided into three categories based on bactericidal mechanisms: (1) Pore-forming type colicins: the formation of pores or channels in the inner-membrane cause (2) leakage of cytoplasmic compounds, destruct electrochemical gradient, ion loss, (3) and cell death.

#### Microcins

Microcins predominantly produced by *Enterobacteriaceae* are low molecular weight ribosomal synthesized hydrophobic antimicrobial peptides (<10 kDa), which is distinguished



by 25–80 kDa high molecular weight colicins protein. Microcins are produced as precursor peptides, including N-terminal leader peptide and core peptides. Microcin precursor peptides may or may not undergo a post-translational modification process in the course of maturation to an active microcin. Microcins are show great tolerance to heat, extreme pH, and proteases (Rebuffat, 2012). The bactericidal mechanisms of microcins are diverse, including the pore-forming type, the nuclease type, such as DNase and RNase functions, and inhibitors of protein synthesis or DNA replication.

### **Bacteriocins from gram-positive bacteria**

Unlike colicins from Gram-negative bacteria, which are plasmid or chromosome encoded 25–80 kDa proteins, the Gram-positive bacteria bacteriocins exert similar characteristics to microcins. These gene-encoded bacteriocins are low molecular weight antimicrobial peptides with less than 60 amino acids. In Gram-positive bacteria, lactic acid bacteria (LAB) are the typical bacteria producing a variety of bacteriocins of different sizes, structures, physicochemical properties, and inhibitory spectrum. Due to the large diversity of bacteriocins, some investigations show different ways to classify bacteriocins from Gram-positive bacteria.

### **Classification of bacteriocins from Lactic acid bacteria**

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|---------------------|---|
| I. Lantibiotics     | Ribosomally produced peptides that undergo extensive post-Translational modification.<br>Small (<5 kDa) peptides containing lanthionine and methyl lanthionine.<br>Ia. Flexible molecules compared to Ib.<br>Ib. Globular peptides with no net charge or net negative charge.   |
| II. Nonlantibiotics | Low-molecular-weight (<10 kDa), Heat stable peptides.<br>Formed exclusively by unmodified amino acids.<br>Ribosomally synthesized as inactive peptides that get activated by posttranslational cleavage of the N-terminal leader peptide.<br>IIa. Anti-listerial single peptides that contain YGNGGVXC amino acid motif near their N termini.<br>IIb. Two peptide bacteriocins.<br>IIc. Bacteriocin produced by the cell's general sec-pathway. |



- III. Nonlantibiotics      High-molecular-weight (>30 kDa), heat labile proteins carrying lipid or carbohydrate moieties, which appear to be required for activity.
- IV Complex bacteriocins      Such bacteriocins are relatively hydrophobic and heat stable (Pithva *et al.*, 2011)

Bacteriocins are now widely used in food science to extend food preservation duration and pharmaceutical industry and medical society.

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