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REVIEW ARTICLE

HEALTH CARE APPLICATIONS OF BACTERIOCIN PRODUCING LACTIC ACID BACTERIAL ISOLATES

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ABSTRACT

The frequent use of antibiotics has led to a rise in the antibiotic resistance of pathogens associated with humans and animals. Antibiotic resistance and the emergence of multi-drug resistant bacterial pathogens have led to the investigation of alternative antimicrobial agents to treat and prevent infections in both humans and animals. Research on antimicrobial peptides, with a special interest on bacteriocins of lactic acid bacteria (LAB), is entering a new era with novel applications other than food preservation. Many scientists are now focusing on the application of these peptides in medicinal and personal care products. Bacteriocins are small, cationic, amphiphillic peptides produced by some strains of LAB that exhibit structural features typical of members of the eukaryotic channel-forming amphipathic peptides and display antimicrobial activity against other bacteria. Some bacteriocins exhibit a much broader spectrum of antimicrobial activity and may extend beyond the borders of bacteria to include protozoa, yeast, fungi and viruses. Use of live probiotic bacteria may have prophylactic applications, but use of purified bacteriocins appears to be more attractive for eradicating an established infection. Some of the studies have also established bacteriocins as potent spermicidal and anti-neoplastic agents with very impressive market value in the form of health care products.

INTRODUCTION

Lactic Acid Bacteria

LAB have an important role as probiotic starter culture due to increasing consumer awareness of the potential risks derived not only from food borne pathogens, but also the artificial chemical preservatives used to control them. LABs include a group of Gram positive bacteria from the genera *Bifidobacterium, Streptococcus, Lactococcus, Lactobacillus, Carnobacterium, Enterococcus and Pediococcus* which are non-spore forming, cocci or rods and produce lactic acid as the major end product of the fermentation of carbohydrates (Rodriguez *et al.,* 2000). LAB are used in the food industry because they have got "GRAS-Generally Recognised As Safe" status. Their growth lowers pH that inhibits growth of most of the other microorganisms and many strains produces antagonistic compounds such as hydrogen peroxide, diacetyl and bacteriocins (Ray and Daeschel, 1994). The biochemical conversions involved in their growth results in enhancement of flavor, improvement in organoleptic and nutritional properties of foods.

Bacterial species primarily used as probiotic cultures are *Lactobacillus acidophilus* (La2, La5, Johnsonii, NCFM, DDS-1, SBT-2062), *L. bulgaricus* (Lb12), *L. lactis* (La1, A164, BH5), *L. plantarum* (299v, Lp01), *L. rhamnosus* (GG, GR-1, 271, LB21), *L. reuteri* (SD2112), *L. fermentum* (RC-14), *Bifidobacterium longum* (BB536, SBT-2928), *B. breve* (Yakult), *B. bifidum* (Bb-12), *B. esselnsis* (Danone{Bio Activia}), *B. lactis* (Bb-02), *B. infantis* (Shirota, Immunitass, 744, 01) (Krishnakumar and Gordon, 2001). Various LAB strains used as probiotic cultures are enlisted in **table 1**.

Bacteriocin Production Trait In Lab

Many probiotic strains exhibit their antimicrobial property by synthesizing proteinaceous toxins that inhibit the growth of similar or closely related bacterial strain(s). Gratia in 1925 frist discovered a antimicrobial bacteriocins of *E. coli* which were designated as 'colicines'. Production of pediocins by several strains of *Pediococci* including *P. acidilactici, P. pentosaceous, P. damnosus* (Kaur and Balgir, 2007), carnocin is produced by a strain of *Carnobacterium* and Nisin by *Lactococcus lactis* (Hurst, 1981) is reported in literature.

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Table 1 Lactic acid bacteria used as probiotic cultures

Strain	Reference
L. salivarius WB1004	Kabir *et al*., 1997
L. acidophilus (johnsonii) La1	Michetti *et al*., 1999
L. acidophilus	Canducci *et al*., 2001
L. acidophilus CRL 639	Lorca *et al*., 2001
L. gasseri OLL2716	Sakamoto *et al*., 2001
L. GG	Armuzzi *et al*., 2001
Bacillus subtilis	Pinchuk *et al*., 2001
L. reuteri	Mukai *et al*., 2002
Weisella confusa	Nam *et al*., 2002
Lactobacilli and Bifidobacteria	Wang *et al*., 2004
L. casei strain Shirota	Cats *et al*., 2003
L. casei strain Shirota and L. acidophilus	Tursi *et al*., 2004
L. casei subsp. DG	Sgouras *et al*., 2004
L. brevis	Linsalata *et al*., 2004
L. rhamnosus (R0011) and L. acidophilus (R0052)	Johnson-Henry *et al*., 2005
Bacillus clausii	Nista *et al*., 2004
L. reuteri and L. paracasei	Pena *et al*., 2005
L. salivarius	Kieran *et al*., 2008

LABs commonly harbour plasmid-borne genetic determinants for bacteriocin production and for maintaining immunity of the producer cells to their bacteriocins (Klaenhammer, 1993). Yet, there have been reports suggesting that chromosomal determinants may be involved as well in the bacteriocin production (Kawai *et al.,* 2000). Most commonly, the bacteriocin structural and immunity genes are organized in a cluster with two other genes that produce dedicated proteins for export of the bacteriocin from the cell. Lactococcins A, B and M are all encoded on one plasmid with separate secretion protein (Belkumm *et al.,* 1992). *Carnobacterium piscicola* LV17 produces at least three class II bacteriocins: carnobacteriocins A and B2, which are encoded on separate plasmids with separate secretion proteins, and carnobacteriocin BM1, with its structural and immunity genes located on the bacterial chromosome (Ahn *et al.,* 2003). Bacteriocins are used as an alternative to antibiotics and may also replace chemical preservatives in food. Few differences between bacteriocin and antibiotics are mentioned in **table 2** given below.

Table 2 Comparision between characteristic of bacteriocin and antibiotics `

Characteristic	Bacteriocins	Antibiotics
Application	Food	Clinical
Synthesis	Ribosomal	Secondary metabolite
Activity	Narrow spectrum	Varying spectrum
Host cell immunity	Yes	No
Mechanism of target cell	Usually adaptation affecting cell	Usually a genetically transferable
Resistance or tolerance	Membrane composition	Determinant affecting different sites depending the mode of action
Interaction requirements	Sometimes docking molecules	Specific target
Mode of action	Mostly pore formation, but in a few cases possibly cell wall biosynthesis	Cell membrane or intracellular targets
Toxicity/side effects	None known	Yes

Mechanism of Bacteriocin Action

Bacteriocin activity is usually lethal to the bacteria. Mode of action of bacteriocin was extensively reviewed by Moll *et al.* (1999). Various mechanisms have been proposed to describe the bactericidal action of bacteriocins. Bactericidal activities include the formation of selective or nonselective, anion carrier pores, inhibition of the outgrowth of spores and modulation of enzyme activity (Edward and Morwood, 1993). The relatively small action spectrum of some bacteriocins suggests the presence of molecular receptors in the membrane of the target cell, although this has not been demonstrated (Van Belkum and Stiles, 2000). Bacteriocins are classified on the basis of their mode of action and structure into V classes. Some Class I members such as nisin have a dual mode of action: they can bind to phospholipid layer in cell membrane (Lipid II), a universal receptor and the main subunit transporter of peptidoglycan from the cytoplasm to the cell wall, thereby blocking proper cell wall synthesis and contributing to cell death. In addition, nisin binds to lipid II by two of its amino terminal rings forming a complex of eight lantibiotic bacteriocins and four lipid II to initiate the process of membrane insertion and pore formation, which leads to rapid cell death (Breukink and De Kruijff, 2006). In general, Class II peptides have a helical amphiphilic structure that allows them to be inserted into the membrane of the target cell, leading to depolarization and cell death (Drider *et al*., 2006). The initial interaction with the heads of the anionic membrane phospholipids takes place at the hydrophilic N-terminus of the peptides. The C-terminus of the peptide is more hydrophobic than the N-terminal and it is thought to be involved in hydrophobic interactions with the membrane. Class III bacteriocins can act directly on the cell wall of Gram-positive target cells, leading to death and cell lysis. Class IV group consists of glycoproteins (lactocin 27) and lipoproteins (lacstrepcins) that require non-protein moieties for their activity (Ennahar *et al.,* 2000). Class V have unique functional activities as well as circular nature make them potential candidates for developing novel antimicrobial agents (Kumar *et al.,* 2012a).

Antimicrobial Spectrum of Bacteriocin

LAB produce a variety of antibacterial factors and their inhibitory spectrum varies between narrow and broad range depending upon the LAB species. Usually, it is limited only to some closely related organisms, but occasionally the antibacterial effect may cover a large group of organisms. A number of pathogenic and non-pathogenic tested Gram-positive bacterial strains were inhibited by the bacteriocin producing Lactic acid bacteria.

- **Gram-positive strains:** *Enterococcus faecalis*; *Leuconostoc mesenteroides*; *Listeria monocytogenes*; *Gardnerella vaginalis*; *Micrococcus flavus*; *Propionibacterium acnes*; *P. acnes*; *Staphylococcus albus*; *S. aureus*; *S. aureus*; *Streptococcus faecalis*; *S. pyogenes*; *S. thermophilus* (Kaur *et al.,* 2012a)
- **Gram-negative pathogenic indicator strains:** *Bacteriodes fragilis*; *B. ovatus*; *B. vulgates*; *E. coli* DH5α; *E. coli* KL 16; *H. pylori*; *K. pneumoniae*; *K. pneumoniae*; *P. mirabilis*; *P. aeruginosa*; *V. cholrae*;*Y. enterocolitica* (Kaur *et al.,* 2012a)
- **Yeast indicator strain:** *C. albicans* (Kaur *et al.,* 2012a)

Health Effects of Probiotics

Probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and

well-being of the host. This definition implies that probiotics do not necessarily need to be viable. Non-viable forms of probiotics have also been shown to have health effects. The definition does not restrict the use of probiotics in foods; several other applications have been reported to have beneficial health effects. Not only whole microbial cells, but also parts of cells have been observed to improve host health. Following are some health effects of probiotics:

1. ***Intestinal Effects:*** Probiotics improve gut health by increasing intestinal absorption and gut movement. They decrease the ability of pathogenic bacteria to colonize gut epithelium of probiotics relieves symptoms of constipation and diarrhea.
2. ***Treatment of Peptic ulcer:*** Peptic ulcers are produced by an imbalance between the gastro-duodenal mucosal defense mechanisms and damaging forces of gastric acid and pepsin, combined with superimposed injury from environmental or immunologic agent or due to bacterial infection. A major causative factor (60% of gastric and up to 90% of duodenal ulcers) is chronic inflammation due to *H. pylori* that colonizes the antral mucosa. Pediocin BA28 produced by *Pediococcus acidilactici* BA28 is reported to inhibit *H. pylori* (Kaur *et al*., 2012a) that is a candidate strain for formulating topical personal care therapeutics aimed at prevention and treatment of many human diseases especially peptic ulcers.
3. ***Treatment of Bacterial vaginosis (BV):*** BV is a polymicrobial, superficial vaginal infection in the reproductive age affecting both pregnant and nonpregnant women and is caused due to disturbance in natural balance of vaginal microflora. BV is classified as *Gardnerella*-associated vaginitis and nonspecific vaginitis**.** *Gardnerella*-associated vaginitis or just vaginitis is an abnormal vaginal condition that results from an overgrowth of opportunistic pathogenic bacteria. Pediocin BA28 and fermenticin HV6b produced by *Pediococcus acidilactici* BA28 and *Lactobacillus fermentum* HV6b respectively are reported to inhibit *G. vaginalis* growth *in vitro* (Kaur *et al*., 2012a; 2012b).
4. ***Spermicidal activity:*** Bacteriocins such as pediocin CP2 and fermenticin HV6b were shown to significantly reduce motility of the human spermatozoa in a concentration-dependent manner. In the starting, sperms have progressive movement which is characteristic of 'grade a' category according to WHO. But upon exposure to higher concentrations of the fermenticin HV6b, coiling, clumping and agglutination of sperms was observed that dropped down to grade d. Coiling of the sperm tails is considered to be an abnormality and may indicate damage to the plasma membrane (Kumar *et al*., 2012b).
5. ***Anticancerous activity:*** Bacteriocin such as pediocin CP2 and fermenticin HV6b have a significantly higher cytotoxicity and damage of chromosomal DNA in bacteriocin tested cell lines. *In vitro* studies conducted against different tissue models have indicated its potential to be used as a component of anticancerous drug therapy as it is reported to induce apoptosis in cancerous cells (Kumar *et al*., 2012b).
6. ***Other health benefits:*** Probiotic LAB strains help to restore a healthy microbial balance in the digestive tract (Karen Collins, 2007). They promote recovery from antibiotic associated diarrhea, constipation, diarrhea and dysentery (Szajewska and Mrukowicz, 2005). They contribute to health by enhancing specific and nonspecific immunity (Schiffrin *et al*., 1997). They are widely recommended to treat milk allergies caused primarily by lactose content (Kirjavainen *et al*., 2003). They are frequently used to treat allergies such as atopic eczema in pregnant women and newborns (Kukkonen *et al*., 2007). Probiotics may help to prevent liver damage caused by excessive alcohol intake (Kirpich *et al*., 2008). They reduce risk of certain cancers (Kulkarni and Reddy, 1994).

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