HEALTH CARE APPLICATIONS OF BACTERIOCIN PRODUCING LACTIC ACID BACTERIAL ISOLATES

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REVIEW ARTICLE

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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, Johnonii, NCFM, DDS-1, SBT-2062), L. bulgaricus (Lb12), L. lactis (La1, A164, BH15), 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. eselnsis (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 first discovered a antimicrobial bacteriocins of E. coli which were designated as ‘colicines’. Production of pediocins by several strains of Pediococci including P. acidilactici, P. pentosaceus, 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.
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. Lactococccins A, B and M are all encoded on one plasmid with separate secretion protein (Belkum 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 1 Lactic acid bacteria used as probiotic cultures**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. salivarius WD1004</td>
<td>Kabie et al., 1997</td>
</tr>
<tr>
<td>L. acidophilus (johnsonii) Lal</td>
<td>Michetti et al., 1999</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>Canuddci et al., 2001</td>
</tr>
<tr>
<td>L. acidophilus CRL 639</td>
<td>Lorca et al., 2001</td>
</tr>
<tr>
<td>L. gasseri OLL2716</td>
<td>Sakamoto et al., 2001</td>
</tr>
<tr>
<td>L. GG</td>
<td>Armuzi et al., 2004</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>Puchuk et al., 2001</td>
</tr>
<tr>
<td>L. reuteri</td>
<td>Makai et al., 2002</td>
</tr>
<tr>
<td>Weissella confusa</td>
<td>Nam et al., 2002</td>
</tr>
<tr>
<td>Lactobacilli and Bifidobacteria</td>
<td>Wang et al., 2004</td>
</tr>
<tr>
<td>L. casei strain Shirato</td>
<td>Cats et al., 2003</td>
</tr>
<tr>
<td>L. casei strain Shirato and L. acidophilus</td>
<td>Tursi et al., 2004</td>
</tr>
<tr>
<td>L. casei subsp. DG</td>
<td>Sgouras et al., 2004</td>
</tr>
<tr>
<td>L. brevis</td>
<td>Linsalata et al., 2004</td>
</tr>
<tr>
<td>L. rhammosus (RO011) and L. acidophilus</td>
<td>Johnson-Henry et al., (RO052) 2005</td>
</tr>
<tr>
<td>L. brevis</td>
<td>Nista et al., 2004</td>
</tr>
<tr>
<td>L. reuteri and L. paracasei</td>
<td>Pena et al., 2005</td>
</tr>
<tr>
<td>L. salivarius</td>
<td>Kieran et al., 2008</td>
</tr>
</tbody>
</table>

**Table 2 Comparison between characteristic of bacteriocin and antibiotics**

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

**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. Bacterial 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 (lactespins) 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**: Bacteroides fragilis; B. ovatus; B. vulgatus; E. coli DH5α; E. coli KL 16; H. pylori; K. pneumoniae; K. pneumoniae; M. mirabilis; P. aeruginosa; V. cholerae; 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 fermentin 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 fermentin 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 fermentin 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 fermentin 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 (Sazewska 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).

References


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