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Review

Lactobacillus acidophilus bacteriocin, from production to their application: An overview

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Antimicrobial proteinaceous compounds such as bacteriocins or bacteriocin-like compounds produced by *Lactobacillus acidophilus* are largely known and have been found to have potent antimicrobial activities toward closely related bacteria and undesirable harmful microorganisms. They are useful in the fields of food preservation or safety, health care, and pharmaceutical applications. The inhibition activity of these substances has been reported to be strain-dependent. Binding to the epithelial cell on the gastrointestinal surfaces is one of the important factors of resident microflora to colonize the intestine. Certain *L. acidophilus* strains are able to produce substances that compete and prevent pathogenic bacteria from adhering to the receptors on epithelial cells of intestinal surfaces. The potential probiotic effects of *L. acidophilus* is well known in the human ecosystem and their production of antimicrobial peptides can contribute to elucidate the precise mechanisms by which *L. acidophilus* can dominate the intestinal microbiota and achieve their probiotic function. This paper presents a review of the antimicrobial proteinaceous compounds produced by various acidophilus strains, the attempts made to purify them, their characterization and useful applications.

Key words: *Lactobacillus acidophilus*, bacteriocin, application.

INTRODUCTION

Lactobacillus acidophilus nonpathogenic and a member of the normal intestinal microflora is widely used in fermented dairy products and is of considerable industrial and medical interest because it has been reported to aid in the reduction of the levels of harmful bacteria and yeasts in the small intestine and to produce lactase, an enzyme which is important for the digestion of milk (Deraz et al., 2007). Therefore *L. acidophilus* group of lactic acid bacteria (LAB) is added as dietary adjuncts to commercial fermented milk products and the intake of these bacteria may have beneficial effects on human health (Kawai et al., 2001).

The properties of *L. acidophilus* have been investigated in order to establish its specific role in the complex microbial intestinal equilibrium, both of man and higher animals. (Sarra et al., 1980) The *L. acidophilus* has been considered to be the predominant lactobacillus in the intestinal tract of healthy humans (Ray, 1996). *L. acidophilus* strains have been widely utilized as a dairy starter culture for their therapeutic activities associated with an intestinal microbial balance, and has been used in fermented foods, and as a probiotic in dietary supplements (Sanders and Klaenhammer, 2001). Oda et al. (1994) also showed that *L. acidophilus* increased iron bioavailability of fermented milk in animal model. Recent *in vitro* studies showed that *L. acidophilus* is a strong Th1 cytokine (IL-12, IFN-γ) inducer (Gackowska et al., 2006). *L. acidophilus* significantly up-regulated surface markers on dendritic cells (DCs), including HLA-DR, CD40, CD86 and CD83 (Zeuthen et al., 2006).

There is an increasing interest in the research of antimicrobial peptides (bacteriocins and bacteriocin-like compounds) produced by lactic acid bacteria (LAB) because of their potential use as antimicrobial agents for

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improving the safety of food products (Yildirim et al., 1999). Among *Lactobacilli*, strains belonging to species of the *L. acidophilus* complexes are most frequently used as probiotics (Klaenhammer and Kullen, 1999). Bacteriocin production is often proposed as a beneficial characteristic of probiotic bacteria (Klaenhammer and Kullen, 1999; Fooks and Gibson, 2002). It may contribute to the colonisation resistance of the host and its protection against gastroin-testinal pathogens (Reid, et al., 2001; Bourlioux, 1997). As the bacteriocin producing strain *L. acidophilus* IBB 801, a dairy isolate, displays antibacterial activity against *Escherichia coli* and *Salmonella*, the authors suggested that it may have potential as a probiotic (Zamfir et al., 1999). Numerous reports have proved the ability of *L. acidophilus* strains to produce bacteriocins (Chumchal-ova et al., 2004). *L. acidophilus* strains exhibiting anta-gonistic activity towards certain types of psychrotrophic microorganisms such as *Listeria monocytogenes*, *Bacillus cereus*, and *Clostridium* sp. are especially impor-tant as these microorganisms even at low levels in food pose a significant spoilage and public health threat (Kantani et al., 1995 Bogovic-Matijasic et al., 1998). Bac-teriocins are lethal to closely related bacteriocin-related species, food-borne pathogens and spoilage bacteria (Tagg et al., 1976; Klaenhammer, 1993).

Among the *Lactobacillus* species, *L. acidophilus* strains have been extensively utilized as probiotic cultures in dairy and pharmaceutical products and numerous reports have proved its ability to produce bacteriocins (De Souza et al., 2005), yet no review has been published summarizing its bacteriocin production. Therefore, the purpose of this paper is to have an overview and summarize the study of previous authors on bacteriocin producing *L. acidophilus* strains.

Since Metchnikoff (1908) proposed a role for lactobacilli in suppressing undesirable intestinal microflora, numerous researchers have investigated the antimicrobial activities of *L. acidophilus*. Broad spectrum inhibition has been clearly demonstrated for organic acids and hydro-gen peroxide produced by *L. acidophilus* (Gilliland and Speck 1977; Tramer 1966). In addition, a number of reports suggest that antimicrobial proteins, or bacte-riocins, either mediate or facilitate antagonism by *L. acidophilus* (Gilliland and Speck 1977; Hamdan and Mikolajcik. 1974; Hosono et al., 1977; Morin et al., 1980). Vincent et al. (1959) first described a bacteriocin-type inhibitor produced in aged liver veal agar cultures of *L. acidophilus*. Crude "lactocidin" was nonvolatile, non-dializable, insensitive to catalyze, active at neutral pH and displayed inhibitory activity against numerous genera, including *Proteus*, *Salmonella*, *Escherichia*, *Staphylococcus*, *Bacillus*, *Streptococcus*, and *L. actobacillus*. No further studies on lactocidin, or similar broad-spectrum bacteriocins produced by *L. acidophilus* have been reported. However previous studies of antagonism by *L. acidophilus* do not specifically address, identify, or confirm the involvement of bacteriocins (Hurst, 1973; Klaenhammer, 1982).

BACTERIOCIN PRODUCTION AND ITS GENERAL PROPERTIES

The first study on the production of bacteriocins by *L. acidophilus* has been reported by Barefoot and Klaenhammer (1983) who mentioned that *L. acidophilus* produces bacteriocin which he named as Lactacin B. *L. acidophilus* was restricted to selected members of the family *Lactobacilliaceae* under conditions eliminating the effects of organic acids and hydrogen peroxide proving that antibacterial activity was only due to bacteriocin. Moreover, in contrast to previous reports, no broad spectrum inhibitory activity was observed for either strain when hydrogen peroxide and organic acids were eliminated (Barefoot and Klaenhammer, 1983). Production of lactatin B was pH dependent, with maximum activity detected in broth cultures maintained at pH 6 (Barefoot and Klaenhammer, 1984). Chromatofocusing and gel filtration high performance liquid chromatography of cell-free filtrates yielded a protein with a pH of 4.1 and a molecular size of 58 kDa that induced lactacin B production (Barefoot and Klaenhammer, 1994). Purified lactacin B was stable under a variety of conditions. Full activity was retained after treatment at 100°C for 3 min at pH 5 or 8.6 in the presence of 1.0% SDS. Addition of 1% 2-mercaptoethanol during the heating step did not decrease activity (Barefoot and Klaenhammer, 1984). Table 1 represents a summary of bacteriocin or bacteriocin-like compounds from acidophilus bacteria that have been purified and named.

Lactacin F, produced by *L. acidophilus* 11088 (NCK88), is more heat resistant and exhibits a broader spectrum of activity than lactacin B inhibiting also *L. acidophilus*, *L. fermentum*, and *Enterococcus faecalis* in addition to the other lactacin B indicators (Muriana and Klaenhammer, 1991). Production of lactacin F is also pH dependent; maximum levels of lactacin F are obtained in MRS broth maintained at pH 7.0, whereas negligible activity is produced in fermentors held at pH 7.5 or 6.5. Purification by ammonium sulfate precipitation, gel filtration, and high performance liquid chromatography resulted in a 474 fold increase in specific activity of lactacin F. The purified bacteriocin was identified as a 2.5 kDa peptide by (SDS-PAGE). Composition analysis indicates that lactacin F may contain as many as 56 amino acid residues (Muriana and Kalenhammer 1991). *L. acidophilus LAPT* 1060 produces heat-labile bacteriocin contrary to lactacin B and Lactacin F. The agent was sensitive to proteolytic enzymes and heat (10 min at 60°C) and is a bacteriocin and designated acidophilucin A (Toba et al., 1991). The genetic determinant for bacteriocin production can be either plasmid or chromosomally encoded (Klaenhammer, 1993). *L. acidophilus* TK8912 produces a plasmid associated bacteriocin, termed as acidocin 8912 which is heat

Table 1. Summary of named bacteriocin purified from acidophilus bacteria.

Species	Peptide	Reference
L. cidophilu N	lactacin B	Barefoot et al. (1983)
L. cidophilu 11088	lactacin F	Muriana and Klaenhammer (1991)
L. cidophilu LAPT 1060	Acidophilucin A	Toba et al. (1991)
L. cidophilu. TK8912	Acidocin 8912	Kanatani et al. (1992)
L. cidophilu M46	Acidocin B	ten Brink et al. (1994)
L cidophilu TK9201	Acidocin A	Kantani et al. (1995)
L. acidophilus CH5	Acidocin B	Chumchalova et al. (995)
L. acidophilus JCM 1229	Acidocin JI229	Tahara and Kanatan (1996)
L. acidophilus JCM 1132	Acidocin JII32	Tahara et al. (1996)
L. acidophilus LA-1	Acidophilicin LA-1	Dave and Shah (1997)
L. acidophilus L. AcidophilusLF221	Acidocins LF221A & B	Bogovic-Matijas˘ic et al. 1998)
L. acidophilus IB801	Acidophilin 801	Zamfir et al. (1999)
L. acidophilus DSM20079	Acidocin D20079	Deraz et al. (2005)
L. acidophilus AA11	Acidocin D20079	Abo-Amer (2007)
L. acidophilus GP1B	Acidocin 1B	Kyoung-Sik et al. (2007)

stable bacteriocin and has been shown to be bactericidal on a limited number of micro-organisms. Of all conditions tested, the production of acidocin 8912 was maximized at 30°C in MRS broth (Tahara et al. 1992; Kanatani et al., 1992; Tahara et al., 1996).

Acidocin B is bacteriocin produced by *L. acidophilus* M46, which combines the inhibition of *Clostridium sporogenes* with a very narrow activity spectrum within the genus *Lactobacillus*. Acidocin B is sensitive to trypsin, heat stable (80°C for 20 min) and can be extracted from the culture supernatant fluid with butanol. Native acidocin B occurs as a large molecular weight complex (100 kDa), while with SDS-PAGE the partly purified activity migrates as a peptide of 2.4 kDa. Optimization of the cultivation conditions resulted in an eight-fold increase of the amount of acidocin B produced during growth. Genetic studies revealed that acidocin B production by *L. acidophilus* M46 is linked to the presence of 14 kb plasmid (Ten Brink et al., 1994; Van der Vossen et al., 1994). *L. acidophilus* CH5, the strain isolated from a commercial dairy starter culture, produced a bacteriocin named acidocin CH5 with suitable properties for the use in food industry. Crude acidocin CH5 was relatively heat stable (retaining 25% of activity after 121°C for 20 min), effective in broad pH range from three to nine, insensitive to catalyze and sensitive to proteolytic and glycosidic enzymes (Chumchalova et al., 1995). Acidocin CH5 exerted wide inhibitory activity spectrum against Gram positive bacteria and food spoilage bacteria, *Arthrobacter* sp., *Bacillus* sp. and *Corynebacterium* sp. Table 2 presents a summary of the acidophilus bacterial strains studied and their spectrum of inhibition towards most sensitive bacteria. Characterization of CH5 bacteriocin revealed that it belongs to the class II bacteriocins with identical N-terminal amino acid sequence described in the literature previously (Chumchalova´ et al., 1995). Acidocin J1229 produced by *L. acidophilus* JCM 1229 was heat stable bacteriocin and its production was in MRS broth was pH dependent, with maximum activity detected in broth culture maintained at pH 5.0. The sequence of the first 24 amino acid residues of the N terminus of acidocin 51229 was determined. The molecular mass of acidocin J1229 as determined by mass spectrometry was 6301 Da (Tahara and Kanatani, 1996a). *L. acidophilus* JCM 1132 produces a heat-stable, two component bacteriocin designated acidocin J1132 that has a narrow inhibitory spectrum. Acidocin J1132 activity was associated with two components, termed α and β. On the basis of N-terminal amino acid sequencing and the molecular masses of α and β components, it is interpreted that the compounds differ by an additional glycine residue in the β component (Tahara and Kanatani, 1996). The crude bacteriocin produced by *L. acidophilus* LA-1 was stable over a wide range of pH and temperature, was active after autoclaving and was not affected by storage at 37, 4 or 18°C. The production of bacteriocin occurred in log phase and was highest in the pH range of 5.5-6. Activity declined considerably when cell entered into death phase (Dave and Shah, 1997). *L. acidophilus* LF221 produced at least two bacteriocins and N-terminal amino acid composition of bacteriocins A and B was found to be different from those of the other bacteriocins, indicating that the two bacteriocins were novel. They were named acidocin LF221 A and acidocin LF221 B. Besides some lactic acid bacteria, the following species were inhibited: *B. cereus*, *Clostridium* sp., *Listeria innocua*, *Staphylococcus aureus*, *Streptococcus* (Bogovic-Matijasic et al., 1998)

A bacteriocin, designated acidophilin 801, produced by *L. acidophilus* IBB 801, has been partially characterized. It is sensitive to several proteases and inhibition occurs under conditions which eliminate the effects of organic

Table 2. Spectrum of antimicrobial activity of the proteinaceous inhibitory compounds obtained from acidophilus bacteria

Species	Inhibitory spectrum*	Reference
L. acidophilus N	Active against member of *lactobacillaceae*	Barefoot et al. (1983)
L. acidophilus 11088	lactobacilli as well as *Enterococcus faecalis*	Muriana and Klaenhammer (1991)
L. acidophilus LAPT 1060	*L delbrueckii, L. helveticus*	Toba et al. (1991)
L. acidophilus TK8912	*Lactobacillus* and *Lactoccus* sp.	Kanatani et al. (1992), Tahara et al. (1996)
L. acidophilus M46	Lactobacillus fermentum, Clostridium sporogenes	Ten Brink et al. (1994)
L. acidophilus CH5	Inhibiting strains of the geni Lactobacillus, Bacillus, Micrococcus and Corynebacterium	Chumchalova et al. (1995)
L. acidophilus TK9201	closely related lactic acid bacteria and *Listeria monocytogenes*	Kantani et al. (1995)
L. acidophilus JCM 1229	*Lactobacillus sp.* and *Lactococcus* sp.	Tahara and Kanatan (1996)
L. acidophilus JCM 1132	Only against *Lactobacillus* sp.	Tahara et al (1996)
L. acidophilus LA-1	Some Species of *L. delbrucki* and *L. casei, L. jugurti*	Dave and Shah (1997)
L. acidophilus LF221	Besides some lactic acid bacteria, *B. cereus*, *Clostridium* sp., *L. innocua*, *S. aureus*	Bogovic-Matijasic et al. (1998)
L. acidophilus IBB 801	only related lactobacilli, *E. coli* and *S. panama*	Zamfir et al. (1999)
L. acidophilus 30SC	Genus *Lactobacilli*, *B. cereus*, *B. subtilis* and *Listeria ivanovii*	Oh et al. (2000)
L. acidophilus YIT 0154	various species of *Lactobacillus* including *L. acidophilus itself*	Yamato et al. (2003)
L. acidophilus DSM 20079	*Against Lactobacillus sp.* including *L. sakei*	Deraz et al. (2005)
L. acidophilus AA11	*salmonella, shigella, E. coli S. aureus, B. cereus, B. subtilis*	Abo-Amer (2007)
L. acidophilus GP1B	*Shigella, E. coli, P. aeruginosa, L. Monocytogenes Yersinia enterocolitica*	Kyoung-Sik et al. (2007)

*Inhibitory spectrum refers to the highest zone of inhibition recorded toward the indicator species.

acids and hydrogen peroxide. Like most of the known bacteriocins produced by *L. acidophilus* strains, acidophilin 801 is a heat stable and low molecular-mass (less than 6500 Da) peptide bacteriocin. Acidophilin 801 displayed a concentration dependent bactericidal effect towards a bacteriocin sensitive strain (*L. helveticus* 102) without causing concomitant cell lysis of the indicator cells. Amino acid composition analysis of acidophilin 801 revealed a strongly hydrophobic peptide. Taking all these biochemical characteristics into consideration, acidophilin 801 produced by *L. acidophilus* IBB 801 appears to belong to the class II lactic acid bacterium bacteriocins according to the classification of Klaenhammer (Zamfir et al., 1999). The bacteriocin produced by *L. acidophilus* 30SC displays antimicrobial effects on bacteria such as *Listeria* and *Bacillus* species; spore-forming bacteria, *B. cereus* and *B. subtilis* were sensitive to the bacteriocin produced by *L. acidophilus* 30SC. The *L. acidophilus* 30SC bacteriocin was active over a wide range of pH, and was stable to various heat treatments. The loss of antimicrobial activity following treatment with proteinase K and proteinase E indicated that the active component secreted extracellullarly by *L. acidophilus* 30SC was proteinaceous in nature. The molecular mass of the *L. acidophilus* 30SC bacteriocin was estimated at 3.5 kDa (Oh et al., 2000). *L. acidophilus* YIT 0154 was found to produce a bacteriocin-like substance in the culture filtrate. The substance produced in growth associated manner, showed heat stability at neutral and acidic pH and exhibited antibacterial activity against various species of *Lactobacillus* including *L. acidophilus* itself. The molecular weight of the substance was in the range of 6.2–9.5 kDa. N-terminal amino acid sequence analysis suggests that the substance may belong to class II b bacteriocin (Yamato et al., 2003). *L. acidophilus* DSM 20079 is the producer of a novel bacteriocin termed acidocin D20079. Like many other bacteriocins from the *L. acidophilus* group (Kanatani et al., 1992; Tahara and Kanatani, 1996a; Tahara et al., 1996b; Zamfir et al., 1999), acidocin 20079 has a rather narrow inhibition spectrum, in principle restricting its action to other strains of *Lactobacilli*. This antimicrobial peptide was extremely heat stable (30 min at 121 ◦C) and was active over a wide pH range. It was found to be sensitive to proteolytic enzymes (trypsin, ficin,

pepsin, papain, and proteinase K). Mass spectrometry was used to determine the molecular mass of the peptide 6.6 kDa (Deraz et al., 2005, 2007). *L. acidophilus* AA11 produced higher antimicrobial activity with a wide range of inhibition. The agent AA11 was sensitive to proteolytic enzymes and retained full activity after 30 min at 100°C. Activity against sensitive cells was bactericidal but not bacteriolytic. 12% SDS-PAGE analysis of 40% ammonium sulphate precipitated agent showed two peptides with molecular weights of 36 and ~29 kDa. No plasmid was identified in *L. acidophilus* AA11 indicating that the genes encoding the inhibitory agent were located on the chromosome (Abo-Amer, 2007). Acidocin 1B, a bacteriocin produced by *L. acidophilus* GP1B, exhibited profound inhibitory activity against a variety of LAB and pathogens, including Gram negative bacteria. It retained approximately 67% of the initial activity after storage for 30 days at 4ºC and 50% of its initial activity after 30 days at 25 and 37ºC. The molecular mass of acidocin 1B was estimated to be 4,214.65 Da by mass spectrometry. Plasmid curing results indicated that a plasmid, designated as pLA1B, seemed to be responsible for acidocin 1B production (Kyoung-Sik et al., 2007).

PURIFICATION OF *ACIDOPHILUS* BACTERIOCIN

Lactacin B was purified by ion-exchange chromatography, ultrafiltration, and successive gel filtrations in the presence of 8 M urea and then 0.1% sodium dodecyl sulfate (Barefoot and Klaenhammer, 1984). Strong hydrophobicity is a characteristic property of the class II peptide bacteriocins. For instance, the proportion of hydrophobic amino acids in the *L. acidophilus* group bacteriocins, namely acidophilin 801, gassericin A, lactobin A and lactacin F, amounts to 50.8, 45.7, 38.0 and 29.0%, respectively (Tahara and Kanatani, 1996; Tahara et al., 1996b; Nissen-Meyer and Nes, 1997). This strong hydrophobic character is one of the main reasons why bacteriocin purification is tedious and cumbersome. The number of chromatographic steps varies from three or more (e.g. acidocin LF221A and B; Bogovic-Matijasic et al. 1998) to only one after concentration of the bacteriocin from the cell-free culture supernatant fluid either via ammonium sulphate precipitation (e.g. lactacin F; Muriana and Klaenhammer, 1991) or through ammonium sulphate precipitation followed by chloroform/methanol extraction (Zamfir et al., 1999). The lactacin F was isolated as a floating pellet from culture supernatants brought to 35 to 40% saturation with ammonium sulfate. Purification by ammonium sulfate precipitation, gel filtration, and high performance liquid chromatography resulted in a 474 fold increase in specific activity of lactacin F (Muriana and Klaenhammer, 1991).

Acidocin J1229 was purified by ammonium sulphate precipitation and sequential cation exchange and reversed-phase chromatography. These purification steps resulted in more than 80 fold increase in the specific activity and a recovery of 2% of the activity (Tahar and Kanatani, 1996). Acidocin J1132 was purified by ammonium sulfate precipitation and sequential cation exchange and reversed phase chromatography. These purification steps resulted in a more than 280 fold increase in the specific activity and a recovery of 5% of the activity (Tahar and Kanatani, 1996). The cell-free culture supernatant fluid (1600 AU $ml^{-}1$) was precipitated with 40% ammonium sulphate saturation. The bacteriocin activity of the pellicle increased to 25600 $AUml^{-}1$. After chloroform/methanol extraction, the activity of the precipitate, re-dissolved in ultra pure water, was 51200 $AUml^{-}1$. This material was considered to be a partially purified bacteriocin preparation. The partially purified bacteriocin was further purified by reversed phase FPLC (Zamfir et al., 1999). Acidocin CH5 was purified using combinations of chromatographic methods based on hydrophobic and cation exchange principles. The recovery of bacteriocin activity was very low in all fractions. The purification procedure resulted in a more than 49 fold increase in the specific activity. RP-HPLC on C4 reverse phase column, the final purification step, resulted in a peak which followed a major protein peak. Acidocin CH5 was purified and eluted from the reversed phase column at about 83% of ethanol (Chumchalav et al., 2004). During purification of the *L. acidophilus* DSM 20079 peptide, optimal recovery was achieved with ammonium sulphate precipitation followed by two chromatographic steps that is by using CM Sepharose column and by Octyl Sepharose column. The recovery after each of these steps is comparable to results for bacteriocins from other *Lactobacilli*, but the advantage here is that the procedure is relatively short (Deraz et al., 2005). Acidocin bacteriocin activity was eluted from Octyl sepharose CL-4B at approximately 45 to 50% ethanol and then from the C18 Sep-Pak cartridge at 60% methanol. The final purification step by HPLC gel filtration yielded a single main peak of the acidocin 1B (Kyoung-Sik et al., 2007).

MODE OF ACTION OF *ACIDOPHILUS* BACTERIOCIN

To test for a bactericidal or bacteriolytic mode of action, we investigated the effects of lactacin B on the viability and lysis of *L. leichmannii* 4797. The addition of increasing concentrations of lactacin B to *L. leichmannii* 4797 cells resulted in proportional increases in cell death. According to a single hit mechanism for bacteriocin action, bacteriocins sequentially adsorb to, penetrate, and kill sensitive cells. Lactacin B adsorbed to sensitive cells and acted in a single hit fashion characteristic of bacteriocinic proteins (Barefoot and Kaenhammer, 1983). Acidocin B has bactericidal effect on the target strain as the optical density remained constant, indicating that lysis did not occur (Ten brink et al., 1994). Acidocin J1229 is a pore forming bacteriocin that creates cell membrane channels

through the 'barrel-stave' mechanism. Taken together, acidocin J1229 has a bactericidal effect on sensitive organisms by dissipating the PMF, inhibiting the transport of amino acids, and causing the leakage of essential compounds by the formation of pores in the cytoplasmic membrane (Tahara and Kanatani, 1996). Acidocin J1132 has a bactericidal effect on sensitive organisms by dissipating the PMF, inhibiting the transport of amino acids, and causing the leakage of essential compounds by the formation of pores in the cytoplasmic membrane (Tahara and Kanatani, 1996). Most bacteriocins exert bactericidal mode of action against the sensitive microorganisms, although some of the bacteriocins have been shown to act in a bacteriostatic manner. Bactericidal activity of bacteriocins may be accompanied by lysis of sensitive cells (bacteriolytic bacteriocins), as shown for acidocin D20079 (Deraz et al., 2007). Acidocin 1B displayed bactericidal action with a maximum viability loss of about 2 log cycles, which was achieved in less than 60 min when it was added to final activity levels of 160 and 320 AU/ml. Electron microscopy of the acidocin 1B treated sensitive cells corroborated that the acidocin 1B induced cell lysis. This was apparently caused by changes in the structure of the cell wall, resulting in the rupture of the cells in several places and the escape of cell contents. The cell wall ultimately disintegrates, leaving debris that was apparently responsible for the granular appearance of the observation field (Kyoung-Sik et al., 2007).

APPLICATION OF *ACIDOPHILUS* BACTERIOCIN

The application of a purified bacteriocin from bifidobacteria directly to food systems has never been reported until now but its potential in such an application has not been disregarded. Many reports mention about the incorporation of acidophilus into different types of foods and beverages and their usefulness to human health. Acidophilus products includes fermented milk such as acidophilus milk, *acidophilus* yoghurt and sweet acidophilus milk (Salminen et al., 1996), fruit juice and vegetable juice (Tsen et al., 2004 and 2008) and soy milk (Apostolidis et al., 2007). Growth of preschool children was improved when fed an iron fortified fermented milk beverage supplemented with *L. acidophilus* was given to them (Silva et al., 2008). Supplementation with a combination of *B. longum* and *L. acidophilus* has been reported to reduce the ecological changes in the intestinal microbiota induced by administration of clindamycin (Horrange et al., 1994).

Researchers have proposed the possibility of incurporating antimicrobial compounds isolated from bifidobacteria directly into food systems and pharmaceutical products. In contrast to most other bacteriocins produced by lactic acid bacteria, acidocin B is more inhibittory towards *Clostridia* than to other lactic acid bacteria, it could also be used to control clostridial activity in fermented products such as cheese or silage (Ten Brink et al., 1994). The ability to survive acidic conditions, bile resistance, and the production of a bacteriocin that is active against food related pathogens and spoilage microorganisms, contributes to the ability of *L. acidophilus* 30SC as a probiotic culture that may have potential applications in the production of cultured foods and dietary adjuncts (Oh et al., 2000). Acidocin CH5 is unique due to its broad activity spectrum and its physico-chemical properties (Chumchalova et al., 1995). Acidocin CH5 is produced not only in various laboratory complex broths but also in milk, so it can be used in fermented milk products to retard the growth of pathogenic bacteria (Chumchalava, 2004). *L. sakei* NCDO 2714 was found to be among the sensitive strain whose growth was inhibited by acidocin 20079. This strain is often present in vacuum-packaged meat, and can cause anaerobic spoilage if specific hydrogen sulphide producing *L. sakei* strains predominate in the environment. Addition of bacteriocins preventing *L. sakei* could therefore be advantageous for meat conservation purposes (Deraz et al., 2005). Acidocin 1B and its producer strain, which can endow broad antimicrobial activity against a variety of pathogens, may have potential value as a biopreservative in the various food systems during long storage periods at different preservatory temperatures and pH conditions (Kyoung-Sik et al., 2007). In conclusion, acidocin AA11 meets many of the requirements proposed by Piard and Desmazeaud for an ideal antimicrobial compound. In addition to its inhibition spectrum, technological properties (heat and storage stability) provide the bacteriocin with an application potential as a biopreservative (Abo-Amer 2007). Currently, there are no bacteriocins from acidophilus that are being applied commercially in food systems as food preservatives. But this is not only true for the bacteriocins from acidophilus but also true for many bacteriocins isolated from lactic acid bacteria. Many bacteriocins have been characterized biochemical and genetically but many aspects of these compounds are still unknown (Cleveland et al., 2001) which explains why till date nisin, approved by the Food and Drug Administration, is the only purified bacteriocin widely used as a food preservative. Toxicity data exist for only a few bacteriocins, none of which are for bacteriocins from acidophilus, but research and their long time intentional use strongly suggest that bacteriocins can be safely used (Cleveland et al., 2001).

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