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ABSTRACT

L. acidophilus is a homofermentative, microaerophilic, short chain gram positive microorganism with rod morphology having its bacteriocins belonging to class II a. Several bacteriocins of L.

acidophilus have been isolated and characterized. These are structurally similar but, their molecular weight varies as well as their spectrum of antimicrobial activity. They exhibit important technical properties, i.e thermostability and retaining of activity at a wide pH range along with strong inhibitory actions against food spoilage and pathogenic bacteria, make them an important class of bio-preservatives. L. acidophilus can be added as an adjunct in many food fermentation processes contributing to unique taste, flavor and texture. It also preserves the products by producing lactic acid and bacteriocins. A lot of new information regarding the bacteriocins of L. acidophilus has emerged during the last few years. In this review, an attempt has been made to summarize and discuss all the available information regarding the sources of bacteriocins production, their characteristics and their antimicrobial action along with their application.

Keywords *L. acidophilus,* taxonomy, bacteriocins, applications

INTRODUCTION

Lactobacillus acidophilus: A Lactic Acid Bacteria

Lactobacillus bacteria exist in a variety of environments from dairy products to the human gastrointestinal tract. These are generally gram positive, non motile, non sporulating, present in round or rod shape and produce lactic acid as an end product of fermentative metabolism (Anonymous, 2010). These bacteria survive at the pH of 4-5 or lower (Ivanova *et al*., 2000). Some strains growing on blood agar (media rich in haeme group) exhibit pseudocatalase activity (Mares *et al*., 1994; Singh *et al*., 2007).

Lactic acid bacteria (LAB) ferment sugars and produce lactic acid as the final product through Embden Meyerhof Parnas (EMP) pathway (Kenneth, 2009). Bacteria that produce only lactic acid as a result of fermentation are referred as homofermentative and those producing CO_2, hydrogen peroxide, acetic acid and alcohol alongwith lactic acid are known as heterofermentative (Gandhi, 2006). These LAB are divided into eight main genera including *Lactobacillus, Leuconostoc, Carnobacterium, Bifidobacterium, Streptococcus, Lactococcus, Enterococcus and Sporolactobacillus*. Each of these can be further divided into species, subspecies, variants and strains (Wessels *et al*., 2004). On the basis of carbohydrate metabolism and growth at different temperature range, microorganisms included in the genus *Lactobacillus* divided into three subgenera: *Streptobacterium, Betabacterium and Thermobacterium*. Kandler & Weiss (1986) classified this genus into three groups:

- Group A: Strict homofermentative species. It includes 15 species classified as *Thermobacterium* e.g *Lactobacillus delbrüeckii* and *Lactobacillus acidophilus*. They do not ferment pentoses and grow at 45^0C but not at 15^0C.

- Group B: Facultative heterofermentative species ferment glucose and produce ethanol, formic acid and acetic acid in addition to lactic acid through the EMP metabolic pathway. Originally, LAB from this group was classified as *Streptobacterium* (recent classification known as *Lactobacillus*) with following examples, *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus sakei*. At 45^0C, they grow poorly or not at all but can show growth at 15^0C.
- Group C: Strict heterofermentative species that produces lactic and acetic acid as their end products e.g. *Lactobacillus brevis*, *Lactobacillus fermentum,* and *Lactobacillus reuteri*. Temperature range varies with species.

***Lactobacillus acidophilus*: Physiological and Biochemical Features**

Among LAB, only *L. acidophilus* has been widely known. Moro described this specie as "*Bacillus acidophilus*" in 1900 from infant feaces, and then Hanson & Moquot (1970) renewed it. According to the phenotypic characteristics, *L. acidophilus, L. amylovorous, L. crispatus, L. gallinarum, L. gasseri* and *L. johnsonii* were identified as *L. acidophilus* group strains (Fujisawa *et al*., 1992). *L. acidophilus* is a gram positive microorganism with rod morphology, approximately 2-10 µm in size. It is a homofermentative anaerobic microorganism classified in group A (Kandler & Weiss, 1986). As *L. acidophilus* is homofermentative, it found to use glycolysis or EMP pathway to ferment hexoses and produced D and L- lactic acids while those present in heterofermentative groups followed phosphoketolase pathway to ferment hexoses and pentoses (Hutkins, 2006).

Lactobacillus spp. are a heterogenous group. A DNA-DNA hybridization study described that they contain several biotypes and genetic groups (Dellaglio *et al*., 1975). The cell wall of gram positive lactobacilli consists of the sacculus made up of peptidoglycan, decorated with (lipo) teichoic acids, surface proteins, and anionic and neutral polysaccharides (Delcour *et al*., 1999). Among surface properties S- layers are of particular interest which covers the bacterial cell wall in a regular, two - dimensional array and this property exists in a number of *Lactobacillus* species, including *L. acidophilus* (Boot *et al*., 1995; Smit *et al*., 2001).

In *L. acidophilus,* the EMP pathway is prescribed in the presence of aldolase and glyceraldehyde-3-phosphate (G3P) that catalyzed important steps to produce pyruvate which under normal conditions reduced to lactate. Then, NAD^+ dependent lactate dehydrogenase (nLDH) further catalyzed this reduction, in turn reoxidizes the NADH formed in the first steps of glycolysis and produce lactic acid as the only end product, thus referred to homo lactic fermentation (Salminen *et al*., 2004). Among several *Lactobacillus* species isolated from fermented cane molasses, the strains of *L. acidophilus* produced succinic acid and diammonium citrate in de Man-Rogosa-Sharpe broth was determined as a precursor of the succinic acid produced (Kaneuchi *et al*., 1988). *L. acidophilus* has the ability to ferment different carbohydrates (Table 1). Cholesterol removing *L. acidophilus* strain found to produce organic acids from the fermentation of mannitol, fructooligosaccharides and inulin (Liong & Shah, 2005). *L. acidophilus* in both free and immobilized forms presented satisfactory rates of survival in milk and acidified milk because the average reduction of the population was only one log cycle after 21 days of storage means its population was slightly affected during storage (Grosso & Trindade, 2004).

L. acidophilus is a thermophilic strain showing growth at a temperature of 30-45 ^{0}C but good growth was found at pH 4-5 (Jones, 1999). *L. acidophilus* has been isolated from many fermented products e.g yoghurt (Anonymous, 2000). On the basis of physiological properties, Johnson *et al*. (1980) found out that *L. acidophilus* produce maximum amount of lactic acid, some acetic acid with no hydrogen and no catalase.

Classical methods are used to differentiate between *Lactobacillus* spp. based on growth requirements, growth at certain temperatures, lactic acid configuration, arginine hydrolysis and patterns of carbohydrate fermentation reactions (Axelsson & Lindgren, 1987). These characteristics are still used as an indication, though more appropriate characterization methods are being implemented, namely peptidoglycan structure, DNA base composition, DNA homology, electrophoretic mobility of L-lactate dehydrogenase, species-specific PCR (derived from rRNA sequences), RAPD-PCR, PFGE, and restriction enzyme analysis (Kandler & Weiss, 1986; Axelsson & Lindgren, 1987). Phenotypic methods together with genetic methods must be used to distinguish different species because many of them are phenotypically very similar but genotypically different (Vandamme *et al*., 1996).

Molecular characterization of LAB is important and usually it is performed by 16S ribosomal RNA (rRNA) encoding gene, the 16S-23S rRNA intergenic spacer region the 23S rRNa encoding, *recA* and *ldhD* genes; randomly amplified polymorphic DNA, restriction fragment length polymorphism (RFLP), denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis, amplification rDNA restriction analysis, restriction enzyme analysis, rRNA, pulse field gel electrophoresis and amplification fragment length polymorphism for identifying and differentiating closely related species and strains of LAB associated with food and industry

(Mohania *et al*., 2008). For determining the gene expression profiles of *L. acidophilus* in yoghurt, seven genes related to stress response (*dnaJ*, *dnaK*, *gadC, groEL*, *groES, grpE* and *hrcA*) and one housekeeping gene (16S rDNA) were selected. Their species-specific primers were designed by using Amplicon (Berthier & Ehrlich, 1998), then the most unique regions to *L. acidophilus* were selected for designing the forward and reverse primers ~~for~~ e.g. forward primer for gene 16S rDNA was AGCAGATCGCATGATCAGCT and its reverse primer was TTGCCTTCGCAGGCTTG. Wang *et al*. (2002) used primer LAA-1(5'-TGAACCAACAGATTCACTTCGGTGATGACGTTGGGAAACGCT-3') for the identification of *L. acidophilus* ATCC 332 from fecal samples. Other specie specific primers have also been found such as NG^3 (5'- GAATCTGTTGGTTCAGCTCGC- 3') that was used in the identification of *L. acidophilus* naturally exist in the intestinal tract (Hensiek *et al*., 1992) and a combination of specific primers such as Aci I (TCTAAGGAAGCGAAGGAT) and Aci II (CTCTTCTCGGTCGCTCTA) used in the identification of *L. acidophilus* strains taken from Burkina Faso fermented milk (Savadogo *et al*., 2004).

LAB may contain ß-D-glucans having negative organoleptic properties (Lauberes *et al*., 1989), acetic acid (Funel, 1999), citrulline, a precursor of carcinogenic ethyl carbamate (Mira De Orduna *et al*., 2000) and biogenic amines (Coton *et al*., 1999; Funel, 2001). The content of biogenic amines such as histamine, tyramine, putrescine, and cadaverine, might found to cause food poisoning hazard that's why these are considered to be important regarding the safety of fermented food products. These are biologically active low molecular weight organic molecules normally produced by bacterial decarboxylation of their precursor amino acids and generally considered to be either psychoactive or vasoactive (Watherson *et al*., 1975; Suzzi and Gardini,

2003). High proteinaceous fermented food products can potentially support the production of biogenic amines (Capillus & Colmenero, 2004). Biogenic amines are produced in the presence of amino-acid decarboxylating microorganisms which are able to synthesize decarboxylases and this characteristic is strain-specific (Silla, 1996). *Lactobacilli* found actively perform in production and consequent accumulation of histamine, tyramine and putrescine (Cid & Holzapfel, 1999).

Pintado *et al.* (2008) reported that biogenic amines are present in Terrincho, a traditional Portuguese cheese, and those produced by LAB in alcoholic beverages was reviewed by Farias *et al.* (1995) and Lucas *et al.* (2003). Among LAB, *L. plantarum* and *L. hilgardii* are known to produce biogenic amines (de Nadra, 2007).

Antibiotic Resistance in *L. acidophilus*

Health care industries have been using antibiotics against bacterial infections for a long time however bacteria, being a highly adaptable creatures develop resistance to antibiotics (Levy, 1997). People having some common bacterial infections can suffer from antibiotic resistance (Levy, 1992). For several decades, clinically relevant species were mainly focused to study for selection and dissemination of antibiotic resistance (White *et al.*, 2002). Recently many investigators found out that LAB may contain reservoir of antibiotic resistance genes as present in human pathogens (Levy and Salyers, 2002) and can transfer and spread them to the pathogenic microbes (Levy, 1997; WHO, 1997).

Bacteria have developed numerous antibiotic resistance mechanisms like mutations in the bacterial genome or through the acquisition of additional genes encoding the resistance mechanism. These genetic mutations change the defensive functions of bacteria by (A) enzyme

inactivation of the antibiotic (Walsh, 2003), (B) extrusion of the antibiotic out of the cell by active transport (active efflux pumps) (Walsh, 2003), (C) alteration of the target site by changing membrane permeability (Davies, 1997) and (D) by directing metabolic pathways around the disrupted point (Poole, 2002). A vast number of data is available on the antibiotic resistance mechanism in clinical bacteria but that on the antibiotic resistance genes present in LAB and other commensal bacteria is scarce (Cataloluk & Gojebakan, 2004; Florez *et al*., 2005). As LAB has the ability to colonize the GI tract, the transfer and spread of genetic material vertically or horizontally to indigenous microflora (and vice versa), is possible (Mathur & Singh, 2005).

According to a research, 68.4% of probiotic isolates exhibited resistance against some antibiotics, particularly many strains of *Lactobacillus* spp. were resistant to chloramphenicol, erythromycin, tetracycline, and kanamycin (Temmerman *et al*., 2002). Penicillin is a well known antibiotic against which *L. acidophilus* ACA-DC 243 from Greek cheese show resistance (Charteris *et al*., 1998) *L. acidophilus* isolated from Nigerian fermented foods and beverages showed 42.5% (Olukoya *et al*., 1993) and those isolated from European probiotic products has 26% resistant (Temmeman *et al*., 2002) against tetracycline. Moverover, the *L. acidophilus* strains also carries intrinsic resistance towards vancomycin due to d-alanine: d-alanin ligase-related enzymes (Elisha & Courvalin, 1995).

Bacteriocin Production from *L. acidophilus* and their General Characteristics

Bacteriocins are natural antimicrobial agents having proteinaceous nature and are lethal to bacteria other than producing strains (Deraz *et al*., 2005). These are named according to the

genus and specie of the strain that produces it e.g. plantaricin produced by *L. plantarum* (Joerger *et al*, 2000). Probiotic bacteria are considered beneficial due to bacteriocin production (Klaenhammer & Kullen, 1999; Fooks & Gibson, 2002) because they colonized in host GI tract and give protection from gastrointestinal pathogens (Reid *et al*., 2001; Bourlioux, 1997).

LAB are known for their production of bacteriocins or bacteriocin like peptides (De Vuyst & Vandamme, 1994) exhibited potential antimicrobial properties towards closely related bacteria and other harmful microbes (Chumchalova *et al*., 2004; Kantani *et al*., 1995). *L. acidophilus* produced majority of bacteriocins that are heat stable, non-lantibiotic peptides having low molecular mass belonging to class II (Zamfir *et al*., 1999). A list of bacteriocins produced by different strains of *L. acidophilus* against pathogenic bacteria is shown in Table. 2 and Table. 3. *L. acidophilus* has a potential probiotic effects in the human ecosystem due to the production of bacteriocins, they get priority over other microbes in the intestine (Zaheer *et al*., 2010).

Bacteriocins production by *L. acidophilus* has been first studied by Barefoot and Klaenhammer (1983) who found out that *L. acidophilus* produced Lactacin B, a bacteriocin against *L. bulgaricus, L. lactis, L. helveticus* and *L. leichmani*. *L. acidophilus* DSM 20079 was another strain that produced a small bacteriocin, named acidocin D20079 having molecular weight of 6.6 kDa. This was found to be extremely heat stable (30 min at 121 ◦C), showed maximum production at pH 6.0, sensitive towards proteolytic enzymes (trypsin, ficin, pepsin, papain, and proteinase K), having a narrow inhibitory spectrum restricted to the genus *Lactobacillus* including *L. sakei* NCDO 2714 and was purified by ammonium sulphate precipitation, sequential cation exchange and hydrophobic interaction chromatography (Deraz *et al*., 2005).

L. acidophilus TSI isolated from indigenous dahi exhibited wider zone of inhibition against *E. coli, E. feacalis, S. aureus* and *S. typhi* by producing bacteriocin having molecular weight of 7.5 kDa with thermostability from 72-110^{0}C for 15 minutes and retained its antimicrobial activity over pH range of 3.0 - 10. It was partially purified by solvent extraction method followed by chloroform precipitation and HPLC (Maqsood *et al*., 2008). One of the sources of *L. acidophilus* strain was the gut of marine prawn that released bacteriocin of 2.5 kDa which have broad range of antimicrobial activity against pathogenic microbes. Maximum bacteriocin production was observed at 50^{0}C, pH 4 and 0.9% NaCl. It was also purified by ammonium sulphate precipitate and ion exchange (DEAE cellulose) chromatography (Karthikeyan & Santosh, 2009). *L. acidophilus* 30SC found to produce bacteriocin that remained active over wide range of pH, stable to various heat treatments and killed number of gram +^{ve} bacteria including *Listeria* and various pathogenic microbes. This bacteriocin was purified by 50% ammonium sulfate precipitation followed by hydrophobic interaction column chromatography (Oh *et al*., 2000).

Bacteriocin named as acidocin 8912 was produced by *L. acidophilus* TK 8912 that inhibited the *L. casei* cells at the cytoplasmic membrane by the dissipation of the protonmotive force and by pore formation (Tahara *et al*., 1996). Acidocin B was produced by *L. acidophilus* strain M46 and its genetic analysis was done by Leer *et al*., (1995). It was found to be active against *L. monocytogenes, C. sporegenes, L. fermentum and L. bulgaricus. L. acidophilus* IBB 801 originated as dairy isolate produce maximum bacteriocin against *L. bulgaricus* LMG 6901 (Avonts *et al*., 2003). Another study was done by Zamfir *et al*. (1999) on a small bacteriocin of 6.5 kDa designated as Acidophilin 801 produced by *L. acidophilus* IBB 801 against *E. coli, Salmonella panama* 1467. It was also heat stable (30 minutes at 121^{0}C) and maintained over

wide range of pH. Crude form of this bacteriocin was isolated as a floating pellicle after ammonium sulphate precipitation (40% saturation) and partially purified by extraction/precipitation with chloroform/methanol followed by reversed phase fast performance liquids chromatography.

One of the strains of *L.* acidophilus YIT 0154 was found to produce a bacteriocin like substance (6.2–9.5 kDa), produced in growth associated manner, remained heat stable at neutral and acidic pH and showed antibacterial activity against various species of *Lactobacillus* including *L. acidophilus* itself. Analysis of its N-terminal amino acid sequence suggested that the substance may belong to class IIb bacteriocin (Yamato *et al*., 2003). *L. acidophilus* LAPT 1060 produced acidophilucin A that is heat labile bacteriocin as compared to lactacin B and Lactacin F. It was found to be sensitive towards proteolytic enzymes and heat treatment for 10 minutes at 60°C (Toba *et al*., 1991). A plasmid associated bacteriocin, known as acidocin 8912 was produced by *L. acidophilus* TK8912 that showed heat stability bacteriocin and bactericidal effect on a limited number of micro-organisms. Its maximum production was achieved at 30°C in MRS broth (Tahara *et al*., 1992). *L. acidophilus* CH5 produced acidocin CH5 which itself isolated from a commercial dairy starter culture. This bacteriocin with suitable properties preferred to use in food industry. Crude acidocin CH5 was relatively heat stable (retaining 25% of activity after 121°C for 20 minutes), effective at 3-9 pH, insensitive to catalyze and sensitive to proteolytic and glycosidic enzymes and inhibited *Arthrobacter* spp., *Bacillus* spp. and *Corynebacterium* spp. (Chumchalova *et al*., 1995).

L. acidophilus JCM 1229 produced acidocin J1129 that was heat stable bacteriocin of 6301 Da and showed maximum activity at pH 5.0 with a narrow inhibitory spectrum (Tahara & Kantani,

1996). *L. acidophilus* LF221 produced two novel bacteriocins named as acidocin LF221 A and acidocin LF221 B that differentiate them from other bacteriocins with their N-terminal amino acid composition (Maijasic *et al*., 1998). *L. acidophilus* AA11 produced a bacteriocin known as acidocin D20079 that retained full activity at 100^0C after 30 minutes but found to be sensitive towards proteolytic enzymes. The agent purified with 40% ammonium sulphate comprised of two peptides with molecular masses of 36 and 29 kDa (Abo-Amer, 2007). Acidocin 1B, a bacteriocin of 4,214.65 Da produced by *L. acidophilus* GP1B exhibited profound inhibition against pathogenic 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. Plasmid curing results indicated that a plasmid, designated as pLA1B seems to be responsible for acidocin 1B production (Kyoung *et al*., 2007).

Application of *L. acidophilus* in Food Production

These days, the majority of people have awareness about their health and they know well which food items are healthy for them and which are not. They intend to consume food which can turn out to be helpful in preventing against different health disorders (Mattila-Sandholm *et al*. 2002). The addition of different probiotic micro-organisms to foods to increase their potential and health advantages is presently of great interest. The micro-organisms which are being most commonly used for this purpose are those belonging to the genera of *L. acidophilus* and these are considered to possess various beneficial effects regarding the health of humans (Saxelin *et al*. 2005). Some of these benefits are decrease in blood cholesterol, decrease of the risk of mutagenicity and carcinogenicity and decrease in the risk of constipation, diarrhea and lactose

intolerance (Holzapfel & Schillinger 2002; Marteau & Boutron-Ruault 2002; Korhonen & Pihlanto 2004; O' May & Macfarlane 2005).

Dairy Products

L. acidophilus is used in number of fermented products (Gilliland *et al*., 2002). The most common are yoghurt and sweet acidophilus milk. Sweet acidophilus milk is manufactured by inoculating *L. acidophilus* bacteria in milk. After that, the milk sets for 24 hrs and yields a type of buttermilk having low content of lactose (Vela, 1997). Mostly, sweet acidophilus milk is consumed by those individuals having problem of lactose maldigestion and intolerance, a condition that effect approximately 75% of the world population. This condition prevails when lactase enzyme unable to break down lactose in the intestine, thus failure to digest lactose results in the occurrence of discomfort, cramps and diarrhea (Sanders, 2000). Foods in which *L. acidophilus* is used in adequate amounts include live yoghurt cultures, miso, and tempeh. These products vary greatly concerning the type of bacteria used and their individual potencies. *L. acidophilus* and *L. casei* were added as adjuncts to yoghurt and cultured buttermilk and their viability was checked during 28 days of refrigerated storage at 5^0 to 7°C. For the enumeration of *L. acidophilus* and *L. casei*, modified LBS (*Lactobacillus* selection) agar was used that helped in the colony formation of the adjunct bacteria only while preventing colony formation of the traditional yoghurt or buttermilk starter cultures. In both cultured products, some strains of *L. acidophilus* survived well but others loss their viability but there was no viability loss of *L. casei* GG in any of the cultured products during storage (Nighswonger *et al*., 1996).

L. acidophilus also has a lot of applications in different types of cheese both at experimental and industrial level. It is used in addition with other probiotics (*Lb. casei*, *Lb. paracasei* or *Bifidobacterium* spp.) in cheddar cheese (Ong *et al*., 2007), minas fresh cheese (Flavia *et al*., 2006), probiotic white cheese (Lu *et al*., 2004) and Gouda cheese (El-Sayed *et al*., 2010) for chemical analysis and sensory evaluation during storage. In soymilk, *L. acidophilus* is used since long time. In the United States in 1933, *L. acidophilus* soymilk became commercially popular when Dr. John Harvey Kellogg, head of the famous Battle Creek Sanitarium, started using it against colitis and various intestinal or digestive disorders. Then, in 1974 Wang, Kraidej (from Thailand), and Hesseltine, all at the USDA Northern Regional Research Center in Peoria, Illinois, developed an acidophilus soymilk. That milk was fermented with *L. acidophilus* strain B-1910, having an ability to consistently produce a better product than other strains tested and contained 4% more sucrose with refreshing sweet-sour taste. A taste panel accepted the milk because the fermentation process followed, largely eliminated any beany flavor found in the original soymilk (Shurtleff & Aoyagi, 2007).

Probiotics are available in dried or liquid cultures of living bacteria and used in a variety of nutritional supplements. These cultures are often marketed as freeze-dried powders, granules, or capsules and suppositories. Once these probiotic products are consumed, *L. acidophilus* begins to colonize the digestive tract (Admin, 2010)**.** In microencapsulation, microcapsules are formed to support the growth of the probiotic and provide protection from harsh external environments. Recently, microencapsulation study was done mainly on probiotic sustainability in the food product with limited experimentation on the probiotics viability during delivery into the body (Akhtar, 2010). Effect of microencapsulation on the viability of probiotics was checked

(Kailasapathy, 2002). Kim *et al*., (2008) also evaluated the effect of microencapsulation on viability and other characteristics in *L. acidophilus* ATCC 43121 and found out that microencapsulation was effectively used as it protected the microorganisms from heat and acid treatment in delivering the viable cells to intestine without any harmful effect on their functionalities.

Currently, research is emphasized on the use of *L. acidophilus* supplements with great health benefits. These supplements are found to be effective against yeast infections, gastrointestinal distress and low immune activity (Johnson *et al*., 1987). Lactic acid producing bacteria like *Streptococci* such as *S. cremoris* and *S. lactis* and *Lactobacilli* such as *L. acidophilus* are found to present in camel milk. All of these strains were tested for lactic acid production from lactose and it was observed that *L. acidophilus* has the ability to convert 23% lactose into lactic acid. Survival of the probiotic species was dependent on the physicochemical characteristics of the cheese, but final numbers were still above the recommended threshold for a probiotic effect. *L. acidophilus* could be used with *B. lactis* as a starter culture for the successful manufacture of a goat cheese with good flavor and texture characteristics (Ana & Malcata, 1998).

In conventional foods (milk, yoghurt, and toddler formula) and dietary supplements, *L. acidophilus* NCFM present as a probiotic strain. It was commercially available in the fermented products in United States since the mid 1970s because of its safety, human probiotic functionality and its amenability to commercial manipulation. Its taxonomic status as a type A1 *L. acidophilus* strain has been verified with phenotypic and genotypic techniques (Buck *et al*., 2005). *L. acidophilus* Oral is a product that contains probiotic bacteria, *L. acidophilus* which is also a natural inhabitant of stomach and intestines. Sometimes antibiotics alter the normal

balance of intestinal bacteria and became the cause of diarrhea. This product is useful against diarrhea caused by antibiotic use, vaginal and urinary tract infections and can help restore the normal balance of intestinal bacteria.

One of its new applications is the acidophilus with goat's milk that contained only *L. acidophilus* that produces lactic acid, hydrogen peroxide, and other products by the break down of nutrients. These byproducts make the environment hostile to undesirable organisms. It also helps the body to produce niacin, folic acid and Vitamin B_6. Still research is done to identify all the possible benefits and applications for *L. acidophilus* and other probiotics that may also help boost the immune system, lower the frequency of vaginal yeast infections, and may decrease cholesterol levels. *L. acidophilus* is also used as a test organism with *E. coli* K12 DH 5 in ultrasound-assisted thermal processing for preservation and quality retention of liquid foods (Zenker *et al*., 2003).

Probiotic ice cream is manufactured at pH 5.0, 5.5, and 6.0 by using probiotic cultures of *L. acidophilus* and *B. bifidum* that ferment standard ice cream mix. The fermented mix was frozen in a batch freezer. After freezing, *L. acidophilus* count was 1.5 x 10^8 cfu/ml and that of *B. bifidum* was 2.5 x 10^8 cfu/ml. Viability of *L. acidophilus* and *B. bifidum* as well as ß-galactosidase activity was monitored during 17 week of frozen storage at -29^{o}C. Probiotic ice cream was prepared to determine consumer preferences and concluded that probiotic microbes such as *L. acidophilus* and *B. bifidum* are beneficial and contribute healthy effect towards consumer (Hekmat & Mc Mahon, 1992). Recently, in Japan, a new type of yoghurt, 'Fit down' was developed. It is fermented by *L. acidophilus* LA67, which was selected by adhesive activity

through their screening system, and to which functional antihypertensive peptides prepared from whey proteins by protease digestion are added (Saito, 2004).

Non Dairy Products

Non dairy products are of significant importance around the world due to emerging trend of vegetarianism and high prevalence of lactose intolerance. Dairy sector which is strongly linked to probiotics, accounts for nearly 33% of the broad market while cereal products products have just over 22% (LFI 2006). A total of 78% of current probiotic sales in the world today are delivered through yoghurt. However, fruit juices, desserts, and cereal based products containing probiotics may be other suitable media for delivering probiotics (Cargill, 2009). Fruits and vegetables are made ideal substrates for probiotic culture by altering their structural characteristics by modification of food components in a controlled way through technological advances (Betoret *et al*., 2003).

Due to traditional and economic reasons and some drawbacks like allergy, lactose intolerance, the use of dairy products is going to be limited in developing countries of China, Japan and Africa, thus makes the development of non dairy probiotic foods essential (Shah, 2007). To fulfill consumer's expectancy for relish and healthy food, the development of new non dairy probiotic products turns out to be increasingly challenging. For developing new product, first of all basic formulation need to be known, secondly, optimum levels of key ingredients is necessary

to be determined in order to obtain suitable sensory and physicochemical characteristics, extended shelf life, chemical stability, and reasonable price (Jousse, 2008).

Bifidobacterium species and lactic acid bacteria, especially *Lactobacillus* strains, are widely used in food production, not only in fermentation of vegetables, sausages, and milk, but also in fruit based and vegetable based products, such as carrot, beet and celery (Karovicova *et al*., 2002), garlic (Castro *et al*., 1998), green olives (Sanchez *et al*., 2000), green cucumber juice (Lu *et al*., 2001), onions and peas (Karovicova *et al*., 1993), alfafa, clover, and galega (Shurkhna *et al*., 2006), and cereals (Angelov *et al*., 2006). Other non dairy products include acidophilus soy-based drink, frozen desserts, vegetable-based drinks, oat based and puddings (Donkor *et al*., 2007; Helland *et al*., 2005; Heenan *et al*., 2005 and Angelov *et al*., 2006).

According to Champagne *et al*., (2009), the development of a fermented product containing probiotics requires strain selection for the ability to grow in the substrate, as well as the ability to compete or even establish a synergy between strains. The main probiotic bacteria studied for growth in soy beverages are *L. acidophilus, L. fermentum, and bifidobacteria*. Soya beverage fermentation with *L. acidophilus* reduces its beany flavor and chalkiness. Soy is also considered a good substrate for functional foods, since fermentation by probiotics has the potential to (1) reduce the levels of some carbohydrates possibly responsible for gas production in the intestinal system, (2) increase free isoflavone levels and (3) favor desirable changes in bacterial populations in the gastrointestinal tract. Soy also benefits bone health (Champagne *et al*., 2009). Moreover, Larkin *et al*., (2007) showed that a combination of soy with either a probiotic or a prebiotic resulted in significant lipid lowering effect for both total and low-density lipoprotein

(LDL). This effect is not related to isoflavone bioavailability, since the bioavailability of daidzein and genistein was not affected by probiotic or prebiotic consumption and were not associated with lipid changes.

Tsen *et al*., (2004) used immobilized *L. acidophilus* to ferment banana puree and observed that the number of viable cells during fermentation was increased significantly relative to free cells. Similarly trend was reported by Nazzaro *et al*.,(2009) when immobilized *L. acidophilus* in an alginate prebiotic mixture and incubated it in carrot juice.

CONCLUSION

L. acidophilus has got numerous applications in the food industry. It has been isolated from various food items like meat, milk, fruits, vegetables and cereal products as being a natural contaminant. It has been commonly used as a starter culture in many food fermentation processes. Moreover, *L. acidophilus* produces various antimicrobial compounds thus contributing to the final product. Probiotic properties of a few strains of *L. acidophilus* have been thoroughly investigated within the last 20 years. The above review clearly reflects the importance of bacteriocins of *L. acidophilus*. Their bacteriocins can be added either directly in food or incorporated into the edible or non edible antimicrobial foods. The methods described in the literature for the production, purification and recovery of the bacteriocin of *L. acidophilus* are suitable for laboratory experiments but they need a systematic approach for the optimum production of bacteriocins at commercial level in a cost effective manner. It is evident that the potential application of *L. acidophilus* in the form of protective cultures either in dairy or non dairy products is significant. This study highlights the potential of the importance of bacteriocins

of *L. acidophilus* that may produced at a large scale and used a food preservative in commercial food products. In addition, probiotic properties for some *L. acidophilus* strains were intensively investigated. One of them is to produce antimicrobial compounds, contributing to the safety of the final product. The capability of *L. acidophilus* or its metabolites to decrease the growth of human pathogens like *E. coli, S. aureus, P. aeruginosa* and some others was well documented. Its antibiotic resistance mechanism was also reported but still there is a need of more research on it. Furthermore, genetic engineering of already identified strains and those newly discovered to make them more efficacious should be pursued.

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Table 1: Some physical, biochemical and genetic characteristics of *L. acidophilus*

Production of lactic acid from: Fructose, galactose, lactose cellobiose, amygdalin,

maltose, glucose, stachiose

Lactic acid were not produced from: mellibiose, raffinose, melezitose, mannitol,

Arabinose

Lactic acid isomers D and L

Hydrolysis of arginine –

Mol % G+C* 36.7 ± 0.7

Antibiotic resistance to Kanamycin, chloramphenicol, erythromycin, tetracycline, penicillin and

vanomycin

*G: Guanine; and C: Cytosine (Nuleotides of DNA).

Table 2: Summary of purified bacteriocin from *L. acidophilus*

Sr. No.	Producer *s*trains	Bacteriocins	References
1	AA11	Acidocin D20079	Abo-Amer, 2007

2	FM22	Bacteriocin	Adenike *et al*., 2007
3	IBB801	Bacteriocin	Avonts *et al.,* 2004
4	N2	Lactacin B	Barefoot and Klaenhammer, 1983
5	CH5	Acidocin B	Chumchuvalova *et al.s*, 1995.
6	LA-1	Acidophillicin LA-1	Dave and Shah, 1997.
7	DSM20079	Acidocin D20079	Deraz *et al.,* 2005
8	ATCC4356	Single Component Bacteriocin	Han *et al*., 2002
9	*Lactobacillus acidophilus*	Bacteriocin	Jagadeeswari *et al*., 2010
10	TK8912	Acidocin 8912	Kantani *et al*., 1992
11	TK9201	Acidocin A	Kantani *et al*., 1995
12	*Lactobacillus acidophilus*	Bacteriocin	Karthikeyar and Sanoth, 2009
13	GP1B	Acidocin 1B	Kyoung *et al*., 2007
14	M46	Acidocin B	Leer *et al*., 1995
15	TSI	Bacteriocin	Maqsood *et al*., 2008
16	LF221A	Acidocin LF221A and LF221B	Maijasic *et al*., 1998
17	1108	Lactacin F	Muriana and Klaenhammer, 1991
18	88	Lactacin F	Muriana and Klaenhammer, 1987
19	30SC	Bacteriocin	Oh *et al*., 2000
20	TL099a	Bacteriocin	Sengul *et al.,* 2003
21	JCM1229	Acidocin J1229	Tahara and Kantani, 1996
22	JCM1132	Acidocin J1132	Tahara *et al*., 1996
23	TK8912	Acidocin 8912	Tahara *et al*., 1992
24	M46	Acidocin B	Ten *et al*., 1994
25	LAPT1060	Acidophulicin A	Toba *et al*., 1991
26	YIT 0154	Bacteriocin like substance	Yamato *et al*., 2003
27	IBB801	Acidophilin 801	Zamfir *et al*., 1999

Table 3: Antimicrobial activity of bacteriocin obtained from *L. acidophilus*

Sr. No	**Producer strains**	**Inhibitory spectrum**	**References**
1	FM22	*B. subtilis* K12, *E. coli* V157 and *Vibrio* IINABA	Adenike *et al*., 2007
2	IBB801	*L. bulgaricus* LMG 6901	Avonts *et al*., 2004
3	N2	*L. bulgaricus* 1489, *L. lactis* 970, *L. helveticus* 87 and *L. leichmani* 4797	Barefoot and Klaenhammer, 1983
4	CH5	*Arthrobacter* spp. , *Bacillus* spp. and *Corynebacterium* spp.	Chumchuvalova *et al*., 1995.
5	DSM20079	*Lb. sakei* NCDO 2714	Deraz *et al*., 2005

6	ATCC4356	Pathogenic microbes	Han *et al*., 2002
7	*Lactobacillus acidophilus*	*B. subtilis, B. cereus, S. aureus* MTCC 96, *Vibrio cholerae, P. aeruginosa* MTCC 424 and *E. coli* MTCC 739	Jagadeeswari *et al*., 2010
8	*Lactobacillus acidophilus*	*L. bulgaricus, S. aureus, B. subtilis, E. coli, S. typhimurium, S. paratyphi 'B', Klebsiella* spp., *Serratia marsacence, P. aeroginosa* and *Vibrio cholerae*	Karthikeyar and Santosh, 2009
9	M46	*L. monocytogenes, C. sporegenes, L. fermentum, Brochothrix thermosphacta* and *L. delbrueckii* subsp. *bulgaricus*	Leer *et al*., 1995
10	TSI	*E. coli, E. feacalis, S. aureus* and *S. typhi*	Maqsood *et al*., 2008
11	88	*L. bulgaricus, L. lactis, L. helveticus* and *L. leichmani*	Muriana and Klaenhammer, 1987
12	30SC	*L. ivanovii* and *S. aureus*	Oh *et al*., 2000
13	TL099a	*Enterobacter cloacae* ATCC 13047	Sengul *et al.,* 2003
14	TK8912	*L. casei* ATCC 7469	Tahara *et al*., 1992
15	JCM1132	*L. casei, L. fermentum* and *L. plantarum*	Tahara *et al*., 1996
16	YIT 0154	*L. acidophilus* A201-7	Yamato *et al*., 2003
17	IBB801	*E. coli, S. panama* 1467	Zamfir *et al*., 1999

