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Role of Lactic Acid Bacteria (LAB) in Food Preservation and Human Health – A Review

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Abstract: Fermentation of various food stuffs by lactic acid bacteria (LAB) is one of the oldest forms of biopreservation practiced by mankind . Bacterial antagonism has been recognized for over a century but in recent years this phenomenon has received more scientific attention , particularly in the use of various strains of lactic acid bacteria One important attribute of many LAB is their ability to produce antimicrobial compounds called bacteriocins. In recent years interest in these compounds has grown substantially due to their potential usefulness as natural substitute for chemical food preservatives in the production of foods with enhanced shelf life and / or safety. There is growing consumer awareness of the link between diet and health. Recent scientific evidence supports the role of probiotic LAB in mediating many positive health effects.Traditional probiotic dairy strains of lactic acid bacteria have a long history of safe use and most strains are considered commensal microorganisms with no pathogenic potential.

Key words: Human health, food preservatin, lactic acid bacteria

Introduction:

The single most important development permitting the formation of civilization was the ability to produce and store large quantities of food . Hunter-gatherer societies lived from day to day either starving or gorging themselves based upon the amount of food they could find in a day. When it became possible for one person to produce more food then they needed, time from gathering food could be apportioned to culture and science. Following this trend, it became beneficial to be able to store as much food as possible in order to minimize the amount of time spent gathering that food. Food storage has always been at odds with food spoilage. Some of the earliest evidence of food preservation comes from the post-glacial era, from 15,000 to 10,000 BC. The first use of biological methods was from 6000 to 1000 BC when fermentation was used to produce beer, bread, wine, vinegar, yoghurt, cheese and butter. In 1864, Louis Pasteur proved that microorganisms in foods were the cause of food spoilage, that heat treatment of food killed these microbes and that sealed containers helped to preserve food by preventing recontamination from atmospheric air. A major development in the distribution and storage of foods came in 1940 with the availability of low cost home refrigerators and freezers. Other developments included the artificial drying, vaccum packaging, ionizing radiations and chemical preservation.

Now-a-days consumers are concerned about the synthetic chemicals used as preservatives in food , and there is resulting trend towards less processed food. These untreated foods can harbour dangerous pathogens which can multiply under refrigeration and without oxygen . A solution to this dilemma is the use of antimicrobial metabolites of fermentative microorganisms . Many antimicrobial chemicals have been in use for a long time without any known adverse effects. Many of the organic compounds which have stirred interest are antimicrobial metabolites of bacteria used to produce , or associated with fermented foods.

In fermentation, the raw materials are converted by microorganisms (bacteria , yeast and molds) to products that have acceptable qualities of food. In common fermented products such as yogurt, lactic acid is produced by the starter culture bacteria to prevent the growth of undesirable microorganisms (Ray and Daeschel 1992). Food fermentations have a great economic value and it has been accepted that these products contribute in improving human health. LAB have contributed in the increased volume of fermented foods world wide especially in foods containing probiotics or health promoting bacteria.

Micro-organisms of genera *Lactococcus, Lactobacillus, Leuconostoc, Streptococcus* and *Pediococcus* are involved in these fermentations. In addition, *Lactobacillus spp.* and species of *Bifidobacterium* which is not LAB in nature are part of normal human intestinal microflora and they exert a positive effect on human health. (Daly and Davis, 1998).

This review will focus on some of the properties of LAB that contribute to their roles in biopreservation and in modulating the health of their hosts.

Development in the biotechnology of LAB: One area where genetic engineering would be of particular benefit to the dairy industry is in the genetic modification of lactic acid bacteria (LAB) which are commonly used as starter cultures in the production of fermented dairy foods . During past 20 years much of the research on LAB focused on dairy *lactococci* , investigations now include different LAB involved in wide variety of fermentation processes and, various *lactobacilli* and *bifidobacteria* belonging to the human microbiota. However, significant development in bacteriophage biology and resistance mechanisms, pyruvate metabolism and production of bacteriocins have also been made (Fitzgerald and Hill, 1996; von Wright and Sibakov, 1998).Where as research on chromosomal genetics of LAB is also progressing rapidly.Physical & genetic map of many of these strains have been constructed (Davidson *et al.*, 1996). New techniques such as ability to sequence large tracts of 16s and 23s rRNA genes using polymerase chain reaction (RAPD-PCR) and the use of pulsed field gel electrophoresis (PFGE) to finger print genomic restriction patterns have contributed enormously in strain identification and classification (Axelsson, 1998). This relates to field of probiotics where the ability to monitor strains through clinical trials and to evaluate their effects on the gastrointestinal tract microflora as well as the protection of their proprietary value depends on exact and reproducible strain identification.

LAB bacteriocins as biopreservatives: Despite improved manufacturing facilities and implementation of effective process control procedures such as Hazard Analysis and Critical Control Points (HACCP) in the food industries, the number of food borne illnesses has increased. Nowadays consumers favour food with few chemical preservatives (Daeschel, 1993). As a result there is increased interest in the preservation through LAB because of their safe association with human fermented foods. Several metabolic products produced by these bacteria have antimicrobial effects, including organic acids, fatty acids, hydrogen peroxide and diacetyl (Holzapfel *et al.*, 1995; Ouwehand, 1998). However attention has focused on the ability of LAB to produce specific proteinaceous substances, bacteriocins that inhibit the growth of pathogens such as *Listeria, Clostridium, Staphylococcus, Bacillus spp.* and *Enterococcus spp.*, therefore they enhance the shelf life of foods.

Bacteriocins produced by LAB are the subject of intense research because of their antibacterial activity against foodborne bacteria. Bacteriocin producing strain of LAB may be very important in competing with other organisms in the intestine. They consists of a biologically active protein moiety, have a bactericidal mode of action and attach to specific cell receptors. Bacteriocins are heterogeneous group of bacterial antagonists that vary considerably in molecular weight, biochemical properties, range of sensitive hosts and mode of action. Klaenhammer (1988) define them as, protein or protein complexes with bactericidal activity directed against species that are usually closely related to the producer bacterium. Both Gram negative and Gram positive bacteria produce them. The Gram-negative bacteriocins are colicin, which are produced by strain of *E.coli* (Braun *et al.*, 1994). These are large, complex proteins, 29-90 kDa, with characteristic structural domains involved in cell attachment, translocation and bactericidal activity. They bind to specific receptors on the outer membrane of the target cell. The bacteriocins produced by Gram-positive bacteria are small peptides 3-6 kDa, in size (Nes *et al.*, 1996), although there are exceptions (Joerger and Klaenhammer, 1990). They fall with in two broad classes,viz the lantibiotics (Jack *et al.*, 1995) and the non- lantibiotic bacteriocins (Nes *et al.*, 1996).Most of the Gram positive bacteriocins are membrane active compounds that increase the permeability of the cytoplasmic membrane (Jack *et al.*, 1995). They often show a much broarder spectrum of bactericidal activity than the colicins. There is currently much interest in the application of bacteriocins in both food preservation and the inhibition of pathogenic bacteria (Liao *et al.*, 1994; Young and Ray, 1994; Delves-Broughton, 1996). Most of the bacteriocins have been isolated from organisms involved in food fermentation. Bacteriocin production and resistance is considered as an important property in strains used as commercial inoculants to eliminate or reduce growth of undesirable or pathogenic organisms.

At present four classes of LAB bacteriocins have been identified (Table 1). Nisin which is produced by *Lactobacillus lactis subsp lactis* strains, belongs to the class 1 lantibiotics (Dodd and Gasson, 1994; Jack *et al.*,1995) and is active against many gram positive bacteria including *Listeria spp.* It prevents the growth of germinating *bacillus* and *clostridial* spores and through the addition of calcium chelator, it is active against some gram negative bacteria (Stevens *et al.*, 1991). The mature nisin molecule is just 34 amino acids long and undergoes certain post translational modifications in which serine and threonine residues are dehydrated and several thio-ether bridges are formed, this results in the formation of five ring structures. The primary target of Nisin's antimicrobial action is the cell membrane. It is thought that nisin interferes with the energy supply, of the cell by creating pores in the membrane and dissipating its potential (Sahl *et al.*, 1995). Another compound, lacticin 3147 was identified from *lactococcal* isolate of Irish Kefir grains used in the manufacture of butter milk (Ryan *et al.*, 1996) This bacteriocin inhibits gram positive food borne pathogens such as *staphylococcus, clostriduim* and *listeria spp.* as well as several mastitis producing *staphylococci* and *streptococci* (Meaney *et al.*, 1997) Lacticin 3147 requires two peptides for activity.

Class II bacteriocins are relatively small cationic peptides (30-100 amino acids) exhibiting a high degree of heat stability. Class III bacteriocins are produced by members of the *Lactobacillus* genera. Helveticin J is the best known compound of this class.

Many bacteriocins of LAB are safe and effective natural inhibitors of pathogenic and food spoilage bacteria in various foods. Nisin is the classic example, it prevents *clostridial* spoilage of processed and natural cheeses, inhibits the growth of some psychrotrophic bacteria in cottage cheese, extends the shelf life of milk in warm countries, prevents the growth of spoilage *lactobacilli* in beer and wine fermentations and provides additional protection against *bacillus* and *clostridial* spores in canned foods. Nisin is a permitted food additive in more than 50 countries including the US and Europe under the trade name Nisaplin (Vandenberg, 1993; Delves- Broughton *et al.*, 1996).

Listeria monocytogenes is common contaminant of raw foods such as milk, meat and vegetables and results in the serious foodborne illness in consumers (Ryser and Marth, 1991). Therefore bacteriocins of sub class IIa pediocin PA-1/AcH produced by *Pediococcus acidilactici* have ability to control *Listeria* in cheese, vegetables and meat. *P. acidilactici* is used as a starter culture in the production of many fermented meat (Vandenberg, 1993; Stiles, 1996). Ryan *et al.* (1996) developed lacticin 3147 producing starter strains for cheese making, these strains effectively controlled the growth of a non starter LAB in cheddar cheese and completely eliminated *L. monocytogenes* from cottage cheese. Lacticin 3147 is effective at neutral pH and starter cultures producing this bacteriocins have good acid producing and bacteriophage resistance properties. Unfortunately the LAB bacteriocins are not effective against gram negative bacteria and yeasts and moulds.

Recently, studies aimed at broadening the bactericidal activity of LAB bacteriocins are focused on the synergistic effects of bacteriocins most notably nisin, with other antibacterial factors such as the lactoperoxides system present in milk, hydrolytic enzymes, various chelating agents (including siderophores)and other bacteriocins (Helander *et al.*, 1997). Now-a-days nisin remains the only LAB bacteriocin legally used as a food additive. Two products ALTA 2431 and Microgard have been developed as shelf life extenders based on crude LAB fermentation products.

ALTA 2341 is produced from *Pediococcus acidilactici* fermentation and have to rely on the inhibitory effects of pediocin PA-1/AcH. It is added to Mexican soft cheese which is susceptible to *listerial* contamination (Glass *et al.*, 1995) Microgard is the result of a *propionibacterium* fermentation. It is active against gram negative bacteria such as *Pseudomonas, Salmonella, Yersinia* as well as yeast and moulds. Microgard's protective action is probably due to the presence of propionic acid. It has been approved by FDA for use in cottage cheese and fruit flavoured yoghurts. Another product Bioprofit is a combination of specific *Lactobacillus* and *Propionibacterium* strains is used in normal starter cultures to inhibit the growth of yeasts, moulds, *Bacillus spp. Clostridium*

Table 1: Properties of some well characterized bacteriocins

Bacteriocin	Producer organism	Properties
Nisin	*Lactococcus lactis subsp. lactis* ATCC 11454	Lantibiotic, broad spectrum,chromosome/plasmid mediated, bactericidal, produced late in the growth cycle
Pediocin A	*Pediococcus pentosaceus* FBB61 and L-7230	Broad spectrum, plasmid mediated
Pediocin AcH	*Pediococcus acidilactici* H	Broad spectrum, plasmid mediated
Leucocin	*Leuconostoc gelidum* UAL 187	Broad spectrum, plasmid mediated, bacteriostatic, produced early in the growth cycle
Helveticin J	*L.helveticus* 481	Narrow spectrum, chromosomally mediated, bactericidal
Carnobacteriocin	*Carnobacterium piscicola* LV17	Narrow spectrum, plasmid mediated, produced early in the growth cycle

spp. and heterofermentative *lactobacili* during dairy fermentations. (Mayra-Makinen and Suomalainen, 1995).
It is proposed that bacteriocins should be used in combination with other physical chemical and microbial preservatives to check the growth of pathogenic or food spoilage bacteria.

LAB and health :Probiotics: As we enter in the new millennium, people are aware that for spending a healthy life style diet play a major role in preventing diseases and promoting health. Therefore there is an increasing trend for foods containing probiotic cultures.
The concept of probiotics was in use in the early 1900s, however, the term was coined in 1965 by Lilly and Stillwell . Probiotic is a preparation of live microorganisms which when applied to man or animal, beneficially affects the host by improving the properties of the indigenous microbiota (Havenaar and Huis in't Veld, 1992).
Probiotics are viable organisms and supportive substances that improve intestinal microbial balance, such as *Lactobacillus acidophilus* and bioactive proteins (Fuller, 1991). The empirical evidence that for many years, linked the use of fermented dairy products such as yogurt and milk with the promotion of intestinal health is today well supported by modern science. The ability of the probiotic *L. acidophilus* to help prevent pathogenic bacteria from proliferating and healthy bacteria from becoming toxic is well documented. (Speck, 1975 ; Wynder, 1977).
Antimicrobial compounds produced by LAB have provided these organisms with a competitive advantage over other microorganisms. Exploitation of antibiosis of LAB is the best choice for not only improving the microbial safety of the food products but as a probiotic preparation because of their natural adaptation to the gut environment. Lactics need to be acid tolerant bacteria and exhibit resistance to lysozyme present in the saliva and other enzymes, gastric juice and duodenal fluids. Many lactics are resistant to the bile salt present in the gut and survive the intestinal motility and adhere well to gastric mucosa. Probiotics act through suppression of viable count by production of antibacterial compounds, competition for nutrients and adhesion sites, alteration of microbial metabolites and stimulation of immunity. (Mishra and Lambert, 1996).
Traditional probiotic dairy strains of LAB have a long history of safe use. There is considerable interest in extending the range of foods incorporating probiotic organisms from dairy foods to infant formulae, baby foods, fruit juice based products, cereal based products and pharmaceuticals (Lee and Salminen, 1995). *Lactobacillus spp.* and *Bifidobacterium spp.* are prominent members of the commensal intestinal flora and are the commonly studied probiotic bacteria. They cause reduced lactose intolerance, alleviation of some diarrhoeas, lowered blood cholesterol, increased immune responses and prevention of cancer (Marteau and Rambaud, 1993, 1996; Gilliland 1996, Salminen *et al.*, 1998a). Salminen *et al.* (1998a) presented a list of successful probiotic strains, including, *L. acidophilus* NCFB 1478, *L. johnsonii* LAI, *L. casei shirota* strain and *L. rhamnosus GG*. The selection criteria for probiotic LAB include: human origin, safety, viability/activity in delivery vehicles, resistance to acid and bile, adherence to gut epithelial tissue, ability to colonise the GIT, production of antimicrobial substances, ability to stimulate a host immune response and the ability to influence metabolic activities such as vitamin production, cholesterol assimilation and lactose activity (Huis in't Veld and Shortt, 1996, Salminen *et al.*, 1996). Probiotic preparations such as *Lactobacillus GG, L. johnsonii* LA1 and NCFB 1748, *L. casei shirota* strain and *L. reuterii* are beneficial in the prevention and treatment of certain GI infections including infantile viral diarrhoea and antibiotic associated diarrhoea (Lee and Salminen, 1995, Salminen *et al.*, 1998a). It is likely that *lactobacilli* suppress the growth of pathogens at the mucosal surface probably by out competing them for nutrients or by producing antibacterial compounds (Salminen *et al.*, 1998a; Isolauri *et al.*, 1998).
Several studies in both animals and humans have showed the ability of LAB to reduce the toxicity of intestinal contents by suppressing the levels of bacterial enzymes such as B-glucoronidase, nitroreductase, azo-reductase and urease, all of which activate procarcinogens (Salminen *et al.*, 1996, 1998a; Isolauri *et al.*, 1998).
In addition many LAB produce metabolic end products (butyrate/ butyric acid) that have antitumorigenic activities in vitro (young, 1996). *Lactobacillus casei shirota* strain when orally administered reduced the recurrence of superficial bladder carcinoma in humans (Aso and Akazan, 1992; Aso *et al.*, 1995). LAB can modulate host immune response. Reports showed increased production of immunoglobulins, interleukins 6 and 10, gamma interferon, tumour necrosis factor-a and increased phagocytic activity. *L. GG* stimulate local and systemic IgA to rota virus during infection of children with this agent (Kaila *et al.*, 1992). *L. salivarius* UCC 118 also exhibits a strong mucosal IgA immune response in humans during clinical trials (Mattila Sandholm, 1997).
Another compound called prebiotics are based on non- or slowly absorbable complex CHO that can be assimilated by beneficial bacteria such as *Bifidobacterium* & *Lactobacillus spp.* Examples of prebiotic substrates are inulin, lactulose, various galacto, fructo, xylo-oligosaccharides and sugar alcohols such as lactitol and xylitol (Salminen *et al.*, 1998b).Many of the functional foods contain a combination of a probiotic culture with a prebiotic substrate that favours its

growth. One fermented drink Fyos (Nutricia) is the synbiotic product which is combination of probiotic culture *L. casei* and prebiotic oligofructose, inulin .Over the years a number of microbes have been utilized as probiotics.

Microorganisms used as probiotics:
Lactobacillus acidophilus
L.plantarum
L. casei
L. casei subsp. rhamnosus
L. delbreuckii subsp. bulgaricus
L. fermentum
L.reuteri
Lactococcus lactis subsp. lactis
L. lactis subsp. cremoris
Bifidobacterium bifidum
B. infantis
B.adolescentis
B. longum
B. breve
Streptococcus salivarius subsp. thermophilus
Enterococcus faecalis
E.faecium
Saccharomyces boulardii
Source: Conway (1996).

The recent advances in biotechnology have significantly increased the production of high quality, nutritious and tasteful foods that remain fresh for long time and are completely safe and less reliant on artificial additives. The potential application of bacteriocins as consumer friendly biopreservatives either in the form of protective cultures are as additives is significant. Besides being less potentially toxic or carcinogenic than current antimicrobial agents , lactic acid bacteria and their by products have been shown to be more effective and flexible in several applications. Evidence is accumulating that confirms that probiotics can benefit the host by improving intestinal well being .In order to have functional probiotic strains with predictable and measurable beneficial effects , strict attention to strain selection is required.

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