

Research Trends in Probiotic Technology

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Chapter - 3

Lactic Acid Bacteria

Harshada Joshi and Deepti Khandelwal

In 1873, ten years after Pasteur studied lactic acid fermentation (between 1857 and 1863) the first pure culture of a lactic acid bacterium ("*Bacterium lactis*") was obtained by Lister. Starter cultures for cheese and sour milk production were introduced in 1890, while fermented food has been used by man for more than 5,000 years (Schlegel, 1999 and Stiles and Holzapfel, 1997). The first monograph by S. Orla-Jensen appeared in 1919. A typical lactic acid bacterium grown under standard conditions (nonlimiting glucose concentration, growth factors and oxygen limitation) is gram-positive, nonsporing, catalase negative in the absence of porphorinoids, aerotolerant, acid tolerant, organotrophic and a strictly fermentative rod or coccus, producing lactic acid as a major end product. Its features can vary under certain conditions. Catalase and cytochromes may be formed in the presence of hemes and lactic acid can be further metabolized, resulting in lower lactic acid concentrations. Cell division occurs in one plane, except pediococci. The cells are usually nonmotile. They have a requirement for complex growth factors such as vitamins and amino acids. Axelsson (2004) suggested that an unequivocal definition of LAB is not possible.

The term lactic acid bacteria were accepted in the beginning of the 20th century (Carol and Leon, 2010). Other terms normally used such as "milk souring" and "lactic acid producing" for the same bacteria causing a slight confusion. Lactic acid bacteria form a group of bacteria that shows morphological, metabolic and physiological similarities, and they are also phylogenetically related. Lactic acid bacteria have been used in food fermentations for more than 4000 years. The term "Lactic acid bacteria" which is usually used has no official status in taxonomy and is only a general term used for convenience to describe the group of functionally and genetically related bacteria. They consist of bacterial genera within the *Firmicutes* comprised of about 20 genera. This is a core group consisting of following four genera; *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*. Recent taxonomic revisions have proposed several new genera and the group now comprises of the following: *Aerococcus*,

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Chapter - 7

Antibacterial Spectrum of Lactobacilli

Harshada Joshi

Lactic acid bacteria exhibit the antagonistic effect against the growth of various organisms. The effect is based on the metabolic products resulting from different biochemical activities of LAB and competitive organisms. Certain metabolites in cultured dairy products may be responsible for increased shelf life of the foods by inhibiting a wide spectrum of food spoilage organisms. The antibacterial principles elaborated by lactic cultures used in preparation of cultural dairy products apparently contribute to the increase in shelf life of food and inhibition of food-borne organisms. The consumption of cultured products containing such antibacterial substances elaborated by LAB may provide the consumer with protection against disease causing organisms.

Evidence has been mounting concerning the inhibition of *Staphylococcus aureus*, *Pseudomonas putrefaciens*, *Escherichia coli*, *Clostridium perfringens*, *Salmonella tennessee*, *Vibrio parahemolyticus* and other spoilage and pathogenic organisms by lactic cultures such as *Streptococcus lactis*, *Streptococcus diacetylactis*, *Leuconostoc cremoris* and several strains of lactobacilli (Shahani and Chandan, 1979). Grosswies *et al.* (1947) observed that antibiotic substance produced by a strain of *Lactobacillus* was found to be effective in *in vitro* study against gram positive and gram negative organisms. In another study, Wheater *et al.* (1952) reported that inhibitory substance of lactobacilli was found active against *Staphylococcus aureus*. In another experiment, definite inhibition of growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was observed when these organisms were streaked on liver veal agar plates heavily grown with lactobacilli isolated from rat intestine (Vincent *et al.*, 1955).

Vincent *et al.* (1959) claimed that antibiotic like substance from certain strains of intestinal *Lb. acidophilus* had a broad antibacterial spectrum in its crude form and inhibited the growth of *Staphylococcus* spp. and *Escherichia coli* isolated from human faeces. Neri (1961) reported that several strains of *Lactobacillus* such as *Lb. casei* 22, *Lb. acidophilus* 1 and *Lb. acidophilus* 3

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Chapter - 8

Bacteriocins of Lactic acid Bacteria

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Several factors that are responsible for the inhibition of harmful bacteria from multiplying on and attaching to the intestinal epithelium include bacteriocins and organic acids (antimicrobial agents). Other factors such as production and secretion (Bezkorovainy, 2001), adherence via competition for the binding sites and steric hindrance (Caplice & Fitzgerald, 1999; Ralfe, 2000 and Schrezenmair & de Vrese, 2001). Reduction in pH as a result of lactic acid production from sugar fermentation process is one of the major factors for the prevention of the proliferation of the undesirable microorganisms (Ivanova *et al.*, 2000 and Kuipers *et al.*, 2000). The production of lactic acid from the fermentation processes lead to the resultant pH reduction resulting in the liposolubility of organic acids thereby enhancing the ease with which they can penetrate the cell membrane and gain entrance into the pathogen cytoplasm (Haller, 2000). Chundawat and Joshi (2015) studied the antagonistic activity of *Lactobacillus* isolates from cow milk on pathogenic microorganisms. Three *Lactobacillus* strains namely *Lactobacillus plantarum* CM1, *Lactobacillus fermentum* CM4 and *Lactobacillus casei* subsp. *casei* CM6 were isolated from cow milk samples and their antagonistic activity was tested against five pathogenic bacteria such as *Serratia marcescens* NCDC 108, *Enterobacter aerogens* NCDC 106, *Proteus vulgaris* NCDC 73, *Pseudomonas aeruginosa* NCIM 5029, and *Micrococcus luteus* NCDC 131 using agar well assay method. Results suggested that the *Lactobacillus* strains namely *Lactobacillus plantarum* CM1, *Lactobacillus fermentum* CM4 and *Lactobacillus casei* subsp. *casei* CM6 were able to inhibit the growth of all the pathogenic bacteria in varying degrees. All the three *Lactobacillus* isolates were found to be the most sensitive against *Micrococcus luteus* than the rest of the pathogenic bacteria used in the study. The inhibition observed in the case of all the isolates that have antagonistic effect may be due to the production of organic acids as well as other compounds such as bacteriocins, hydrogen peroxide etc. Various factors that contribute for antibacterial activity are discussed in the previous chapter.