

Antimicrobial Activity and Characterization of *Lactobacillus Reuteri* Isolated From Human Milk

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ABSTRACT

Breast milk is the primary food for an infant. Chief ingredients of breast milk include lactobacillus strains. *Lactobacillus* strains isolated from breast milk were investigated for their antimicrobial activity against pathogenic microorganisms. The antibacterial activity is attributed to bacteriocin secreted by *Lactobacillus*. Bacteriocin present in cell free supernatants of *Lactobacillus reuteri* exhibited inhibitory activity against microorganisms such as *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus* spp. and *Vibrio cholera* by agar well diffusion method. Optimization of bacteriocin production and characterization of extra cellular bacteriocin were studied. The bacteriocin produced by *Lactobacillus reuteri* was stable at 60°C for 10 minutes, but inactivated by heating at 80°C for 10 minutes. It was stable at pH 4.0 to 7.0 but sensitive to pH 9.0. 1% and 2% sodium chloride enhanced the production of bacteriocin from 25% to 75% in MRS broth. Molecular weight of bacteriocin purified by ammonium sulphate precipitation method was 2.5 kDa. Thus, bacteriocin of *Lactobacillus reuteri* strains isolated from human milk exhibited antibacterial activity against common clinical pathogens. It also exhibited properties of a good probiotic.

R. Ilayaraja and Radhamadhavan (2010). Antimicrobial activity and characterization of *Lactobacillus reuteri* isolated from human milk. *Internat. J. Med. Sci.*, 3(1 &2):27-33

Key words :

Bacteriocin,
Antimicrobial
activity,
Lactobacillus
reuteri, Human
health

Human milk is a complex species-specific biological fluid perfectly adapted to satisfy the nutritional and immunological needs of neonates. It has been demonstrated that breast milk confers protection against different infectious diseases since the incidence of these disorders is lower in breast-fed than in formula-fed infants. This anti-infective effect is due to several bioactive compounds present in colostrums in mature milk. (Saavedra, 2007).

Recent studies have shown that human milk, far from being a sterile fluid, constitutes an excellent and continuous source of commensal bacteria for the infant gut. These bacteria also play an important role in the reduction of incidence and severity of infectious diseases in breastfed children. This is supported by relatively older studies reporting the loss of antimicrobial activity in pasteurized human milk. (Ford, 1997).

The predominant bacterial species found in human milk include *Staphylococcus*, *Lactococcus*, *Enterococcus* and *Lactobacillus*. (Martin, 2003). Recently, there is an increased focus on some of the breast milk lactobacilli such as *L.gasseri*, *L.salivarius*, *L.rhamnosus*, *L.plantarum* and *L.fermentum*,

because they are considered as potentially probiotic species.

Bacteriocins of *Lactobacilli* are shown to belong to the four major classes of antimicrobial proteins produced by lactic acid bacteria. Class-I (Lantibiotics) was only recently discovered in the *Lactobacillaceae* by Mortvedt (1991). Lantibiotics are small membrane active peptides (<5kDa) containing the unusual amino acid lanthionine. Class-II is represented by small heat stable, non-lanthionine containing membrane active peptides (<10kDa). Class-III bacteriocins, include heat labile proteins of large molecular mass. Class-IV is a complex bacteriocin group. These proteins are associated with other lipid or carbohydrate moieties, which appear to be required for activity. (Klaenhammer, 1993).

Probiotic is a live microbial supplement which affects host's health positively by improving its intestinal microbial balance. The high concentration of LAB in milk from healthy mother may play an important biological role during the first months of life. The probiotic preparations use for traveller's diarrhoea, antibiotic associated diarrhoea and acute diarrhoea which is shown that they have

Accepted :
September, 2010