

Evaluation of Antimicrobial Activity of Probiotic Microflora from Neonatal Origin.

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Abstract: The antimicrobial effects of the bacteriocin obtained from nine Lactic Acid Bacteria isolated from the fecal sample of breastfed neonates were tested against three pathogenic indicator strains, *Shigella flexineri* NCIM5265, *Bacillus cereus* NCIM2079 and *Proteus vulgaris* NCIM2027. 20 hrs old cultures supernatant was used to perform the antimicrobial activity using well diffusion method. All the nine isolates showed good inhibition of *Shigella flexineri* and *Bacillus cereus*. While seven isolates of nine showed moderate inhibition of *Proteus vulgaris*. Two isolate showed no inhibition to *Proteus vulgaris*.

Keywords: antimicrobial effects, breastfed neonates, well diffusion method

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Introduction: Lactic acid bacteria (LAB) are Gram-positive, acid -tolerant and non-spore forming cocci, coccobacilli and rods. One common feature of the LAB is their ability to produce lactic acid as a major end product of their fermentation of hexoses. As fermenting organisms, LAB lack electron transport systems and cytochromes, and they do not have a functional Krebs cycle. Based on the products of glucose metabolism, LAB can be divided into two groups, namely homofermentative and heterofermentative. LAB are widespread organisms and they may be found in many environments rich in carbohydrates including human gut. LAB of neonatal origin were belong to genera Lactobacilli, Bifidobacteria, Enterococci and Streptococci (Yoshioka, H.1983).

The inhibitory power of Lactococcus was first observed in 1930 when the inhibition of commercial cheese starter cultures by similar dairy bacteria was reported. Mode of antagonistic activity of lactic acid bacteria depends upon their metabolic products and its action on test organisms such as i) Carbon dioxide inhibit decarboxylation and thus reduces membrane permeability of test bacteria ,ii) Diacetyl interacts with arginine - binding proteins of test organisms and thus inhibits the test bacteria,iii) Hydrogen peroxide oxidize basic proteins iv) Lactic acid dissociated the membrane and hence lowered the intracellular pH of test organisms, and v) Bacteriocins, affect membrane, DNA synthesis and protein synthesis (Review by Siamansouri, M.,2013). Lactic acid bacteria (LAB) especially, Lactobacilli and bifidobacteria have gained particular attention nowadays, due to the production of bacteriocins. The aim of this study was to evaluate the antimicrobial activity of lactic acid bacteria isolated from breastfed neonate against pathogenic

Shigella flexineri, *Bacillus cereus* and *Proteus vulgaris*

(The study protocol was approved by Institutional Ethics Committee of Bharati Vidyapeeth Deemed University, Pune, India.)

Material and method

Sample collection: Stool samples were collected with the help of swabs, transported in Carry Blair medium and further processed within two hrs. Sample dilutions were made with MRS liquid medium and 100µl were placed on MRS media. All the plates were incubated anaerobically at 37°C for 24 hrs in 5% CO₂. Suspected LAB colonies were picked up and selected for this study which were non motile, non oxidase and catalase negative and non H₂S producing. The isolates were maintained in Glycerol buffer at -20°C.

All the test organisms were purchased from National collection of industrial microorganisms NCIM, National Chemical Laboratory, Pune. Working stocks of test organisms were maintained on nutrient agar.

Antibacterial activity: The antibacterial activity of the isolates was checked against test organisms *Shigella flexineri* NCIM5265, *Bacillus cereus* NCIM2079 and *Proteus vulgaris* NCIM2027 by agar well diffusion technique. In short Supernatants of LAB isolates were monitored for antibacterial activity against indicator bacteria inoculated on nutrient agar. A volume of 100µl of cell free supernatants was filled in 8-mm diameter wells cut in the nutrient agar seeded with appropriate test organism. The diameter of the inhibition zone was measured after 24 hrs of incubation. The results were confirmed with repeating the experiments thrice.

Result and discussion:

Results of Antimicrobial activity of probiotic organisms in Agar-well diffusion method

Name of the LAB isolate	Zone of inhibition in diameter		
	<i>Shigella flexineri</i>	<i>Bacillus cereus</i>	<i>Proteus vulgaris</i>
S.S.201	10	12	No inhibition
S.S.83	12	13	9
S.S.141	11	14	9
S.S.192	10	11	10
S.S.322	12	12	9
S.S.95	13	14	No inhibition
S.S.172	11	14	9
S.S.251	14	12	10
S.S. 72	12	10	9

The use of bacteriocins and/or bacteriocin-producing strains of LAB are of great interest as they are generally recognized as safe organisms and their antimicrobial products as biopreservatives. As per report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food (2002), one of the most important parameters by which potentially new probiotic strain must be characterized is the production of antimicrobial activity against potentially pathogenic bacteria. All Lactic acid bacteria isolated from breastfed neonate showed good inhibition against *Shigella flexineri* causing food spoilage. This is similar to the findings of Ogunbanwo *et. al.* (2003). All the cell free supernatant of isolated LAB show inhibition to

Bacillus cereus. The similar results were observed in the findings of Mohammed, S. *et.al.*, 2013 where the LAB was isolated from fermented milk. *Proteus vulgaris* which was well known UTI pathogen was inhibited by seven out of nine isolates.

In conclusion antimicrobial compounds produced by LAB were effective against the test pathogens used in this study. These bacteriocins produced could serve as alternative to chemical preservatives/additives used in food preservation. After checking safety of the LAB on the basis of criteria laid down by WHO for potential probiotics these organisms may be used as human or animal probiotics.

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