

ORIGINAL RESEARCH ARTICLE

Microbial Product Act As a Probiotic against Human Intestinal Pathogens

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Received 15 May 2011; Revised 29 Jul 2011; Accepted 05 Aug 2011

ABSTRACT

Bacteriocins produced by *L.acidophilus* (LAB) have been in focus of many investigations because of their particular importance in dairy industry. Antibacterial activity of the isolated LAB (cell free filtrate) was tested against selected gram positive and gram negative bacterial species by well diffusion assay. Bacteriocin produced by *L.acidophilus* was showed higher antibacterial activity against gram negative pathogenic bacteria, such as *E.coli*, *K.pneumoniae*, *S.typhimurium* and *V.cholerae*. Whereas gram positive pathogenic bacteria showed lower antibacterial activity against *S.aureus* and *B. cereus*. The results obtained in the present study suggest that bacteriocin produced by *L.acidophilus* inhibits the growth of selected gram positive and gram negative bacterial sp. Regular consumption of probiotic food will prevent gastrointestinal infection in adult.

Key words: *Lactobacillus acidophilus*, Bacteriocin, Carbohydrate source, Lactic acid bacteria and Yoghurt.

INTRODUCTION

A custardlike food with a tart flavour prepared from milk curdled by bacterium especially *L.bulgaricus*, *Str.thermophilus* and often sweetened or flavoured with fruit are known as yoghurt. *L. acidophilus* strains are widely used as probiotic cultures in dairy and pharmaceutical products because the species is one of the dominant lactobacilli in the human intestine [1]. A probiotic is defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance [2]. Intestinal LAB in human are closely associated with the host’s health because it’s an important biodefense factor in preventing colonization and subsequent proliferation of pathogenic bacteria in intestine [3].

Bacteriocins, are proteinaceous compounds produced by bacteria that exhibit a bactericidal mode of action against related as well as unrelated organisms [4]. Bacteriocins are bacterial peptides that inhibit or kill microorganisms that are usually but not always closely related to the producer strain [5]. Although several bacteria produce bacteriocins, LAB have been extensively studied with the perspective of its use as natural biopreservative in the food industry [6]. A large

number of studies have been carried out only using lactobacillus sp. Therefore in the present study *Lactobacillus acidophilus* has been targeted to isolate it from yoghurt samples based on their potential as antimicrobial agent.

MATERIALS AND METHODS

Collection of sample

Yoghurt sample were purchased from Sholinganallur local market, Chennai, Tamilnadu. They were immediately transported to the laboratory and used for the present study.

Isolation of bacteria

Lactic acid bacteria were isolated from the yoghurt sample for which 10gm was mixed with 90ml of normal physiological saline solution and was homogenised for 2 mts. Serial dilution were performed from 10⁻¹ to 10⁻⁹. From the dilution factor (10⁻⁶ to 10⁻⁹) 0.1 ml of the sample were plated on the De Man Rogosa and Sharpe agar medium (Hi-media, Mumbai). Triplicate plates were incubated at 37°c for 48 hrs in an anaerobic jar. After 48hrs isolated colonies were gram stained and identified using microbiological methods. The isolated bacterial colonies were stored in MRS slant at 4°c and sub cultured periodically for future use [7].

Maintenance of test inoculums

Bacterial species used for the antibacterial activity were *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 2940), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 139), *Vibrio cholerae* (MTCC 3096), *Salmonella typhimurium* (MTCC 98). All the stock cultures were obtained from the Institute of Microbial Technology (IMTEC) Chandigarh, India. Then they were maintained in nutrient slants in the dept. of Microbiology at Mohammed Sathak College of Arts and Science, Sholinganallur, Chennai-119. A loopful of bacterial cultures were inoculated in the Brain Heart Infusion broth and incubated at 37⁰ c for 24 hrs.

Maintenance of lactobacillus culture

MRS broth culture was prepared and inoculated with *Lactobacillus .sp* and incubated anaerobically at 37⁰c for 48 hrs using anaerobic jar.

Preparation of cell-free filtrate

10ml of MRS broth was inoculated with *L.acidophilus* strain and incubated at 37⁰c for 48hrs in anaerobic condition. After incubation, cell free solution was obtained by centrifugation with 6000 rpm for 15min, followed by filtering supernatant solution with 0.2mm pore size whatmans no.1 filter paper. This cell free filtrate was used for antimicrobial activity [8].

Antibacterial activity of *L.acidophilus* by well diffusion assay

Antibacterial activity of the isolated LAB (cell free filtrate) were tested against *S.aureus*, *B.cereus*, *E.coli*, *K.pneumoniae*, *V.cholerae* and *S.typhimurium* bacterial species by well diffusion assay. The test strains of pathogenic bacteria were

Table 1: Antibacterial activity of *Lactobacillus acidophilus* by well diffusion assay

Bacterial strains	Tested Organisms	Zone of inhibition in mm	
		BHA broth filtrate(100µl)	<i>L.acidophilus</i> Control (100µl)
Gram positive	<i>B. cereus</i>	-	1.766 ± 0.173
	<i>S.aureus</i>	-	2.7 ± 0.145
Gram negative	<i>E.coli</i>	-	3.13 ± 0.187
	<i>V.cholerae</i>	-	1.70 ± 0.115
	<i>K. pneumoniae</i>	-	1.90 ± 0.208
	<i>Sal.typhimurium</i>	-	1.90± 0.057

Values are expressed as standard mean ± SEM from triplicates in each microorganism.

Keys: BHA control- Brain Heart Infusion broth, (-) No zone of Inhibition.

DISCUSSION

Antimicrobial proteinaceous compounds such as bacteriocin like compounds produced by *L. acidophilus* are largely known and have been found to have potent antimicrobial activities

inoculated in Brain Heart Infusion broth at 37⁰c for 24hrs incubation. Muller Hinton Agar plates were prepared and inoculated with 0.1ml of 24 hrs brain heart infusion broth culture of test bacterial strains. The petri plates were allowed for solidification. Two wells were made by using a sterile borer of 5mm diameter, one for the test and other for the control (sterile brain heart infusion broth).

100µl of the cell filtrate of *L.acidophilus* were loaded in the test well and the same concentration of control wells were loaded with sterile Brain Heart Infusion broth medium. The petriplate was incubated at 37⁰c for 24hrs. After incubation the diameter of zone of inhibition was measured using callipers in mm then antibacterial activity was determined [9].

Statistical analysis of data

Data was analysed by one way analysis of variance (ANOVA) followed by fischer's LSD post hoc test using spss 10.0 software (spss Inc,Chicago). The values are expressed as mean ± SEM for triplicates in each bacterial strain.

RESULTS

Results of the antibacterial activity of *L.acidophilus* against gram positive and gram negative bacterial species were represented in table 1. Bacterial strain *L.acidophilus* produce bacteriocin that shows higher antibacterial activity against gram negative organisms *E.coli*, *K.pneumoniae*, *S.typhimurium* and *V.cholerae*. But they show lower inhibiting activity against gram positive species *S.aureus* and *B.cereus*. Results of the study as recorded by measuring zone of inhibition in mm varied between 1.7-2.5mm. When compared to the control wells of sterile brain heart infusion broth.

towards bacteria and harmful microorganisms [10]. The inhibition activities of these substances have been reported to be strain dependant. In general strains of *L.acidophilus* exhibit more acid and bile resistance than other LAB [11]. Addition of

L.acidophilus in to various types of food and beverages is useful to human health [12]. Those products include fermented milk, such as acidophilus milk, acidophilus yoghurt, sweet acidophilus milk, fruit juices and vegetable juice [13,14]. Results of the study showed the antimicrobial activity of the bacteriocin isolated from yoghurt samples. This may be due to the production of acetic acid and lactic acid that lowers the p^H of the medium [15].

It is concluded that bacteriocin produced by *Lactobacillus acidophilus* inhibits the growth of selected Gram positive strains such as *S.aureus*, *B.cereus* and Gram negative Spp. *E.coli*, *V.cholerae*, *Sal.typhimurium* and *K.pneumoniae*. So regular consumption of probiotic food will prevent gastrointestinal infections in adults. Growth of preschool children was improved, when fed on fermented milk beverage supplemented with *L.acidophilus*. Intake of fermented probiotic drink or products may strengthen the immune system and kill the intestinal pathogens. Further studies are needed for characterization, purification of *L.acidophilus* for developing new antibacterial drugs.

ACKNOWLEDGEMENTS

The authors are thankful to Dr.J.Prabakaran M.B.B.S.,D.P.H. Asst. Professor, Community Medicine Department. Annapoorana Medical College, Seeragapadi, Salem, Tamilnadu. For his valuable suggestion and constant encouragements. We thank our laboratory faculty members of our college for the help and support throughout the course of study.

REFERENCES

- Gerhard Reuter
The *Lactobacillus* and *Bifidobacterium*. Microflora of the Human Intestine: Composition and Succession. *Cur.Issu. in Intest. Micro* 2001, 2(2):43-53.
- Fuller R. Probiotics in man and animals. *J.Appl.Bacteriol* 1989,66:365-378.
- Oh S, Kim SH, Worobo RW. Characterization and Purification of a Bacteriocin Produced by a Potential Probiotic Culture, *Lactobacillus acidophilus* 30SC. *Journal of Dairy Science* 2008, 83(12):2747-2752.
- Ahern M, Verschuere S, van Sinderen D. Isolation and characterization of a novel bacteriocin produced by *Bacillus thuringiensis* strain B439, *FEMS Microbiol. Lett* 2003, 220:127-131.
- Tambekar DH, Bhutada SA. Studies on Antimicrobial Activity and Characteristics of Bacteriocins Produced by *Lactobacillus* strains Isolated from Milk of Domestic Animals. *The Inter J. of Micro.* 2010, 8(2).
- Sullivan LO, Ross RP, Hill C. Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. *Biochem* 2002,84(5-6):593-604.
- Williams and Wilkins. 9th ed. Bergey's Manual of Determinative bacteriology 1994.
- Kalalou I, Faid M, Ahami AT. Extending the shelf life of fresh minced camel meat at ambient temperature by *Lactobacillus dlbruecki* 2004, 7:1-6.
- Schillinger U, Lucke FK, Fuller R. Antibacterial activity of *Lactobacillus sake* isolated from meat. *Appl. Environ. Microbiol* 2006, 55:1901-1906.
- Luc De Vuyst, Frederic Leroy. Bacteriocins from Lactic Acid Bacteria: Production, Purification, and Food Applications. *J. Mol. Micro. Biotec* 2007, 13:194-199.
- Gilliland SE. Beneficial interrelationships between certain microorganism humans: Candidate microorganisms for use as dietary adjuncts, 1979; *J.F. prot*, 42:164-167.
- Soomro AH, Masud T, Kiran Anwaar. Role of Lactic Acid Bacteria (LAB) in Food Preservation and Human Health – A Review. *Paki. J. of Nutr* 2002, 1(1):20-24.
- Silva MR, Dias M, Sarra ME. Growth of preschool children was improved when fed an iron fortified fermented milk beverage supplemented with *Lactobacillus acidophilus*. *Appl.Environ.Microbiol* 2008, 62:675-721.
- Coffey AG, Daly C, Fitzgerald. The impact of biotechnology on the dairy industry. *Biotechnol, Adv* 1994, 12:625-633.
- Bezkorovainy HC, Con AH, Craven NA. Comparison of two methods used for measuring antagonistic activity of lactic acid bacteria. *J.Nutr* 2001, 4:237-243.