Antibacterial Activity of Bacteriocinogenic Lactic Acid Bacteria Isolated from Idli Batter

Khandare S. S.[1] **and Patil S. D.**[2]
[1]Department of Microbiology, J. B. College of Science, Wardha, Maharashtra, (INDIA), 442001
[2]Department of Microbiology and Biotechnology, ShriShivaji Science College, Amravati Maharashtra, (India) – 444603
ksuhas21@gmail.com

Abstract:

Idli batter samples were collected from market. Samples were properly diluted and were grown on MRS agar at 37^0 C for 2 to 3 days. Depending on colony characteristics, ten random colonies were selected and grown in MRS broth. The antibacterial activity of cell free supernatant of ten isolates against the food borne pathogens *S. aureus* and spoilage organism *P. aeruginosa* isolated from meat was studied. On the basis of zone of inhibition the highest potential bacteriocinogenic LAB-A, LAB-B and LAB-C were selected.Antibacterial activity of LAB isolates were compared with the indicator organism *L. acidophilus* MTCC 10307 obtained from MTCC Chandigarh.LAB-A, LAB-B and LAB-C showed 26.31, 15.78 and 26.31% increased bacteriocin production respectively tested against *S.aureus* and 10, 20 and 20% respectively tested against *P. aeruginosa*than *L. acidophilus* MTCC 10307. All LAB isolates exhibited antagonistic effect with highest spectrum of activity in LAB-A, LAB-B and LAB-C.

Keywords:
Bacteriocinogenic Lactic acid bacteria, pathogens, antagonistic activity, bacteriocin.

Introduction:

In spite of modern advances in technology, the preservation of food is still a debated issue not only for developing countries but also for industrialized world. One of the concerns of food industry is the contamination by pathogens which are frequent cause of food borne diseases. Over the past decade recurrent outbreaks of food borne diseases has been observed. The problem of resistant bacteria to antibiotics and the increasing demand for safe foods with less chemical addetives has increased the interest in replacing these compounds by natural products which do not injure the host or the environment (Chopra et al.,1998, Rao 1998, Kapil 2005).

Lactic acid bacteria (LAB) which are used throughout the world for manufacture of wide variety of traditional fermented food known to man for millennia. LAB are frequently naturally present in food products and are often strong competitors by producing a wide range of antimicrobial metabolites such as organic acids, diacetyl, acetoin, hydrogen peroxide, reuterin, bacteriocins and antifungal compounds (Vuyst et al., 2007, Collins et al ., 2010, Holzapfel et al .,1995). Hence last two decades have seen intensive investigation on LAB and their metabolites to discover new LAB strains that can be used in food

preservation(Galvez et al .,2007,Cortesi et al ., 2009) It is assumed that most representatives of this group do not pose any health risk to man and have GRAS(generally regarded as safe) status(Vijayendra et al .,2010,Jivaratnam et al ., 2005). Bacteriocinsof LAB are considered as safe natural preservatives or biopreservatives as it is assumed that they are degraded by the proteases in gastrointestinal tract (Clevland et al., 2001Jagadeeswariet al.,2010).

Material and methods:

The indicator organisms *L. acidophilus* MTCC 10307was procured from Microbial Type Culture Collection(MTCC) Chandigarh, India. LAB were isolated from idli batter.Food borne pathogens isolated from meat obtained from local market. Culture media and chemicals were obtained from HI media Mumbai.

Isolation of LAB:

1gm of ofidli batter was suspended in 10 ml 0.85% sterile saline solution and subjected to serial dilutions. 100 µl of the diluted sample was spread on MRS medium containing Protease peptone10 g L^{-1}, Beef extract10 gL^{-1} ,Yeast extract 5.0 gL^{-1} , Dextrose 20 gL^{-1},Polysorbate 80 1.0 gL^{-1}, Ammonium citrate 2.0 gL^{-1}, Sodium acetate 5.0gL^{-1}, Magnesium sulphate 0.10 gL^{-1}, Manganese sulphate 0.05 gL^{-1}, Diapotassium phosphate 2.0 gL^{-1}, Agar 15 gL^{-1},pH 6.5 $\pm$0.2and incubated anaerobically at 37^{0}C for 2-3 days. Ten well developed random colonies were picked up and transferred to MRS medium. These ten isolates were stored in MRS soft agar (0.5%) overlaid with 50% glycerol at -10 ^{0}C (Pal et al.,2005) and used for further studies.

Isolation of food borne pathogens

Meat sample obtained from the local market and analysed for presence of food borne pathogens.1gm of meat sample was suspended in 10 ml of 0.85% of sterile saline solution.The required dilutions were performed. The diluted sample inoculated in Brain heart infusion broth, incubated at 37^{0}C for 24 h. Further the culture was inoculated in differential and selective medias (Malleshaet al.,2010, Bilge et al.,2005,Ibrahim et al.,2009). Mannitol salt agar, Pseudomonas isolation agarwere used for the isolation of food borne pathogens and spoilage organism.

Morphological and biochemical characterization of food borne pathogens

The food borne pathogens isolated were studied by performing Gram staining, motility, optimum growth temperature was determined. Carbohydrate fermentations, indole, MR, VP and H_2S tests, nitrate reduction, hydrolysis of urea, liquification of gelatine, catalase, oxidase and coagulase tests were carried out.

Evaluatation of antibacterial activity

Agar well diffusion assay was used for testing the antibacterial activity of the LAB isolates and *L. acidophilus* MTCC 10307. Ten well developed colonies were transferred to 100 ml of MRS broth and incubated at 37 ^{0} C for 48 h . Cell

free supernatant (CFS) obtained by centrifugation at 12000 rpm ,4^{0}C for 13 minutes. To eliminate the possible inhibitory effect of either hydrogen peroxide or lactic acid, pH was neutralized and catalase treated supernatant of overnight cultures was used. CFS was sterilized by passing through 0.22 mm. millipore membrane filter and evaluated for antibacterial activity against the food borne pathogens and spoilage organism

Result and discussion:

Out of ten Isolates, 1, 2 5 ,6, 7, 9 contain cocci in tetrads whereas isolate 3, 4, 8, 10 contain rod shaped bacilli.All the isolates and indicator strain *L. acidophilus* MTCC 10307 were found to be gram positive, catalase negative, oxidase negative. CO_2 was not produced from glucose and Nitrate was not reduced (Table1).

Pseudomonas isolation agar showed bluish green coloured colony. Gram staining showed gram negative bacillus, motile, strongly aerobic with optimum growth temperature 37 ^{0} C. In broth it showed turbidity with pellicle formation. Glucose was utilized forming only acid. Indole, MR, VP and H_2S tests were negative. Nitrates were reduced to nitrites. catalase and oxidase test were positive. The organism showed production of bluish green pigment which was soluble in water and chloroform. From the morphology, cultural and biochemical characteristics the organism was identified as *P. aeruginosa*.Mannitol salt agar showed yellow coloured colony contain gram positive cocci arranged in grape like clusters, non motile, strongly aerobic with optimum growth temperature 37 ^{0} C. Glucose ,Lactose and Mannitol fermented with only acid production. They were catalase positive, hydrolyse urea, reduce nitrate to nitrite, liquefy gelatine, Indole negative, MR and VP positive, coagulase positive and produced golden yellow pigment. This organism was identified as *S. aureus.*

Out of ten LAB isolates three isolates were selected on the basis of highest antimicrobial activity against food borne pathogen *S.aureus* and food spoilage organism *P. aeruginosa.* Isolates LAB-A, LAB-B and LAB-Cshowed maximum zone of inhibition viz. 12, 11 and 12 mm respectively against *S.aureus*and 11, 12 and 12mmrespectively against *P. aeruginosa L. acidophilus* MTCC 10307 showed 9.5 and 10 mm zone of inhibition against *S.aureus* and *P. aeruginosa* respectively (Table 2). LAB-A, LAB-B and LAB-C showed26.31, 15.78 and 26.31% increased bacteriocin production respectively against *S.aureus* and 10, 20 and 20% respectively against *P. aeruginosa* than *L. acidophilus* MTCC 10307. Isolates LAB-A, LAB-B and LAB-C were found to be more efficient than indicator *L. acidophilus* MTCC 10307and selected as highest bacteriocinogenic Lactic acid bacteria.

Table 1: Biochemical characterization of the LAB Isolates

Isolates	Gram reaction	Catalase activity	CO2from glucose	Oxidase	Nitrate reduction
I-1	Gram positive Cocci in tetrad	-	-	-	-
I-2	Gram positive Cocci in tetrad	-	-	-	-
I-3	Gram positive Rod shaped bacilli	-	-	-	-
I-4	Gram positive Rod shaped	-	-	-	-
I-5	Gram possitiveCocci in tetrad	-	-	-	-
I-6	Gram positive Cocci in tetrad	-	-	-	-
I-7	Gram positive Cocci in tetrad	-	-	-	-
I-8	Gram positive Rod shaped	-	-	-	-
I-9	Gram positive Cocci in tetrad	-	-	-	-
I-10	Gram positive Rod shaped	-	-	-	-
L.acidophilus MTCC 10307	Gram positive Rod shaped	-	-	-	-

Table 2: Antibacterial activity of the LAB isolates

Isolates	Antibacterial activity against S.aureus (zone of inhibition in mm.)	Antibacterial activity againstP, aeruginosa (zone of inhibition in mm.)	Selection of Highest bacteriocinogenic LAB isolate
I-1	12	10	
I-2	9	10	
I-3	9	9	
I-4	8	9	
I-5	12	11	LAB-A
I-6	11	12	LAB-B
I-7	12	12	LAB-C
I-8	10	9	
I-9	9	8	
I-10	10	9	
L. acidophilus MTCC 10307	9.5	10	

References:

Bilge H.C. andSumru C. (2005)A comparison of two methods used for measuring antagonistic activity of Lactic acid bacteria. Pak J Nutri 4 : 237-241.

Chopra I., Hodgson J., Metcalf B.and Poste G. (1998) The search for antimicrobial agents effective against bacteria resistant to multiple antibiotics .Antimicrob Agents Chemoter 41:497-503.

Clevland J., Montville T.J., Nes I.F., andChikindas M.L. (2001) Bacteriocins: safe, natural antimicrobials for food preservation.Int J Food Microbiol 71:1-20.

Collins B., Cotter P., Hill and Paul Ross R. (2010) Applications of Lactic acid bacteria produced bacteriocins. In Mozzi F, Raya R, GM V. Biotechnology of Lactic acid bacteria Novel applications : Blackwell publishing 89-109.

Cortesi M.L., Panebianco A., Giuffrida A. and Anastasio A. (2009) Innovations in seafood preservation and storage.Veternairy Research Communications supplement. 1: S15-S23.

Galvez A., Abriouel H., Lopez R.L.and Omar N.B. (2007) Bacteriocin based strategies for food biopreservation. IntJ FoodMicrobiol 120: 51-70.

Holzapfel W.H., Geisen R. And Schillinger U.(1995) A review paper: biological preservation of foods with reference to protective cultures,bacteriocins and food grade enzymes. Int J Food Microbiol. 24:343-36.

Ibrahim S. M.and Desouky S.G. (2009) Effect of antimicrobial metabolites produced by Lactic acid bacteria on quality aspect of frozen tilapia (*Oreochromisniloticus*) fillets. World J fish and marine sciences.1 : 40-45.

Jagadeeswari S., Vidya P.,Mukeshkumar D.J. and Balakumaran M.D. (2010) Isolation and characterization of bacteriocin producing *Lactobacillus sp.* from traditional fermented food. EJEAF Che.9 :575-581.

Jivaratnam K., Jamuna M.andBawa A.S. (2005) Biological preservation of foods-bactertiocins of lactic acid bacteria. Indian J Biotechnol. 4:446-454.

Kapil A. (2005) The challenge of antibiotic resistant : need to contemplate. Indian J Med. Res. 121: 83-91.

Mallesha., Shylaja R., Selvakumar D. andJagannath J.H. (2010) Isolation and identification of Lactic acid bacteria from raw and fermented products and their antimicrobial activity.Recent research in sci and technol.2 :42-46.

Pal V., Jamuna M. andJeevaratnam K. (2005) Isolation and characterization of Bacteriocin producing lactic acid bacteria from a south Indian special dosa (appam) batter.J culture collection.4 : 53-60.

Rao G.G. (1998) Risk factors for the spread of antibiotic resistant bacteria. Aids Int. 55: 323-330.

Vijayendra S.V.N., Rajashree K., andHalami P.M. (2010) Characterization of heat stable anti –listerialbacteriocin produced by vancomycin sensitive *Enterococcus faecium* isolated from idli batter.Indian J Microbiol. 5: 243-246.

Vuyst L.D. and Leroy F. (2007) Bacteriocins from lactic acid bacteria ,production, purification and applications. J MolMicrobiolBiotechnol. 13:194-199.