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International Journal of Current Research in Life Sciences
Vol. 07, No. 04, pp.1528-1532, April, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF BACTERIOCIN FROM LACTIC ACID BACTERIA AGAINST FOOD BORNE BACTERIAL PATHOGENS

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Received 09th February, 2018; Accepted 28th March, 2018; Published Online 06th April, 2018

ABSTRACT

Bacteriocin producing lactic acid bacteria was isolated from cheese sample . The lactic acid bacterial strain was identified based on morphological, cultural,and biochemical characters and carbohydrate utilization pattern identified as *Lactbacillus brevis* LABC1. Antimicrobial activity of crude bacteriocin of *Lactbacillus brevis* LABC1 was tested against food borne bacterial pathogens such as *Listeria monocytogenes* MTCC 657 ,*Bacillus cereus* MTCC 1272, *Escherichia coli* MTCC 2939, *Staphylococcus aureus* MTCC 740, *Salmonella typhi* MTCC 531 . The methods used for testing antagonistic effect on the pathogen well diffusion assay ,Minimal inhibitory concentration, Minimal bactericidal concentration test. This study revealed that inhibition was obtained in 100µl in the well diffusion test. In MIC Among the food borne bacterial pathogens *Listeria monocytogenes* MTCC 657 recorded lower MIC (1.2ml) followed by *staphylococcus aureus* MTCC 740 (1.4ml) *Bacillus cereus* MTCC 1272 (1.6ml) , *Escherichia coli* MTCC 2939 (1.8 ml) and *Salmonella typhi* MTCC 531(2.0ml) whereas; In MBC *Listeria monocytogenes* MTCC 657 recorded lower MIC and MBC (1.8ml) followed by *Staphylococcus aureus* MTCC 740(2.0ml) *Bacillus cereus* MTCC 1272 (2.2ml) , *Escherichia coli* MTCC 2939 (2.4 ml) and *Salmonella typhi* MTCC 531(2.6ml) respctively.

Key words: bacteriocin, cheese, antimicrobial activity, lactic acid bacteria.

Citation: Jaya Chitra, J. and Siva Kumar, K., 2018. "Antimicrobial activity of bacteriocin from lactic acid bacteria against food borne bacterial pathogens" *International Journal of Current Research in Life Sciences*, 7, (04), 1528-1532.

INTRODUCTION

Lactic acid bacteria (LAB) are ubiquitous in nature and as a consequence are present as natural contaminants on a variety of food (Axelsson, 1998). Genera belong to the LAB family include *Lactococcus* sp., *Lactobacillu* sp., *Leuconostoc* sp., *Weissela* sp. *and Pediococcus* sp., as well as *Streptococcus* sp. and *Enterococcus* sp., They are responsible for the contribution for the variety of the organoleptic properties characteristic of fermented food.such as meat (Fontana et al., 2005), vegetables (Randazzo et al., 2004) and dairy products (Marilley and Casey, 2004). The use of Lactic acid bacteria and their metabolites to improve microbiological safety and extend the shelf life of foods is defined as Biopreservative (De Martinis et al., 2001). Antagonistic properties of LAB allied to their safe history of use in traditional food fermented products make them very attractive to be used as biopreservatives (Parada, 1984). The LAB, generally considered as "food grade" organisms, show special promise for selection and implementation as protective cultures. In addition, some LAB exhibit potent antimicrobial activities in the form of small, heat- stable, antimicrobial peptides called bacteriocins (Riley and Wertz, 2002). Bacteriocins are extra-cellularly released peptides or protein molecules, with a bactericidal or bacteriostatic mode of action against closely related species. Although bacteriocins may be found in many Gram positive and Gram negative bacteria, those produced by LAB has received particular attention in recent years due to their potential application in the food industry as natural preservatives. Several types of bacteriocins from food associated LAB have been identified and characterized, of which the important ones are Nisin, Diplococcin, Acidophilin, Bulgarican, Helveticins, Lactacins and Plantaricins (Nettles and Barefoot, 1993). The bacterici-dal activity of bacteriocin is attributable to destabilization of the function of the cytoplasmic membrane of the target cells, and altering the permeability properties of the membrane. Bacteriocins differ from most therapeutic antibiotics in being proteinaceous agents that are rapidly digested by proteases in the human digestive tract. To inhibit patho-genic or spoilage microorganisms, bacteriocinogenic strains or partially purified bacteriocins can be added to foods (Muriana, 1996). However, the effectiveness of bacteriocins in foods may be reduced by different factors (Hanlin et al., 1993; Muriana, 1996). First, the Minimum Inhibitory Concentration (MIC) differs widely among bacteriocins and sensitive strains. Secondly, the activity

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spectrum of bacteriocins produced by Gram positive bacteria is usually limited and does not include Gram negative bacteria. Harris et al. (1992) also demonstrated that bacteriocin resistant variants may appear and grow in the presence of a bacteriocin, and therefore limits its efficacy (Gaenzle et al., 1999).

MATERIALS AND METHODS

Isolation of bacteriocin producing microorganism

About 10 g of cheese sample into a sterile sample dish. The sample was homogenized with 90 ml diluents (normal saline/NS) in a homogenizer and serially diluted to 10^{-2} , 10^{-3}, 10^{-4} . About 1 ml of appropriate dilution of the sample was pipette into sterile petridishes. MRS agar media were poured and incubate at room temperature for 48 hrs. The LAB was identified on the basis of growth on selective MRS agar (pH 5.2), cell morphology, gram staining, catalase activity and biochemical identification of LAB. Further identification of the species of these LAB was performed according to carbohydrate fermentation patterns and growth on MRS broth (HI Media) as described in Bergey's manual of systematic bacteriology. The isolated LAB were subcultured and the purified cultures maintained at MRS agar slants.

Test microorganisms

The pathogenic organisms were brought through the Institute of Microbial Technology (IMTECH), Chandigarh, India. They were maintained as pure cultures in TSB Agar slants with periodic sub culturing every 4-8 days. The different pathogenic strains used in the present study are *Listeria monocytogenes* MTCC 657 , *Bacillus cereus* MTCC 1272, *Escherichia coli* MTCC 2939, *Staphylococcus aureus* MTCC740 *Salmonella typhi* MTCC 531 .

Preparation of bacteriocin

Bacteriocin fermentation was accomplished without controlling the pH. One liter of DB medium (non – fat dry milk -1% ; whey-2%; Yeast extract -1%; Tween 80- 0.2%; manganese sulphate-0.005 % and magnesium sulphate – 0.005%) was sterilized. After cooling the media, 1 % of a 16-18 hrs old culture Tryptone Glucose extracts (TGE) culture broth of the bacteriocin producer strain was inoculated through the inoculation port of the bioreactor. Fermentation for Bacteriocin production was carried out at 37 ^{0}C. During fermentation, a small portion of a culture medium was taken from the fermentor through draining port and analyzed for pH, cell growth and Bacteriocin activity. After fermentation, DB medium with culture of *Lactobacillus brevis* LABC1 were centrifuged at 5000 rpm for 15 min at 4 o C. The supernatant were the filtered through 0.22 µl filters (Hi-media; India) and neutralized to pH 6.5 with 1 mol NaOH, to eliminate the inhibitory effect caused by the decrease of pH. This is followed by treatment with catalase to remove the inhibitory action of hydrogen peroxide and dissolved in phosphate buffer at pH 7.0 at 1 mg/ml final concentration and incubated for 30min at room temperature (Juan C. Neito – Lozano, 2006). Supernatant were then concentrated by evaporation (Lyoplization) crude bacteriocin are prepared used for further assays. The bacteriocin activity was determined and expressed as AU ml – 1 (Rongguang Yang andYanling, 1999).

Antimicrobial activity

For determination of antagonistic activity of crude bacteriocin of *Lactobacillus brevis* LABC1 was tested by, Well diffusion assay, Minimal inhibitory concentration and Minimal bactericidal concentration testes was determined and followed by (Tagg and McGiven, 1971).

Well Diffusion Assay

One ml of the cell suspension (10^{-7} cell ml $^{-1}$) of the bacterial strain, are *Listeria monocytogenes* MTCC 657, *Bacillus cereus* MTCC 4079, *Escherichia coli* MTCC 2939, *Staphylococcus aureus* MTCC 3160, *Salmonella typhi*MTCC531 were prepared separately, mixed with the 100 ml Mueller Hinton agar medium (seeded medium) and plated on the surface of the medium, well were made by using sterile cork borer (6 mm size). The crude bacteriocin of *Lactobacillus brevis*LABC1 at different levels *viz;* 20, 40, 60, 80 and 100 µl mixed with sterile water to make up to total volume to 100 ml was discharged into the well. The well with sterile water served as control. The plate were incubated for 2 days at 30 ^{0}C. The inhibition zone (in mm) was measured around the well using antibiotic zone scale. (Tobba *et al.,* 1991). The test for determined of antagonistic activity was performed by agar well diffusion method as followed.

Minimal inhibitory concentration and Minimal bactericidal concentration methods

Bacterial cell suspension of one ml was mixed with 100 ml nutrient broth. The seeded nutrient broth (along with the inoculum) was poured separately into the test tube and different concentrations of *Lactobacillus brevis* LABC1(aqueous solution) of crude bacteriocin of @ 0.2, 0.4,0.6,0.8,1.0,1.2,1.4,1.6,1.8,2.0,2.2,2.4,2.6,2.8 and 3.0 ml and tube without inoculation served as control ,were added to the test tubes. The test tubes were incubated for 2 days at 30 °C and the growth or inhibition was observed by turbidity developed in the test tubes. The MIC is the lowest concentration in a serial dilution of that inhibits the growth of the test organism. To determine minimum bactericidal concentration (MBC), material from tubes showing no growth in MIC tests are plated on to a solid medium that lacks antibiotics. Organisms that have been killed fail to grow. The lowest antibiotic concentration that kills the test organisms is the MBC. Minimal inhibitory concentration and Minimal bactericidal concentration testes was determined and followed by (Tagg and McGiven, 1971).

RESULTS AND DISCUSSION

Bacteriocins of lactic acid bacteria have the potential as food biopreservatives to control pathogenic and spoilage bacteria. In this study, lactic acid bacteria was isolated from cheese. Microscopic identification of the isolate could determine the rod shaped cells, gram positive, catalase negative, non-motile rods and oxidase negative which indicated the typical basic characteristics of *Lactobacilli*. Based on the carbohydrate utilization pattern of bacterial isolates were identified as *Lactobacillus brevis* LABC1. The results are tabulated in Table 1. Similar characters for lactic acid bacteria observed earlier by Kandler and Weiss (1986).. Inthe present study crude bacteriocin of *Lactobacillus brevis*.LABC1 was tested for their inhibitory activity over some food borne pathogens *Listeria monocytogenes* MTCC 657 ,*Bacilus cereus* MTCC 1272 *E. coli* MTCC 2939 *S. aureus* MTCC740 and *S.*

Table 1. Characterization of lactic acid bacterial isolate

Morphological characters	LABC1
Gram reaction	+
Spores	-
Shape	Rods
Size	0.7 µm X 2µm
Motility	-
Biochemical characters	
Catalase test	-
Oxidase test	-
$NH_3$from arginine	+
Gas production from glucose	+
Carbohydrates	
Arabinose	+
Cellulose	-
Esculin	D
Fructose	+
Galactose	D
Glucose	+
Lactose	D
Maltose	+
Mannitol	-
Mannose	-
Melezitose	-
Melibiose	+
Raffinose	D
Rhamnose	-
Ribose	+
Salicin	-
Sorbitol	-
Sucrose	D
Trehalose	-
Xylose	D
Identified as	*Lactobacillus brevis*

+positive, -negative ,D-delayed reaction

Table 2. Inhibitory effect of bacteriocin *Lactobacillus brevis* against food borne bacterial pathogens(well diffusion method)

Test organisms	Diameter of inhibition zone (mm) Crude Bacteriocin *Lactobacillus brevis* in (µl)					
Listeria monocytogenes MTCC 657	control	20	40	60	80	100
	-	12	13	15	21	25
Bacillus cereus MTCC 1272	-	11	13	15	20	22
Escherichia coli MTCC 2939	-	7	9	12	16	20
Staphylococcus aureus MTCC 740	-	8	10	11	14	18
Salmonella typhi MTCC 531	-	4	6	9	13	16

typhi.MTCC531. Almost all pathogens were inhibited by *Lactobacillus brevis*. The results are tabulated in Table 2 .The higher inhibition zone was recorded at *Listeria monocytogenes* MTCC 657 showed 25mm/100µl inhibition zone follwed by *Bacillus cereus* MTCC 1 2722 /100 µl, *Escherichia coli* MTCC 2939 20/100 µl, *Staphylococcus aureus* MTCC 740 18/100 µl, *Salmonella typhi* MTCC 531 16/100 µl.Our results was in agreement with Rajaram *et al*. (2010) found that crude bacteriocin of *Lactobacillus lactis* inhibited the various gram positive and gramnegative bacteria. The Minimum Inhibitory concentration of crude bacteriocin of *Lactobacillus brevis* LABC1 against food borne bacterial pathogens *viz*., *Listeria monocytogenes* (MTCC 657), *Staphylococcus aureus* (MTCC 740), *Bacillus cereus* (MTCC 1272), *Salmonella typhi* (MTCC 531) and *Escherichia coli* (MTCC 2939) was studied and results are given in Table 3. Among the food borne bacterial pathogens *Listeria monocytogenes* MTCC 657 recorded lower MIC (1.2ml) followed by *staphylococcus aureus* MTCC 740(1.4ml) *Bacillus cereus* MTCC 1272 (1.6ml), *Escherichia coli* MTCC 2939 (1.8 ml) and *Salmonella typhi* MTCC 531(2.0ml) .Similar result was obtained earlier by Ashokkumar *et al*. (2011) the antibacterial activity of cell free supernatant of *Lactobacillus paracasei* against the pathogens. The MIC index of 128 µl was seen against *S. typhi*, 256 µl against *Pseudomonas aeruginosa* and *Klebsiella pneumonia*, 512 µl against *E.coli* and *Staphylococcus aureus*. These results are in agreement with the above findings on antibacterial activity of crude bacteriocin of lactic acid bacteria against food borne bacterial pathogens. The Minimum Bactericidal concentration of crude bacteriocin of *Lactobacillus brevis* LABC1against food borne bacterial pathogens *viz*., *Listeria monocytogenes* (MTCC 657), *Staphylococcus aureus* (MTCC 740), *Bacillus cereus* (MTCC 1272), *Salmonella typhi* (MTCC 531) and *Escherichia coli* (MTCC 2939) was studied and results are given in Table 4. Among the food borne bacterial pathogens *Listeria monocytogenes* MTCC 657 recorded lower MIC and MBC (1.8ml) followed by staphylococcus aureus MTCC 740(2.0ml) *Bacillus cereus* MTCC 1272 (2.2ml) , *Escherichia coli* MTCC 2939 (2.4 ml) and *Salmonella typhi* MTCC 531(2.6ml).

Table 3. Inhibitory effect of bacteriocin Lactobacillus brevis against food borne bacterial pathogens (minimal Inhibitory concentration)

Test organisms (1ml/100ml)	Crude bacteriocin of *Lactobacillus brevis* LABC1 of different concentration(ml)															
	control	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0
Listeria monocytogenes MTCC 657	-	+++	+++	++	+	+	-	-	-	-	-	-	-	-	-	-
Bacillus cereus MTCC 1272	-	+++	+++	++	++	+	+	+	-	-	-	-	-	-	-	-
Escherichia coli MTCC 2939	-	+++	+++	+++	++	++	+	+	+	-	-	-	-	-	-	-
Staphylococcus aureus MTCC740	-	+++	+++	++	++	+	+	-	-	-	-	-	-	-	-	-
Salmonella typhi MTCC 531	-	+++	+++	++ +	++	++	++	+	+	+		-	-	-	-	-

Table 4. Inhibitory effect of bacteriocin Lactobacillus brevis against food borne bacterial pathogens (minimal bactericidal concentration)

Test organisms (1ml/100ml)	Crude bacteriocin of*Lactobacillus brevis* LABC1 of different concentration(mg)															
	control	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0
Listeria monocytogenes MTCC 657	-	+++	++ +	++	+ +	+	+	+	+	-	-	-	-	-	-	-
Bacillus cereus MTCC 1272	-	+++	++ +	++	++	++	++	+	+	+	+	+	+	-	-	-
Escherichia coli MTCC 2939	-	+++	++ +	++ +	++	++	+	+	+	+	+	+	-	-	-	-
Staphylococcus aureus MTCC740	-	+++	++ +	++	++	+	+	+	+	+	-	-	-	-	-	-
Salmonella typhi MTCC 531	-	+++	++ +	++ +	++	++	+	+	+	+	+	+	-	-	-	-

+++= more growth,++ moderate growth,+ growth,-=no growth

This test got significance in determining the antibacterial property of *Lactobacillus brevis* LABC1 crude bacteriocin at a lowest concentration levels. Hence the test provided that *Lactobacillus brevis* could be used as a biologcal preservative. Devos *etal*., 1993 recorded MBC of 0.015 mg /ml and 0.3 mg/ml of nisin were determined for *Micrococcus flavours.* This results suggests need to monitor bacterial concentrations for longer periods when ever antimicrobial substances are being evaluated in order to investigate the recovery of surviving cells.

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