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ANTIMICROBIAL ACTIVITY OF BACTERIOCIN FROM LACTIC ACID BACTERIA AGAINST FISH BACTERIA

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A B S T R A C T

Lactic acid bacteria (LAB) are most commonly used microbiology for preservation of foods. Bacteriocins are proteinaecous substances produced by many bacterial strains and exhibit bactericidal activity against the closely related organisms. They have been the subject of extensive studies in recent years because of their prospective use as natural food preservatives (Villiani *et al*., 2001). Lactic acid bacteria (LAB) are widespread in nature and predominate in microflora of milk and its products. LAB is known to produce bacteriocins and have great potential as food bio preservations (Gilliland, 1990, Jamuna, Jeeveratnum, and Avonts *et al*., 2004). Bacteriocins are peptides with antimicrobial activity that are secreted by some bacteria to inhibit the growth of other competing microorganisms.

Antimicrobial activity of crude Bacteriocin lactic acid bacteria (LAB) isolation of *Lactobacillus acidophilus* was tested for its antimicrobial activity of *Pseudomonas* sp, *Flavobacterium* sp, *Shewanella* sp, *Vibrio parahaemolyticus* and *Vibrio vulnificus* isolated from fish. The methods used for testing the antagonistic effect on the pathogens are agar diffusion assay, Minimal inhibitory concentration, Minimal Bactericidal concentration test. This study revealed that inhibition of growth for fish bacteria was obtained in 100µl in the well diffusion test. In MIC the higher inhibition of growth was found in 2.8ml for *Vibrio parahaemolyticus*, 2.4ml for *Vibrio vulnificus*, 2.2ml for *Pseudomonas* sp, 2.0ml for *Shewanella* sp, and 1.8ml for *Flavobacteria* sp, whereas; In MBC the higher inhibition of growth was found in 3.0ml for *Vibrio parahaemolyticus*, 2.8ml for *Vibrio vulnificus*, 2.6ml for *Pseudomonas* sp, 2.4ml for *Shewanella* sp, and 2.2ml for *Flavobacteria*, respectively.

INTRODUCTION

Lactic acid bacteria (LAB) are most commonly used microbiology for preservation of foods. Bacteriocins are proteinaecous substances produced by many bacterial strains and exhibit bactericidal activity against the closely related organisms. They have been the subject of extensive studies in recent years because of their prospective use as natural food preservatives (Villiani *et al*., 2001). LAB are widespread in nature and predominate in microflora of milk and its products. LAB is known to produce bacteriocins and have great potential as food bio preservations (Gilliland, 1990, Jamuna, Jeeveratnum, and Avonts *et al*; 2004).

Bacteriocins are peptides with antimicrobial activity that are secreted by some bacteria to inhibit the growth of other competing microorganisms. Currently, bacteriocins produced by lactic acid bacteria (LAB) arose most interest because LAB posses the status of QPS (Qualified presumption of safety) i.e. they are regarded as safe microorganisms for human consumption because that and their metabolites have been consumed in fermented foods for countless generation without adverse effects in the population. Moreover, from the earliest days after birth LAB constituted the natural biota of the human digestive trace bacteriocins produced by LAB have found preservatives. In 1933 the description of a protinaceous molecules with antimicrobial activity produced by some strains of the species *Lactobacillus lactis* sub sp *lactis* was reported, which later was named as nisin (Mattick and Hirsh, 1947). This study was understand the ability of *in vitro* antagonistic effect of crude bacteriocin of *Lactobacillus acidophilus* against to inhibit the fish bacteria.

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MATERIALS AND METHODS

Sources of food pathogen

Pseudomonas sp, *Flavobacteria* sp, *Shewanella* sp, *Vibrio parahaemolyticus and Vibrio vulnificus* were isolated from the fish sample.

Culture collection

The strain *Lactobacillus acidophilus* (MTCC 447) was obtained from MTCC (Microbial type culture collection) Chandigarh was transferred to sterile Mueller Hinton broth and streaked on Muller Hinton agar.

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 acidophilus* 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 30 min 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 and Yanling, 1999).

Antimicrobial activity

For determination of antagonistic activity of *Lactobacillus acidophilus* crude bacteriocin such as methods, 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 *Pseudomonas* sp, *Flavobacteria* sp, *Shewanella* sp, *Vibrio parahaemolyticus* and *Vibrio vulnificus* 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 acidophilus* 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 acidophilus* (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 control with Bacteriocin and without Bacteriocin served as control ,were added to the test tubes. The test tubes were incubated for 2 days at 30 ^{0}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).

RESULT AND DISCUSSION

Bacteriocins of lactic acid bacteria have the economical and regulatory purposes, these bacteriocins should be produced in potential as food bio preservatives to control several pathogenic and spoilage bacteria for economical and regulatory purposes; these bacteriocin should be produced in large amounts and preferably in a medium composed of food grade ingredients already attempts have been made to produced bacteriocin from different organisms such as *Lactobacillus* sp, *Leuconostoc* sp, *Lactococcus* sp, *Pediococcus* sp *Streptococcus* (Aktypis 1998, Rongguang Yang and Yanling,1999).

Prasad and Ghodeker (1990) reported that *Lactobacillus acidophilus* isolated from dahi should antimicrobial activity against fish bacteria the results are presented in (Table-1). The higher inhibition zone was recorded at the highest concentration, such as *Vibrio parahaemolyticus* showed 23 mm/ 100 µl inhibition zone followed by *Vibrio vulnificus* recorded 22mm/ 100 µl, *Pseudomonas* sp recorded 19mm/ 100 µl, *Shewanella* sp recorded sp 18mm/ 100 µl, and *Flavobacteria* sp recorded 17mm/ 100 µl diameter of zone of inhibition was recorded.

The Minimal inhibition concentration of crude bacreriocin of *L.acidophilus* against the sensitive strains of fish bacteria are *Pseudomonas* sp, *Flavobacteria* sp, *Shewanella* sp, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* were studied and results are presented in (Table-2). The MIC was carried out and it was found that the increased in the level of concentration of the crude

Bacteriocin of *Lactobacillus acidophilus* (aqueous solution) from 0.2 to 3.0 ml increased the inhibition of growth of the fish bacteria. The higher inhibition of growth was obtained at 2.8ml for *Vibrio parahaemolyticus*, followed by 2.4ml for *Vibrio vulnificus*, 2.2ml for *Pseudomonas* sp, 2.0ml for *Shewanella* sp, and 1.8ml for *Flavobacteria* sp. similar result was documented by (Baron *et al*., 1994) the turbidity was matched with 0.5 ml. Farland standard.

3). The MBC was carried out and it was found that the increased in the level of concentration of the crude Bacteriocin of *Lactobacillus acidophilus* (aqueous solution) from 0.2 to 3.0 ml increased the inhibition of growth of the fish bacteria. The higher inhibition of growth was obtained at 3.0ml for *Vibrio parahaemolyticus*, followed by 2.8ml for *Vibrio vulnificus*, 2.6ml for *Pseudomonas* sp, 2.4ml for *Shewanella* sp, and 2.2ml for *Flavobacteria* sp. This test has got significance in determining the antibacterial property of *L. acidophilus*

Table 1 Inhibitory Effect of Bacteriocin *Lactobacillus acidophilus* against Fish bacteria (Well Diffusion Method)

Test organisms (1 ml / 100ml)	**Diameter of inhibition zone (mm) Crude Bacteriocin *Lactobacillus acidophilus* in (µl)**					
	Control	**20**	**40**	**60**	**80**	**100**
Pseudomonas sp	-	6	8	11	15	19
Flavobacteria sp	-	4	6	9	13	17
Shewanella sp	-	5	7	10	14	18
Vibrio parahaemolyticus		10	11	15	20	23
Vibrio vulnificus	-	8	10	14	18	22

Table 2 Inhibitory effect of crude bacteriocin of *Lactobacillus acidophilus* against Fish bacteria (Minimal inhibitory concentration)

Test organisms (1 ml /100 ml)	**Crude Bacteriocin of *Lactobacillus acidophilus* 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**
Pseudomonas sp	-	+++	+++	+++	+++	++	++	++	++	+	+	-	-	-	-	-
Flavobacteria sp	-	+++	+++	++	++	++	+	+	+	-	-	-	-	-	-	-
Shewanella sp	-	+++	+++	+++	++	++	++	+	+	+	-	-	-	-	-	-
Vibrio parahaemolyticus	-	+++	+++	+++	+++	++	++	++	++	++	+	+	+	+	-	-
Vibrio vulnificus	-	+++	+++	+++	++	++	++	++	+	+	+	+	-	-	-	-

+++ = More growth, ++ = Moderate growth, - = Growth, - = No growth

Table 3 Inhibitory effect of crude bacteriocin of *Lactobacillus acidophilus* against Fish bacteria (Minimal Bactericidal concentration)

Test organisms (1 ml /100 ml)	**Crude bacteriocin of *Lactobacillus acidophilus* 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**
Pseudomonas sp	-	+++	+++	+++	+++	++	++	++	++	+	+	+	+	-	-	-
Flavobacteria sp	-	+++	+++	++	++	++	+	+	+	+	+	-	-	-	-	-
Shewanella sp	-	+++	+++	+++	++	++	++	+	+	+	+	+	-	-	-	-
Vibrio parahaemolyticus	-	+++	+++	+++	+++	++	++	++	++	++	+	+	+	+	+	-
Vibrio vulnificus	-	+++	+++	+++	++	++	++	++	+	+	+	+	+	+	-	-

The Minimal Bactericidal Concentration of crude Bacteriocin of *L. acidophilus* against the sensitive strains of fish bacteria are *Pseudomonas* sp, *Flavobacteria* sp, *Shewanella* sp, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* were studied and results are presented in (Table crude Bacteriocin at a lowest concentration levels. Hence the test provided that *L. acidophilus* could be used as a biological preservative (Devos *et al*., 1993) recorded MBC of 0.015 mg ml-1 and 0.3 mg ml-1 of Nisin were determined for *Micrococcus flavours*. This result suggests the need to monitor bacterial concentrations for longer

periods whenever antimicrobial substances are being evaluated, in order to investigate the recovery of surviving cells.

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