

Research Paper-

**Crude Bacteriocin from *Lactobacillus Acidophilus*-NCDC 015 Used for
Antimicrobial Screening against GIT Pathogens.**

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Abstract:

Lactic acid bacteria have an important role for the preservation of foods and fermented products owing to its probiotic characteristic, with many species from this bacterial group producing several antibacterial compounds including bacteriocins. Antimicrobial screening of crude bacteriocin of *Lactobacillus acidophilus* NCDC – 015 was analyze against the selected food pathogen (*Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli* *Staphylococcus aureus*, and *Salmonella typhi*). The inhibitory spectrum evaluated was found to exhibit maximum activity towards gram positive i.e., *Bacillus cereus* and *Staphylococcus aureus* with slight activity for *Listeria monocytogenes* whereas for gram negatives the crude bacteriocin was ineffective. The two fold arbitrary range for bacteriocin activity with the upper limit of 640 AU/ml against *Bacillus cereus* and *Staphylococcus aureus*, with the reduction in the inhibition for *Listeria monocytogenes* to 40 AU/ml were observed. The results thus give insight of *Lactobacillus acidophilus* NCDC – 015 as a promising bacteriocin producer possessing antagonistic properties against food - borne pathogens.

KEY WORDS: Antimicrobial, Bacteriocin, *Lactobacillus acidophilus*

INTRODUCTION

Consumers' growing awareness of the risks associated with synthetic preservatives that are used to control food pathogens as well as food pathogens themselves has sparked a renewed interest in "Green Technologies," which include innovative methods for minimally processing and utilizing bacteriocins for bio-preservation^[1].

LAB, which are typically referred to as safe (GRAS), are crucial for the long-term storage of food and fermented goods. The primary cause of the preservation effect is the acidic environment that these bacteria produce in food as they grow, in addition to producing an array of inhibitory substances including H₂O₂, ethanol, diacetyl, CO₂, antifungal peptides, bacteriocin or antibiotic like substances^[2].

Certain gramme +ve and gramme -ve bacteria create bioactive peptides called bacteriocins, which have bactericidal effects on bacteria that are closely related. The bacteriocin from LAB has been studied extensively by many coworker. They are converted into four different classes: class I, Lantibiotics, class II. Small (>30KDa), heat llabiles proteins, and class IV is complex bacteriocins containing carbohydrate moieties or lipid. The class II bacteriocin are further converted into Listeria - active peptides with the N – terminal consensus sequence (class IIa), 2 peptide complexes (class IIb) and thio – activated peptide (class IIc)^[3]. Among Lactobacilli, strains belonging to species *Lactobacillus acidophilus* are most frequently used as probiotic. Being non – pathogenic and members of the normal intestinal micro flora it is widely used in fermented dairy product as a starter culture and considerable medical and industrial interest. Bacteriocin production is often proposed as beneficial quality of probiotics bacteria.

Bacteriocins produced by LAB are subjects of intense research because of their antibacterial characteristics against food borne pathogens. LAB's have been recognized as bacteriocins and safe produced by these microorganisms may be a suitable solution to the problem of resurgence of resistant strain to antibiotic. There is a requirement to attract the consumer attention to the existence of natural substances that can protect against food borne disease ^[4]. Further research is needs to gain insight into the activity of bacteriocins preparation which is must for effective and safe exploitation of Lactic acid bacteria bacteriocins, either in feed or food processing industries.

MATERIAL AND METHODS

The National Dairy Research Institute in Karnal, India's Culture Collection Centre provided the *Lactobacillus acidophilus* NCDC - 015 strains. Culture of *Lactobacillus acidophilus* NCDC – 015 was maintained in deManRogosa Sharpe agar slants (MRS) and held at 4±1°C between transfers. The strain was subcultured 2 – 3 times prior to use and routinely checked for purity. The test pathogens used in the study was collected from MTCC Chandigarh India. The bacterial pathogens were maintained in Nutrient and Trypticase soy agar slants. Sub culturing of test pathogens was done periodically at the interval of 15 days. For study only 18 h old broth culture was taken.

For the evaluation of inhibitory effect of crude bacteriocins obtained from *Lactobacillus acidophilus* NCDC – 015, the following strains were selected in the course of study *Bacillus cereus* - MCCB 0027, *Listeria monocytogenes* - MCCB 0028, *Staphylococcus aureus* –MCCB 0045, *E.coli* - MCCB 0018 and *Salmonella typhi* - MCCB 0021.

Screening for antimicrobial activity of *Lactobacillus acidophilus* NCDC – 015

Initial screening was performed by using the agar well diffusion assay with slight modifications. A 24-hour-old culture of *Lactobacillus acidophilus* NCDC-015 was added to

MRS broth, and it was then incubated an aerobically for eighteen hours at 37°C. Centrifugation at 4000xg/30min./4°C was used to get the cell-free supernatant. CFS recovered was set to pH – 6.0 with 1N NaOH 25ml of cooled and melted nutrient agar media was poured into sterile petriplates and allowed to solidify at room temperature. Bacterial pathogens were swabbed on the agar base. Wells of 6 mm was made using cork borer and 100 µl of two fold serial dilutions was mixed properly and kept at 4°C for 24h and incubate at 37°C for 24 – 48 h. Antimicrobial activity was expressed Arbitrary Unit (AU) per ml. The bacteriocins activity was calculated in AU/ml using the formula as proposed by-

$$\text{Bacteriocin Activity (AU/ml)} = \frac{\text{Dilution}}{100} \times 1000$$

The data determine in the present analysis was subject to statistical analysis (ANOVA) using two and three way classification.

RESULTS AND DISCUSSION

Screening of antimicrobial activity

The range of inhibitory activity of crude bacteriocins obtained was evaluated against Gram negative and Gram positive pathogens. The supernatant showed inhibitory activity on Gram positive, with no effect on Gram negative pathogens. As indicated in (table1; fig.1; plate 1,2,3) the cell free supernatants was found to exhibit maximum inhibitory activity towards *Bacillus cereus* (32mm) and *Staphylococcus aureus* (30mm) whereas slight activity was observed for *Listeria monocytogenes* (14mm). On analyzing the data statistically, significant difference (p < 0.05) was observed between inhibition and pathogens in terms of bacteriocins activity.

On calculating the effectiveness in terms of arbitrary unit the bacteriocins activity was found with upper limit of 640 AU/ml for *Bacillus cereus* and *Staphylococcus aureus*, however minimum activity of 40 AU/ml was observed against *Listeria monocytogenes* (table2). The supernatant therefore obtained was ineffective against gram negative bacteria^[5].

TABLE 1: INHIBITORY EFFECT OF CRUDE BACTERIOCINS OBTAINED FROM LACTOBACILLUS ACIDOPHILUS NCDC- 015 AGAINST TEST PATHOGEN

Test Pathogens	MRS	Crude Bacteriocin	Zone of Inhibition (mm) at different dilutions					
	Control		2 ²	2 ⁴	2 ⁸	2 ¹⁶	2 ³²	2 ⁶⁴
<i>Bacillus cereus</i>	00	32	30	24	19	15	14	00
<i>L.monocytogenes</i>	00	14	11	00	00	00	00	00
<i>S.aureus</i>	00	30	28	20	19	15	14	00
<i>Escherichia coli</i>	00	00	00	00	00	00	00	00
<i>S.typhi</i>	00	00	00	00	00	00	00	00

Fcal 5%, 5.299> Ftab 5%, 2.71 (Due to bacterocin), Fcal 5%, 48.86> Ftab 5%, 2.37(Due to pathogens)

TABLE 2: ARBITRARY UNIT OF BACTERIOCIN ACTIVITY OBTAINED FROM *LACTOBACILLUS ACIDOPHILUS* NCDC- 015 AGAINST TEST PATHOGENS

Test Pathogens	MRS Control	Crude Bacteriocin	Bacteriocin Activity (AU/ml) at different dilutions					
			2 ²	2 ⁴	2 ⁸	2 ¹⁶	2 ³²	2 ⁶⁴
<i>Bacillus cereus</i>	00	32	40	80	160	320	640	00
<i>L.monocytogenes</i>	00	14	40	00	00	00	00	00
<i>S.aureus</i>	00	30	40	80	160	320	640	00
<i>Escherichia coli</i>	00	00	00	00	00	00	00	00
<i>S.typhi</i>	00	00	00	00	00	00	00	00

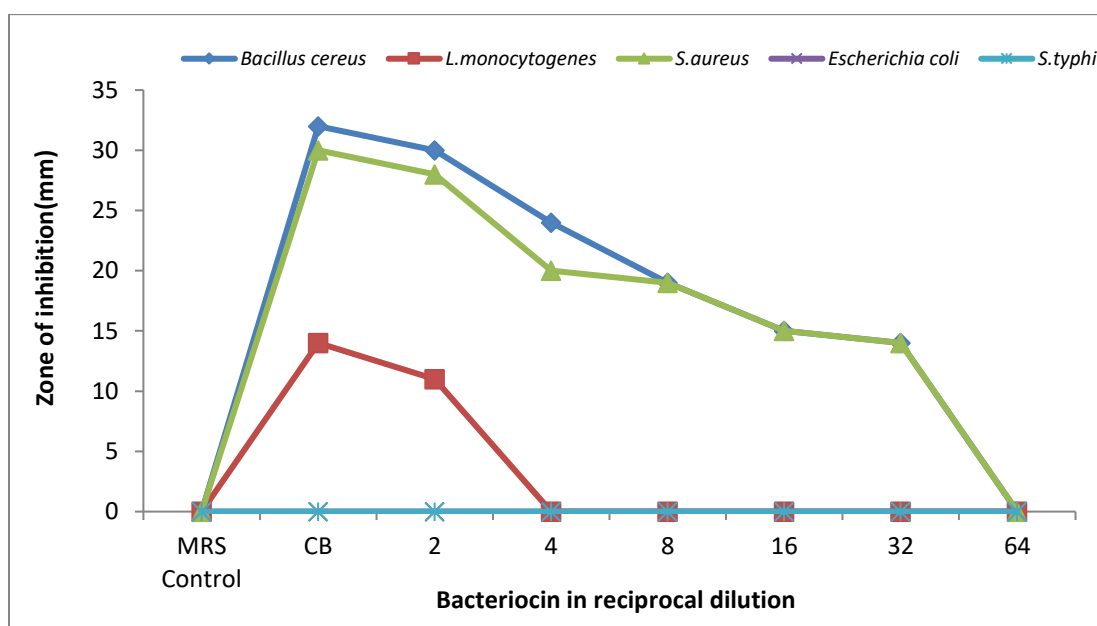


Fig.1: Antagonistic activity of crude bacteriocins obtained from *Lactobacillus acidophilus* NCDC-015 against test pathogen

The LAB can display a relatively broad spectrum of antimicrobial activity. This result are in line with the report of displaying the antimicrobial activity of *Lactobacillus acidophilus* against gram +ve pathogenic bacteria including *Listeria ivanovii*, *Bacillus cereus*, *Listeria monocytogenes* Scott A and *Staphylococcus aureus*^[6].

These antagonistic compounds' spectrum of activity against some significant food-borne pathogens, such as *S. aureus* and *L. monocytogenes*, looked extremely promising. *S. aureus* is thought to pose a risk to the public's health because it produces enterotoxins that cause food poisoning, and *L. monocytogenes* is known to cause listeriosis in humans when it is transmitted through food^[7]. Similar research found that because lactic acid bacteria produce antimicrobial chemicals and have a low pH, they have a strong antagonistic action against food-contaminating

microorganisms, such as spoilage and pathogenic ones [8]. The generation of lactic acid and the decrease in acetic acid, pH, diacetyl, hydrogen peroxide, aldehydes, fatty acids and other substances like bacteriocin are thought to be the causes of LAB's antimicrobial action [9].

Previous finding of consistent with the present observation exhibiting negative result for the inhibitory effect of bacteriocin produce by *Lactobacillus acidophilus* on gram negative bacteria [10]. On contrary demonstrated the inhibitory spectrum of antimicrobial agent isolated from *Lactobacillus acidophilus* a vaginal isolate and *Lactobacillus acidophilus* IBB 801 being active towards gram -ve pathogenic strain of *Salmonella panama* 1467 and *Escherichia coli* [11].

CONCLUSION

It was recorded that the crude bacteriocin obtained from *Lactobacillus acidophilus* NCDC – 015 was potentially effective showing wide zone of inhibition against gram positive food – borne pathogens. Therefore it can be concluded that *Lactobacillus acidophilus* NCDC – 015 can be considered for its safety potential in food industry.

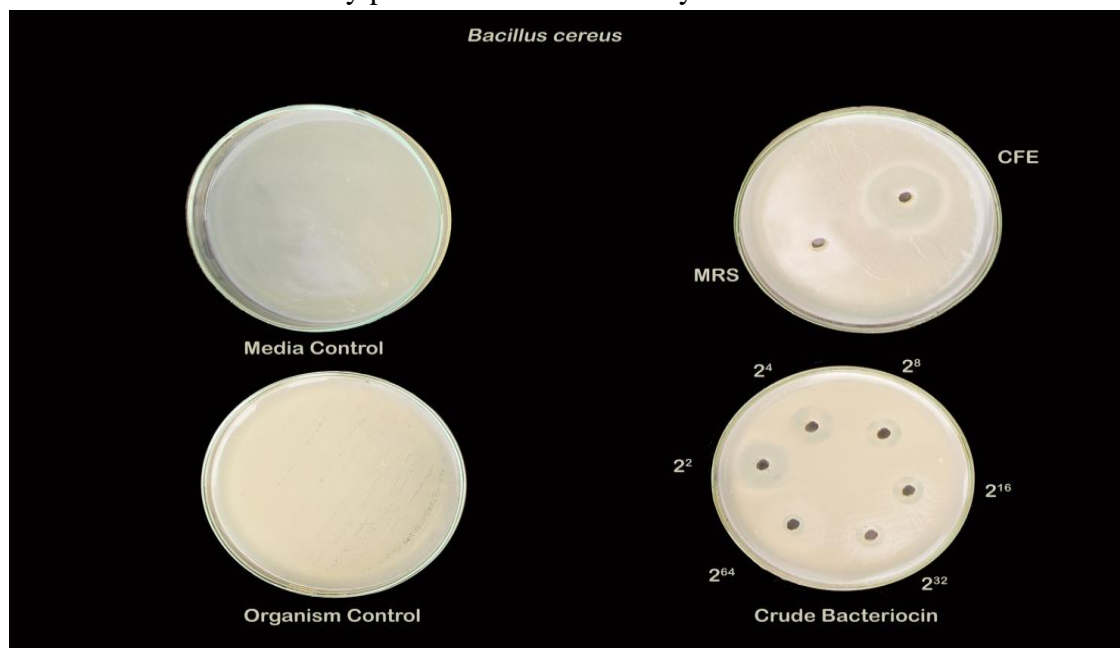


Fig. 2: Inhibitory effect of crude bacteriocin obtained from *Lactobacillus acidophilus* NCDC- 015 against *Bacillus cereus*

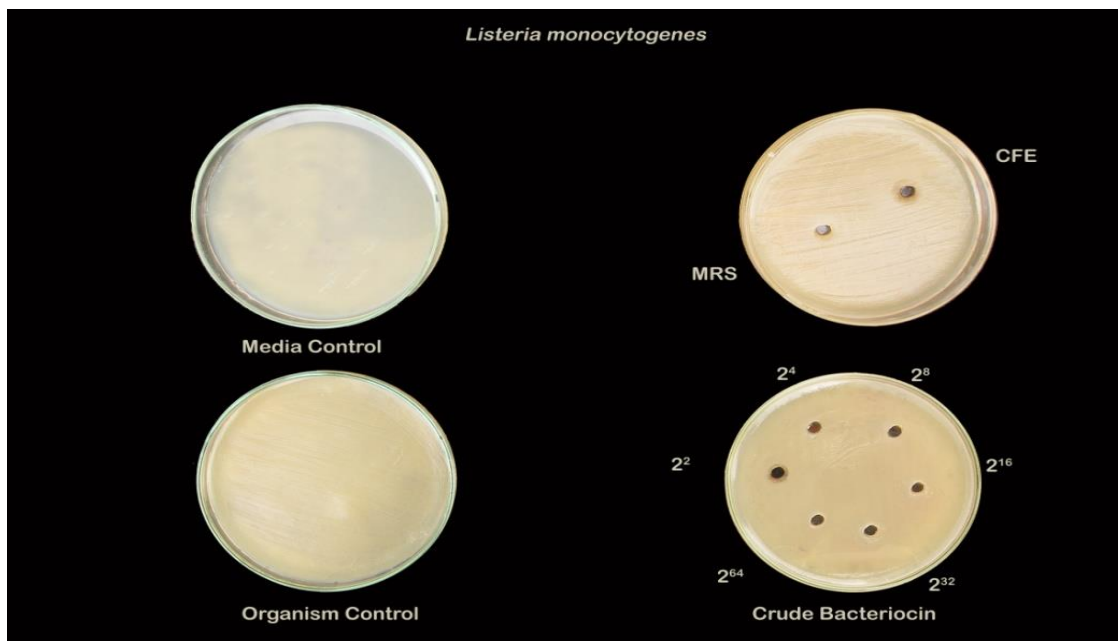


Fig. 3: Inhibitory effect of crude bacteriocin obtained from *Lactobacillus acidophilus* NCDC- 015 against *Listeria monocytogenes*

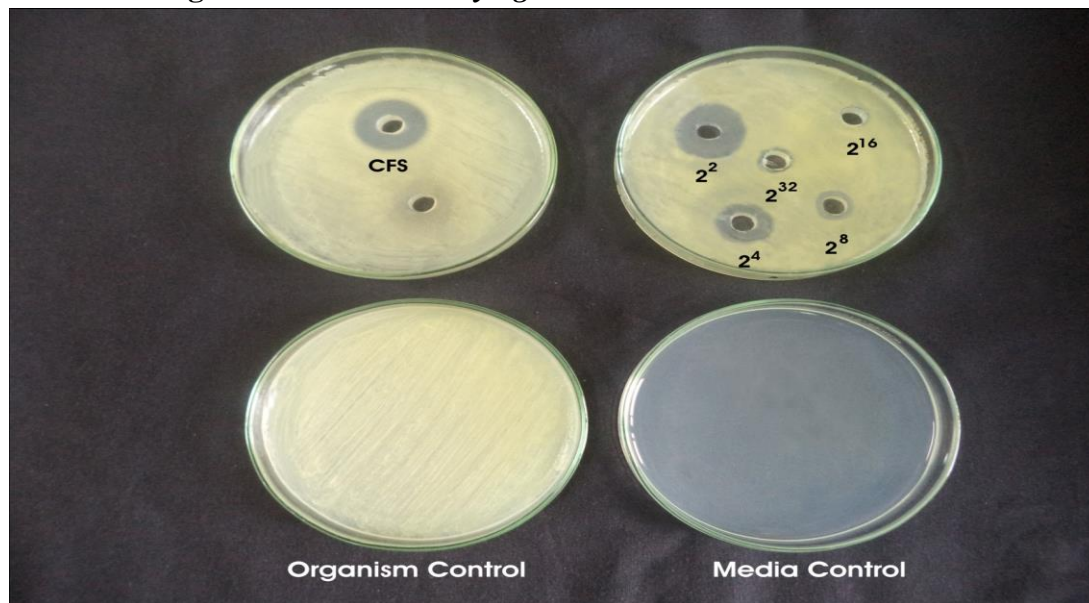


Fig. 4: Inhibitory effect of crude bacteriocin obtained from *Lactobacillus acidophilus* NCDC- 015 against *Staphylococcus aureus*

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