International Journal of Scientific Research in Biological Sciences **Research Paper**
Vol.7, Issue.2, pp.69-76, April (2020) **E-ISSN:** 2347-7520

Bacteriocin Induced Milk as a Vehicle against Dental Cavities

Parul Thapar[1]*, R.K. Malik[2], M.K. Salooja[3]

[1]School of Pharmaceutical and Health care Sciences, CT University, Ludhiana, India
[2]Dairy Microbiology Division, National Dairy Research Institute, Karnal, India
[3]School of Agriculture, Indira Gandhi National Open University, New Delhi, India

**Corresponding author: parul.thapar@yahoo.com, Tel-+91-9953972850*

Available online at: www.isroset.org

Received: 02/Apr /2020, Accepted: 15/Apr/ 2020, Online: 30/Apr/2020

Abstract- The prevalence of dental caries is consistently increasing, in every age across the globe, and today the concern for the "Oral health" is almost at the same level as for "General health". The oral health care demands are beyond the capacities of the health care systems in most low and middle income countries. Therefore, it was thought to explore the natural ways by which the problems of dental caries can be prevented in a cost-effective manner. It was found that a few studies have set the path through use of natural bacteriocins against inhibition of caries causing organisms. The inhibition of caries causing organisms can be done by naturally occurring substances like bacteriocins found in lactic acid bacteria. Bacteriocins are potent protein toxins produced by virtually every bacterial and archeal species. In the present study, prevention of dental caries by utilization of bacteriocins from lactic acid bacteria has been done by incorporating bacteriocin in milk. The bacteriocin producing five strains of *Lactobacillus brevis* isolated from curd were used for the production of bacteriocin. The minimum inhibitory concentration (MIC) of the bacteriocins was carried out to find the best bacteriocin producing strain. Out of five bacteriocin producers, the strain LB11(1) showed maximum percentage of MIC. The characterization of the bacteriocin was done using various optimization conditions. The partial purification was done using ammonium sulphate precipitation and centrifugal filteration. The purified bacteriocin was then incorporated in the milk to check the effect of inhibition on caries causing organisms (*Streptococcus mutans* and *Streptococcus sobrinus* isolated from dental cavities). It was confirmed that the bacteriocin induced milk can be applied to prevent dental caries directly by the consumer and the milk itself can become a vehicle against dental cavities.

Keywords–*Bacteriocins, lactic acid bacteria, Streptococcus mutans, Streptococcus sobrinus, Lactobacillus brevis*

I. INTRODUCTION

Dental caries is the most common disease occurring in all age groups, which is preventable and is the major cause of oral pain and tooth loss [1]. Children suffering with tooth decay, also suffer with improper growth and development which severely affects their health. India is a diverse country with different ethnic, geographic and cultural diversity. Therefore, it is quite expected to have different disease patterns in various regions or states of the country [2]. According to the report of World Health Organization, a large number of clinical treatments for dental caries have been developed with improved technologies in most of the countries of the world, but still the disease of dental caries has become a global burden. The oral health care demands are beyond the capacities of the health care systems in most low and middle income countries [3]. Keeping in view of the above data, it was thought to explore the natural ways by which the problems of dental caries can be prevented in a cost-effective manner. It was found that a few studies have set the path through use of natural bacteriocins against inhibition of caries causing organisms. Bacteriocins are potent protein toxins produced by virtually every bacterial and archeal species [4] which can be applied to prevent dental cavities. Bacteriocins have a fast acting mechanism and they work even at extremely low concentrations. They form pores in the target membrane of bacteria, thus inhibiting the cell-wall biosynthesis [5]. Bacteriocins possess certain characteristics like narrow target range, stability, high activity and low toxicity. The bacteriocins of lactic acid bacteria can also be applied to food and medicine, where they can play a major role in preservation, packaging, food additives and prevention of certain diseases. The objectives of the present study included: a) Isolation and screening of bacteriocins from the strains of *Lactobacillus brevis*. b) Partially purification of the bacteriocin. c) Incorporation of bacteriocin in milk.

Rest of the paper is organized as follows, Section I contains the introduction of the paper, Section II contains the methodology performed to obtain each objective highlighted in the paper, Section III describes the results and discussion, and Section IV concludes research work with future directions.

II. METHODOLOGY

A) Isolation and screening of bacteriocins from the strains of Lactobacillus brevis

Isolation of bacteriocin- The five strains of *Lactobacillus brevis* [LB11(1), LB13(3), LB13(4), LB13(5), LB14(3)] isolated from curd were grown in De Man Rogosa and Sharpe (MRS) broth for 24 h at 24°C. Cells were removed by centrifugation at 12,000 rpm for 5 min in 1.5 ml sterilized Eppendorf tubes [6]. The culture supernatant

thus obtained was transferred to another sterilized Eppendorf tubes.

Determination of anti-bacterial activity- This supernatant (crude bacteriocin) was employed for determining the antibacterial activity. The indicator organisms used were- ten strains of *Streptococcus mutans* [SM10(a), SM13(a), SM14(a), SM22(a), SM23(a), SM28(a), SM29(a), SM33(a), SM35(a), SM36(a)] and four strains of *Streptococcus sobrinus* [SS19(b), SS23(b), SS24(b), SS36(b)] (dental caries causing organisms). These organisms were isolated from the dental cavities. The anti-bacterial activity was determined by Spot on Lawn method.

Minimum Inhibitory Concentration (MIC) of the Crude Bacteriocin- Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation [7]. To determine the MIC of the bacteriocins, the standard method as described by the [8] was followed. Spot on lawn assay was done against the fresh indicator cultures grown at 24 h at optimum conditions. Different concentrations (as given in the Table 1) of the crude bacteriocin solution was put as drop on each plate of the isolate. The incubation was done at 37°C for 24 h. MIC was calculated as, AU/ml= 200 x MIC. The statistical analysis of the MIC was performed using ANOVA- single factor.

Table 1. Different Concentrations

S.No.	Concentration	CFCs	Distilled Water
1.	2x	100µl	100µl
2.	4x	100µl	300µl
3.	6x	100µl	500µl
4.	8x	100µl	700µl
5.	10x	100µl	900µl
6.	15x	100µl x (6)	50µl
7.	20x	100µl x (6)	100µl
8.	30x	100µl x (6)	200µl
9.	40x	100µl x (6)	300µl
10.	50x	50µl x (6)	450µl
11.	100x	100µl x (11)	100µl
12.	200x	100µl x (12)	100µl

Growth Curve of Bacteriocin Producing Strain- To determine the growth curve of bacteriocin producing strain, the method as described by the [9] was followed. The selected bacteriocin producing cultures- LB11(1) was inoculated in sterilized De Man Rogosa and Sharpe (MRS) broth and incubated at 37°C. Optical density (O.D.) of the bacterial growth was measured at 600 nm after 2, 4, 6, 8 10, 24, 30 and 36 h of incubation. To determine the maximum growth of the cells at a particular incubation hour, a growth curve was plotted against the time of incubation and O.D.

Optimization of the Conditions for Maximum Production of Bacteriocin-

- Medium- De Man Rogosa and Sharpe (MRS) and M-17 flasks inoculated with the cultures were incubated at 42ºC.
- Initial pH- De Man Rogosa and Sharpe (MRS) broth adjusted to various initial pH values (5.0, 6.0 and 7.0) was inoculated with the cultures and incubated at 42°C.
- Temperature- De Man Rogosa and Sharpe (MRS) broth inoculated with the producer strain was incubated at 37°C, 42°C and 50°C.

In all the above experiments, the samples were drawn at 0, 8, 16, 24 and 36 h intervals and tested for pH and bacteriocin activity units. The results obtained were represented in the form of table and graphs. The statistical analysis for the parameter pH for all the conditions were statistically analyzed using ANOVA- single factor.

Protein Estimation- Protein content of the samples was estimated by the method of the [10-12] using bovine serum albumin (BSA) as the standard. The concentrations of the test solution were noted under absorbance at 600 nm which were analyzed by graphical representation. The amount of protein was calculated as-

mg protein/ml= mg(protein) (Dilution)/ Reagent (ml)

B) Partial purification of the bacteriocin

Ammonium Sulfate Precipitation- The procedure as described by the [13] was followed. To 100 ml of supernatant, 37.32 g of ammonium sulfate was added slowly with constant stirring at 4- 5°C to achieve 60 per cent saturation and stirring continued for another 4 h at 5-7°C. The mixture was then kept overnight in refrigerator at 0°C. It was then centrifuged at 13,000 rpm for 20 min and the precipitates were dissolved in 0.1N phosphate buffer (pH 6.8). This partially purified bacteriocin was further subjected to purification by centrifugal filters.

Centrifugal Filters- The bacteriocin solution obtained, was further purified using 3kDa MWCO (molecular weight cut off) centrifugal filter. The method as described by the [14] was used with some modifications. 0.5 ml of partially purified bacteriocin was added in two filters of 3kDa each in sterilized conditions. These were centrifuged at 13,000 rpm for 10 min. The purified sample was recovered in another sterile Eppendorff tubes. The specific activity and yield of the bacteriocin were calculated from the protein content and activity units of the samples. The Bacteriocin yield (%) was calculated as- Weight of sample before filtration x 100/ Volume of sample used for filtration.

Enzyme Sensitivity- To know the sensitivity of the bacteriocin against enzyme, the proteolytic enzyme used was Protease. 5 ml of bacteriocin was treated with enzyme (1 mg/ml) at pH 7.0. Incubation was done at 37°C for 24 h. It was then heated at 100°C for 3- 5 min to denature the enzyme. This was then assayed by agar well-diffusion method for determining the antimicrobial activity. The standard method as described by the [15] was followed.

C) Incorporation of bacteriocin in milk

The purified bacteriocin was also incorporated in milk (Fat- 3.0% and Solids Not Fat- 8.5%) (Experimental

Dairy, National Dairy Research Institute). Taken 1ml of skim milk in four different sterile tubes and incorporated with different concentrations of bacteriocin- 0.05 ml, 0.1 ml, 0.5 ml and 1 ml. Agar well assay was used to know the bacteriocin activity while being incorporated in the products. 50 µl of the incorporated bacteriocin were filled in agar wells on the solidified mat of indicator organism. These were incubated at 37°C for 24 h to observe for the presence or absence of zone. A control sample of milk without bacteriocin was also kept along for testing.

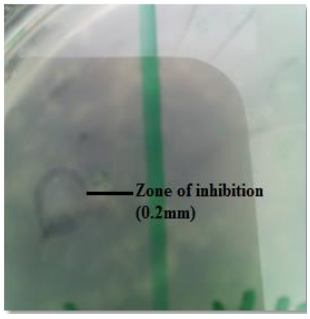
III. RESULTS AND DISCUSSIONS

A. *Isolation and screening of bacteriocins from the strains of Lactobacillus brevis*

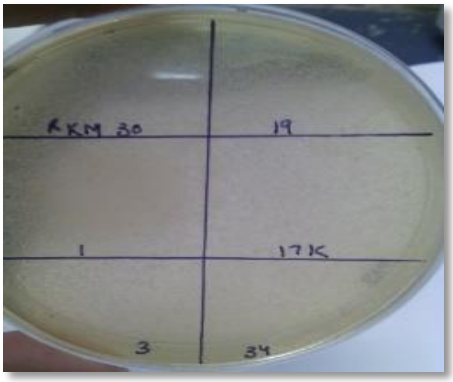
Anti-microbial assay against *Streptococcus mutans* and *Streptococcus sobrinus*- The five strains of *Lactobacillus brevis* were used against the indicator organisms- nine strains of *Streptococcus mutans* and five strains of *Streptococcus sobrinus* to determine the antibacterial activity. All the five strains of *Lactobacillus brevis* showed zones of inhibition against six isolated strains of *Streptococcus mutans* and *Streptococcus sobrinus* species. This is represented in the Figure 1. The statistical analysis of the diameter of zone of inhibition were statistically analyzed using ANOVA. This showed that the maximum percentage of inhibition is shown by the isolate LB11(1)- 26% against the isolates of *Streptococcus mutans* and *Streptococcus sobrinus*. All the values were average of three trials. The p-value within the isolates is 1.71E-09 and showed no significant difference at $p \leq 0.05$. In the Figure 2, the graphical representation of the zone of inhibition has been presented showing the maximum inhibition by the isolate LB11(1) against the *Streptococcal* isolates.

The maximum inhibition shown by the strain LB11(1) may be due to the presence of optimal growth conditions for bacteriocin production.

The study by the [16], had also shown similar results as the diameter zones are included between 2 to 10 mm and the biggest clear zones were recorded by the extracts of *Pediococcus pentosaceus* NRC AM1 and *Pediococcus pentosaceus* NRC AM4 with *Escherichia coli* 0157:H7, but the lowest clear zones were obtained with the extracts of *Lactobacillus brevis* NRC AM2 and *Lactobacillus plantarum* NRC AM7 against *Pseudomonas aeruginosa*.



(a)



(b)

a) Zone of inhibition formed against *Streptococcus mutans* and *Streptococcus sobrinus*
b) No zone of inhibition

Figure 1. Anti-bacterial activity of the Strains against Streptococcal species

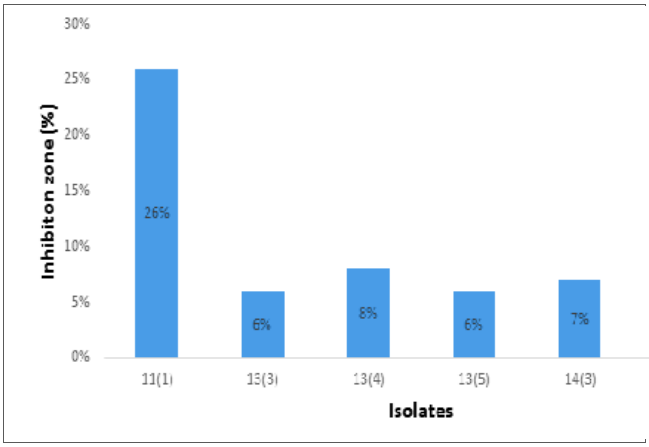


Figure 2. Percentage of Inhibition by Strains

Minimum Inhibitory Concentration (MIC) of the Crude Bacteriocin- To determine the lowest concentration of an antimicrobial (bacteriocin), the Minimum Inhibitory Concentration (MIC) of the crude bacteriocin was determined by spot-on-lawn assay [18]. MIC was calculated as - AU/ml= 200 x MIC.The minimum inhibitory concentration was 0.02 µl as shown by the strain LB11(1) with maximum antimicrobial activity of 10,000 AU/ml against the Streptococcal strain of SM28(a). The dilution of culture supernatant to 50X dilution did not show any zone of inhibition. The diameter of zone of inhibition obtained for all the strains was 0.1mm. The MIC of the crude bacteriocin were statistically analyzed using ANOVA-single factor. This represented the minimum inhibitory concentration as percentage and the maximum percentage of MIC is shown by bacteriocin producing strain LB11(1)- 2% against the isolates of *Streptococcus mutans* and *Streptococcus sobrinus*. All the values were average of three trials. The p-value within the strains is 0.002 and did not show any significant difference at $p \leq 0.05$.

The presence of optimal conditions required for the maximum activity of bacteriocin for the strain LB11(1)

favored this strain to produce maximum percentage of inhibition at the minimum concentration.

In a study by the [17], similar results were shown- MIC of various isolates of lactic acid bacteria producing bacteriocins isolated from fermented milk was determined. The MIC (µl) observed was as follows- *Lactococcus* spp.- 0.05, *Lactococcus* spp.- 0.6, *Leuconostoc*- 0.2, *Pediococcus* (4 different strains)- 0.6, 0.5, 0.5 and 0.05.

Growth Curve- To obtain the optimum growth conditions of the bacteriocin producing strain, a growth curve of isolated lactic acid bacterial strain LB11(1) in MRS broth was plotted against time of incubation and optical density (O.D.) at 600 nm which was incubated at 37°C for 36 h. It was observed that the maximum bacteriocin was produced at 24 h and production remained stable till 30 h. After reaching 36 h, the bacteriocin production started to decline and pH of MRS broth decreased from 5.97 to 3.6 (Figure 3).

In a study by the [19] the values obtained were somewhat similar- the growth curve of isolated strain of *Lactobacillus brevis* from Jiaoke (natural fermented cream in China) was plotted, which showed that the maximum bacteriocin production was reached after 24 h and production remained stable from 24 h to the end of the growth. During 48 h of growth, pH of MRS broth decreased from 5.8 to approximately 3.6.

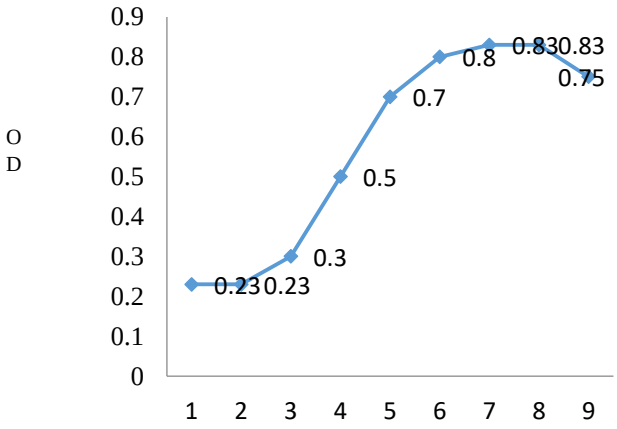


Figure 3. Growth Curve

Optimization of Conditions for Maximum Production of Bacteriocin-

- **Influence of Culture Medium on Bacteriocin Production-**The optimization of conditions for different media was done using De Man Rogosa and Sharpe (MRS) and M-17 media. A gradual decrease in the initial pH of the growth media (MRS and M17) was observed with increase in incubation period varying from 0-36 h. The bacteriocin production was observed maximum in MRS media at 24 h at pH 5.9 against the Streptococcal strain SM28(a) (Figure 4(a)). Statistical analysis: The optimization of conditions for different media for pH were statistically analyzed using ANOVA- single factor. The p-value within the strains for MRS media is 5.87E-15 and did not show any significant difference at $p \leq 0.05$. Similarly, the p-value within the strains for M-17 media is 2.56E-12 and did not show any significant difference at $p \leq 0.05$.

In a study by the [20], the activity of the bacteriocin produced from *Lactobacillus brevis* isolated from curd, was evaluated against three bacterial species in MRS media supplemented with various nitrogen and carbon sources. MRS media supplemented with soy bean meal recorded maximum anti- bacterial activity against the tested bacterial strains which is in support to the present study.

- **Influence of Initial pH on Bacteriocin Production-** The optimization of conditions for initial pH was done using pH 5.0, 6.0 and 7.0. De Man Rogosa and Sharpe (MRS) broth was adjusted to various initial pH values (5.0, 6.0 and 7.0) and inoculated with the cultures and incubated at 42°C. A gradual decrease in the initial pH range of 5.0, 6.0 and 7.0 was observed in the growth media at incubation period varying from 0-36 h. The bacteriocin activity was observed at 24 h and it showed decrease in the activity at 36 h in the pH of 5.0, 6.0 and 7.0. The bacteriocin production was shown to be maximum at pH 5.8 at 24 h against the Streptococcal strain SM28(a) (Figure 4(b)). Statistical analysis: The statistical analysis of the strains for comparison of the parameter pH was done using ANOVA-single factor. The p-value within the strains at pH 5.0 is 1.64E-06 and did not show any significant difference at $p \leq 0.05$. At pH 6.0, the p-value within the strains is 4.78E-13 and did not show any significant difference at $p \leq 0.05$. For pH 7.0, the p-value within the strains is 1.84E-10 and did not show any significant difference at $p \leq 0.05$.

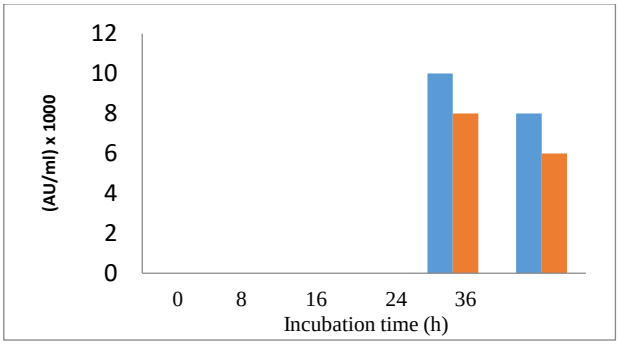
In a study, the bacteriocin strains of *Lactobacillus* species isolated from dairy products, showed that all the strains were grown well at pH 4, 6 and 8 but only strains of *Pediococcus pentosaceus* NRCAM1, *Lactobacillus brevis* NRCAM2 and *Pediococcus pentosaceus* NRCAM4 were also grown at pH 2.0 [16] which is in support to the present study.

- **Influence of Incubation Temperature on Bacteriocin Production-** The optimization of conditions has been done using different temperatures- 37°C, 42°C and 50°C. De Man Rogosa and Sharpe (MRS) broth inoculated with the culture was incubated at 37°C, 42°C and 50°C. It was observed that with gradual rise in incubation period from 0 to 36 h, there was a

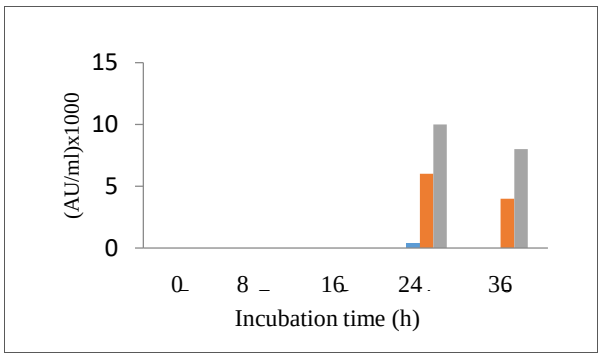
gradual decrease in the initial pH. The rise in the incubation period from 0 to 36 h, at 37°C, there was maximum bacteriocin activity at 24 h which gradually decreased at 36 h of incubation. Whereas, at 42°C and 50°C, there has been consistent decrease in the activity of bacteriocin. The maximum activity of bacteriocin was shown at 37°C and pH 5.9 for 24 h against the Streptococcal strain SM28(a).

Statistical analysis: Statistical analysis of the isolates for the parameter pH at different temperatures was performed. For temperature 37°C, the p-value within the strains is 3E-15 and did not show any significant difference at $p \leq 0.05$. At temperature 42°C, the p-value within the strains is 2.4E-14 and did not show any significant difference at $p \leq 0.05$. Also, for temperature 50°C, the p-value within the strains is 5.41E-15 and did not show any significant difference at $p \leq 0.05$.

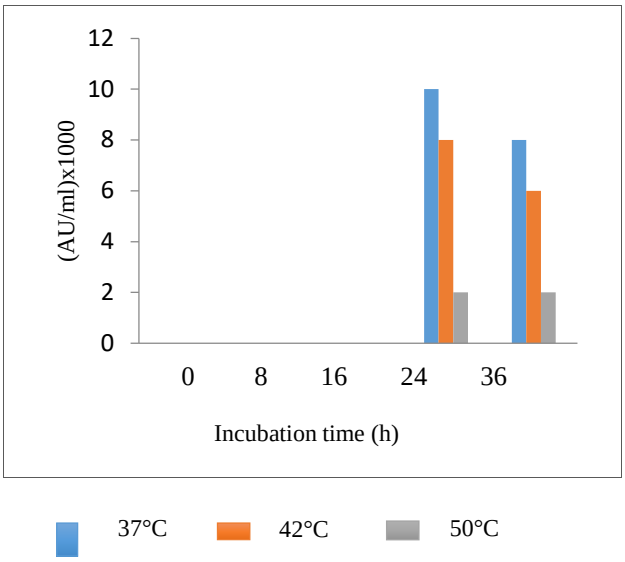
In a study, the bacteriocin strains of *Lactobacillus* species isolated from dairy products including the strains of *Lactobacillus brevis* NRCAM2 showed the growth at 30°C and 37°C. On the other hand, strains of *Lactobacillus brevis* NRCAM2, *Lactococcus lactis* sub sp. *lactis* NRCAM3 and *Lactobacillus pentosaceus* NRCAM5 were not able to grow at temperature 45°C. On the other hand, the species of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* can grow at 45°C [16] which is in support to the present study.



(a)



(b)



(c)

Figure 4. Influence of different conditions

B) Partial purification of the bacteriocin

The partial purification of the bacteriocin was done by using ammonium sulphate precipitation. The partially purified bacteriocin was passed through centrifugal filter of 3 kDa. After purification, the specific activity of the purified bacteriocin was determined by spot-on-lawn assay against the isolated strains of *Streptococcus mutans* and *Streptococcus sobrinus*. The activity units of the bacteriocin LB11(1) was 20,000 AU/ml against the isolated strain of *Streptococcus sobrinus*- SS19(b). The diameter of the zone of inhibition was 0.2 mm. The molecular weight cut-off of the purified bacteriocin after centrifugal filteration was 3kDa and the bacteriocin yield obtained was 100%.

A study by [19], showed similar results: the molecular weight of the bacteriocin of *Lactobacillus brevis* isolated from Jiaoke (natural fermented cream in China) was approximately 3.8 kDa according to tricine SDS-PAGE which is in support of the present study.

Protein Estimation of the Crude and Partially Purified Bacteriocin- The total protein content of the crude and purified bacteriocin of the strain LB11(1) was determined by the method described by the Lowry *et al*, 1951. The total protein content in crude bacteriocin was calculated as 1.0 mg/ml and that of purified bacteriocin was 0.2 mg/ml.

In a study by the [21], similar results were obtained which showed that the purified bacteriocin produced from the lactic acid bacteria had protein content of 0.21 mg/ml.

Enzyme Sensitivity of Bacteriocin- To determine the proteinaceous nature of the bacteriocin, the bacteriocin of the strain LB11(1) was treated with the enzyme- protease. It was observed that the bacteriocin had lost its inhibitory activity upon treatment with the proteolytic enzyme. The enzyme protease had cleaved the bacteriocin and this

bacteriocin could not show its antimicrobial action against the indicator organism. This confirmed the proteinaceous nature of the bacteriocin.

The bacteriocin like substance produced by *Lactobacillus brevis* B23 showed no antimicrobial activity or it was unstable after heat treatment with all the proteolytic enzymes (protease and trypsin). The completely inactivated inhibitory activity indicated that it was proteinaceous in nature [22] which is in support of the present study.

C) Incorporation of bacteriocin in milk

Agar well assay was performed on solidified mat of indicator organism. The diluted concentrations for 100X and 200X of 50 µl milk was poured into the wells of the medium and incubated at 37°C for 24 h. After incubation, these were observed for the presence or absence of zones. It was observed that the zone of inhibition was absent in the control sample (without bacteriocin) and the zone was formed in the bacteriocin induced milk (Figure 5). The zone of diameter for the milk with bacteriocin at concentration of 100X was 0.04 mm (average of three trials) and at concentration of 200X was 0.08 mm (average of three trials).

Statistical analysis: Statistical analysis of the zone of diameter for the bacteriocin induced milk at concentration 100X and 200X, was performed using ANOVA-single factor. The p-value within the isolates is 6.54E-07 and did not show any significant difference at $p \leq 0.05$. The comparison of zone of diameter for bacteriocin induced inhibition by milk is shown in the Figure 6.

Any literature in support for the present work has not been published till now.

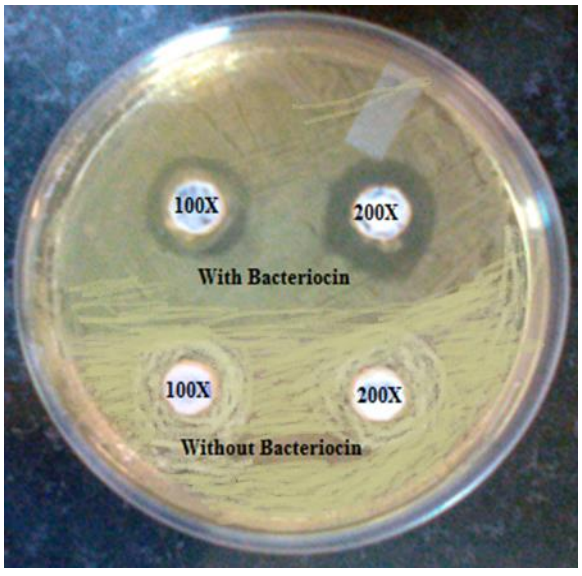
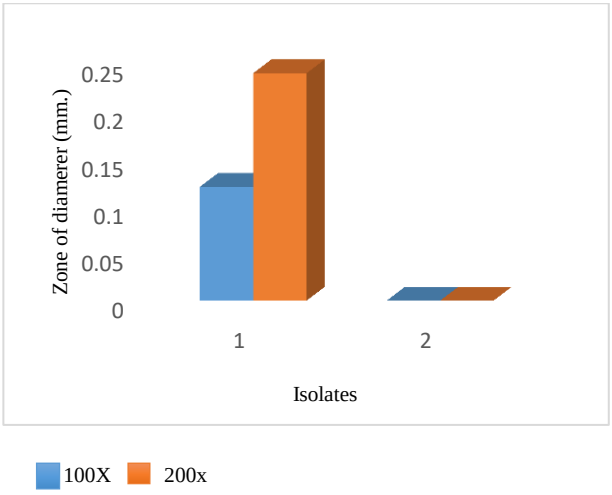


Figure 5. Inhibition of Bacteriocin Induced Milk against Indicator Organism and No Inhibition by the Control milk sample



1- With bacteriocin 2- Without bacteriocin

Figure 6. Zone of Diameter for Inhibition by Milk

IV. CONCLUSION

The five strains of *Lactobacillus brevis* isolated from curd, were used for the production of bacteriocins. The crude bacteriocins obtained were applied to test the antimicrobial activity against the isolated Streptococcal species of dental cavities. All the five strains showed antimicrobial activity against the six indicator organisms of Streptococcal species. This indicated the presence of natural inhibiting substance called bacteriocin within the isolated *Lactobacillus brevis* strains. It was found that the minimum inhibitory concentration was obtained by the bacteriocin of the strain LB11(1)-0.02 µl with maximum antimicrobial activity of 10,000 AU/ml against the Streptococcal strain of SM28(a) and LB11(1) was regarded as the best bacteriocin producing strain as the optimal conditions for its growth were suitable. The statistical analysis revealed De Man Rogosa and Sharpe (MRS) to be superior to M-17 media at 24 h at pH 5.9 against the Streptococcal strain SM28(a). This is because the MRS media contains all essential nutrients which is required for the bacteriocin production whereas there is absence of such nutrients in M-17 medium. It was concluded that the bacteriocin producing strain LB11(1) showed maximum antimicrobial activity at 37°C for 24 h between pH 6.0-7.0. The molecular weight cut-off of the purified bacteriocin after centrifugal filteration was 3kDa and the bacteriocin yield obtained was 100%. The total protein content in crude bacteriocin- 1.0 mg/ml and that of purified bacteriocin- 0.2 mg/ml. The proteinaceous nature of the bacteriocin was also confirmed by the application of enzyme protease. Thus, bacteriocins from *Lactobacillus brevis* can help in enhancing the prevention of dental caries. This can be achieved by inoculating the bacteriocin in milk so that the milk can itself become a vehicle against dental caries when consumed directly by the consumers and patients of dental caries in easy and cost-effective manner.

ACKNOWLEDGEMENT

Words, in fact, cannot express my deepest sense of gratitude and regards to *Dr. R.K. Malik, Emeritus Scientist*, Division of Dairy Microbiology, National Dairy Research Institute, Karnal, for his benevolent guidance, openness, sustained encouragement throughout the research work and for all the financial support during research. I, sincerely thank *Dr. M.K. Salooja*, *Professor* and *Program Coordinator (PHDDR)*, School of Agriculture, Indira Gandhi National Open University, New Delhi, for his guidance, support, consideration, constructive counsel, motivation and care bestowed upon me during this period of the course. I am thankful to all the project staff, research fellows and other staff members for their timely help and support.

REFERENCES

[1] S. Mathews and G.V. Mathew, "War inside the mouth," *Journal of Dental and Medical Sciences*, Vol. **13**, Issue. **1**, pp. **23-25**, **2014**.

[2] C. Janakiram, B. Antony, J. Joseph and V. Ramanarayanan, "Prevalence of dental caries in India among the WHO index age groups: a meta- analysis," *Journal of Clinical Diagnostic and Research*, Vol. **12**, Issue. **1**, pp. **8-13**, **2018**.

[3] A. R. Hosseinpoor, L. Itani and P. E. Petersen, "Socio-economic inequality in oral healthcare coverage: results from the World Health Survey," *Journal of Dental Research*, Vol. **91, Issue. 3**, pp. **275-281**, **2012**.

[4] L. R. Dorit, M. S. Roy and A. M. Riley, "The bacteriocins current knowledge and future prospects book," *Caister Academic Press*, **USA**, pp. **103-126**, **2016**.

[5] H. P. Rodney, Z. Takeshi and S. Kenji, "Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications," *Microbial Cell Factories*, Vol. **13**, Issue **5**, pp. **1-3**, **2014**.

[6] L. L. Burlanek, and A. E. Yousef, "Solvent extraction of bacteriocins from liquid cultures," *Journal of Applied Microbiology*, Vol. **31**, Issue. **1**, pp. **193-197**, **2000**.

[7] J. M. Andrews, "Determination of minimum inhibitory concentrations," *Journal of Antimicrobial Chemotherapy*, Vol. **48**, Issue. **3**, pp. **5-16**, **2002**.

[8] P. Jean, W. Melvin, E. George, J. Stephen, L. James, L. Brandi, M. Amy, M. Tony, P. Robin, R. Sandra, S. Michael, S. Jana, T. Maria and Z. Barbara, "Performance standards for antimicrobial susceptibility testing," *Clinical and Laboratory Standards*, Vol. **37**, No. **1**, pp. **144-249**, **2017**.

[9] B. G. Hall, H., Acar, A. Nandipati and M. Barlow, "Growth rates made easy," *Journal of Molecular Biology and Evolution*, Vol. **31**, Issue. **5**, pp. **232-238**, **2013**.

[10] E. E. Hartree, **"**Determination of protein: a modification of the lowry method that gives a linear photometric response," *Analytical Biochemistry*, Vol. **48**, Issue. **1**, pp. **422**, **1972**.

[11] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, "Protein measurement with the Folin phenol reagent," *Journal of Biological Chemistry*, Vol. **19**, Issue. **3**, pp. **265**, **1951**.

[12] K. Wilson, and J. Walker, "Practical Biochemistry: Principles and Techniques," *Cambridge University Press*, **USA**, pp. **50**, **2000**.

[13] R. Yang, M.C. Johnson and B. Ray, "Novel method to extract large amounts of bacteriocins from lactic acid bacteria," *Applied Environmental Microbiology*, Vol. **58**, Issue **5**, pp. **3355-3359**, **1992**.

[14] N. Gautam and N. Sharma, "Purification and characterization of bacteriocin produced by strain of *Lactobacillus brevis* MTCC 7539," *Indian Journal of Biochemistry and Biophysics*, Vol. **46**, Issue **1**, pp. **337-341**, **2009**.

[15] S. F. Barefoot, and T. R. Klaenhammer, "Purification and characterization of *Lactobacillus acidophilus* bacteriocin lactacin B," *Antimicrobial Agents and Chemotherapy*, vol. **26**, Issue. **7**, pp. **328-334**, **1984**.

[16] M. M. Ahmed, A. M. Baher, I. M. Zainab, N. F. Tawfiek and Z. M. R. Hassan et al., "Antibacterial activity of some lactic acid bacteria isolated from egyptian dairy products," *International Journal of Chemical Technology and Research*, Vol. **6**, Issue. **1**, pp. **1139-1150**, **2014**.

[17] N. Agrawal and A. Prakash, "Isolation of lactic acid bacteria from fermented milk products and their antimicrobial activity against *Staphylococcus aureus*," *Journal of Food Safety*, Vol. **15**, Issue. **4**, pp. **39-42**, **2013**.

[18] C. R. Toro, Use of probiotic lactic bacteria in feeding *Litopenaeus vannamei* shrimp as inhibitors of pathogenic microorganisms and immune system stimulants. PhD Thesis, Federal University of Paraná, Curitiba, Brazil, 2005.

[19] H. Wang, H. Gong, X. Meng and L. Man, "A Bacteriocin Produced by *Lactobacillus brevis* KLDS1.0373 isolated from "Jiaoke", a traditional fermented cream from China". *Advanced Materials and Research*, Vol. **18**, Issue **3**, pp. **1132-1136**, **2011**.

[20] S. K. R. Namasivayam, J. C. R. Angel, R. S. A. Bharani and M. Y. Karthik, "Effect of Media on bacteriocin production by *Lactobacillus brevis* and

evaluation of anti-bacterial activity," *Research Journal of Pharmaceutical, Biological Chemical Sciences*, Vol. **5**, Issue. **10** pp. **1129-1136**, **2014**.

[21] M. Mohankumar and M. Murugalatha, "Characterization and antibacterial activity of bacteriocin producing *Lactobacillus* isolated from raw cattle milk sample," *International Journal of Biology*, Vol. **3**, Issue. **1**, pp. **128-143**, **2011**.

[22] A. A. Rushdy at. al. "Antimicrobial compounds produced by probiotic *Lactobacillus brevis* isolated from dairy products," *Annal of Microbiology*, Vol. **63**, Issue **6**, pp. **81-90**, **2013**.

AUTHORS PROFILE

Dr. Parul Thapar (born 20 December 1989), persued B.Sc. in Biotechnology from Kurukshetra University, Kurukshetra, India in 2010, M.Sc. in Microbial and Food Technology form Punjabi University, Patiala, India in 2012 and Ph.D. in Dairy Science and Technology (sp. Dairy Microbiology) from Indira Gandhi National Open University, New Delhi, India in 2019. She is currently working as Assistant Professor in School of Pharmaceutical and health care sciences, CT University, Ludhiana since 2019. She is Editorial board member of Journal of Acta Scientific Microbiology since 2018 and Editorial board member of World Journal of Food Microbiology since 2019. She has published more than 10 research and review papers in reputed national and international journals including Thomson Reuters (SCI & Web of Science) and conferences including IEEE and it's also available online. Her main research work focuses on Food and dairy microbiology, molecular biology including DNA isolation and Polymerase chain reaction, use of bioinformatics databases and tools like NCBI, BLAST, PDB and UCSC Browser. She has 1.5 years of teaching experience and 2 years of research experience.z

Dr. Ravinder Kumar Malik, Ex-Joint Director (Research), National Dairy Research Institute, Karnal. He is currently working as Emeritus Professor at ICAR- NDRI, Karnal since 2012. He has published more than 50 research papers reputed national and international journals including Thomson Reuters (SCI & Web of Science) and conferences. His main research work focuses on Bacteriocin based biopreservatives and food safety. He has more than 25 years of teaching and research experience.

Dr. Mohinder Kumar Salooja, M.Sc., Ph.D. in Dairy Technology from Kurukshetra University, Kurukshetra in 1979, 1983 respectively and Post-Doctorate from CSIRO, Australia in 1984. He is currently working as Professor at Indira Gandhi National Open University, New Delhi since 9+1997. He is Life member of Indian Dairy association, life member of Association of Food Scientists and Technologies (AFSTI), Mysore and member of All India Food Processors' Association (AIFPA), New Delhi since 2018. He has published more than 20 research and review papers in reputed national and international journals including Thomson Reuters (SCI & Web of Science) and conferences and more than 10 book chapters. His main research work focuses Applications on Food and dairy sciences. He has more than 30 years of teaching and research experience.

