

# “PRODUCTION OF A BACTERIOCIN FROM HALOPHILIC ARCHAEON NATRINEMA SPECIES SSBJUP-1 ISOLATED FROM LONAR LAKE”

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## Abstract

A haloalkaliphilic archaeon *Natrinema* sp.SSBJUP-1 was isolated from Lonar Lake situated in Buldhana District, Maharashtra, India. It produced bacteriocin which was active against gram positive and gram negative bacteria viz., *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and *Proteus vulgaris* respectively. The growth and bacteriocin production was maximized when the organism was grown at a temperature of 40°C at the pH of 8.5 and when supplemented with 20% w/v NaCl to the halophilic broth medium. The results of stability studies indicated that the bacteriocin became thermostable working at the temperature >80°C and was found unstable over extreme acidic and alkaline values below pH 3.0 and above pH 9.0 respectively. The maximum effectiveness of this bacteriocin was observed at 15% salt concentration while it was absent with 1% salt concentration and low to negligible at 5%. These results indicate that the haloalkaliphilic archaeon *Natrinema* sp.SSBJUP-1 produces a bacteriocin that may have potential for application as preserving agents in food industry, leather industry and in control of infectious bacteria.

**Figure: 03**   **References: 13**   **Table: 01**

**Key words:** Bacteriocin, Haloalkaliphilic archaea, Lonar Lake, *Natrinema* sp.

## Introduction

Halophilic archaea (haloarchaea) of the order Halobacteriales are members of the euryarchaea, which thrive in NaCl saturated

environments. To cope with high osmotic pressure, halophilic archaea keep a very high concentration of salts internally, thus remaining isoosmotic with the environment. To remain soluble and functional in intimate contact with salts, haloarchaeal proteins have a high content of acidic amino acid residues accompanied by an increase of negative charge on the protein surface 3, 9. To date, several unique characteristics of halophilic archaea were found to be of considerable biotechnological interest. These include halophilic enzymes able to perform their function at saturated NaCl; bacteriorhodopsin, a light driven proton pump; production of biopolymers, carotenoid pigments, and gas vesicles and bacteriocins. Haloarchaea were the first members of Archaea found to produce bacteriocin 10. Termed as halocins; these proteinous antibiotics act against related species and are universally produced by halophilic archaea 13.

Bacteriocins are plasmid borne and heat resistant peptides arose from a need for survival in overpopulated environment or culture. Thus cells containing plasmid encoding for bacteriocins have capability of destroying surrounding cells without bacteriocin plasmids.

Bacteriocins are specific in their action. Bacteriocins are secreted across cell envelope to extracellular medium; they recognize specific receptors located on surface of sensitive cells and are subsequently translocated to their specific intracellular targets. Bacteriocins induce toxicity by variety of mechanisms mostly by formation of

membrane pores, a mechanism which is increasing attention to bacteriocin for their potential use as preservative in food industry 5.

The formation of bacteriocins by a member of the Halobacteriaceae, *Haloferax mediterranei* was first described in 1982 10. Halocins (bacteriocin produced by halophilic bacteria) always reduce competition among haloarchaeal strains. Moreover, proteinaceous antimicrobials that can lyse competitors enrich the environment for the producer 4,7, 11. While lots of halocin producer strains are known; only few of them have been characterized at the protein level (halocin H4, H6 and R1) and halocin H4 has been characterized at both the gene and mRNA transcript levels 2,6,8.

Halocins are under investigation as antimicrobials for use in controlling spoilage during industrial processes such as in leather production 1. Though several species of haloarchaea were studied for halocin production, reports on bacteriocin production by *Natrinema* sp. are rather limited. In this context, herein we report the production of halocin by *Natrinema* sp. an extreme haloalkaliphilic archaea isolated from Lonar Lake, India.

## **Materials and Methods**

### **Microorganisms**

*Natrinema* sp. SSBJUP-1 is one among six extremely haloalkaliphilic archaea isolated from alkaline Lonar lake. All the six were tested for production and activity of bacteriocin against each other as well as laboratory cultures of common eubacteria such as *E. coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus* sp

The six isolates were cultivated in liquid haloalkaliphilic medium of composition containing (g/l): casamino acid, 7.5; yeast extract, 10; Trisodium citrate, 3; MgSO<sub>4</sub>.7H<sub>2</sub>O, 1; KCl, 2; FeSO<sub>4</sub>.7H<sub>2</sub>O, 0.05; NaCl, 200; Na<sub>2</sub>CO<sub>3</sub>, 18.5. The pH was self adjusted (8.5 as measured on a Toshcon digital pH meter) at 40°C for 21 days. The cells were removed from

the broth by centrifugation at 10000 rpm at 40°C for 15 min. The cell free extracts were used as crude bacteriocin preparations.

### **Testing against microorganisms**

The testing of the action of bacteriocin against test microorganism was done by the agar diffusion method employing nutrient agar plates for the bacteria. Wells 10 mm in diameter were drilled in solid media in petri dishes preinoculated with the test organisms. Bacteriocin preparations were added in 1 ml quantities in wells and the plates incubated at 37°C for two to three days. The growth around the wells was observed for inhibition and diameters of zones (if any) were measured in mm.

### **Effect of temperature on bacteriocin activity**

The bacteriocin was treated at various temperatures ie. 50°C, 60°C, 70°C, 80°C, 90°C, 100°C, 121°C in water baths for 15 minute and cooled to room temperature. It was tested against the test culture by agar diffusion technique. Control was kept containing untreated broth as bacteriocin.

### **Effect of pH on bacteriocin activity**

pH of bacteriocin preparation was adjusted to values from 3 to 10 with addition of sterile 1N NaOH and 1N HCl and tested against the test organism by agar diffusion method.

### **Effect of salt concentration on bacteriocin activity**

Salt concentration in the original bacteriocin solution was adjusted by dilution with sterile distilled water and tested against the test organism using the agar diffusion method.

## **Results and Discussion**

### **Screening of bacteriocin producers:-**

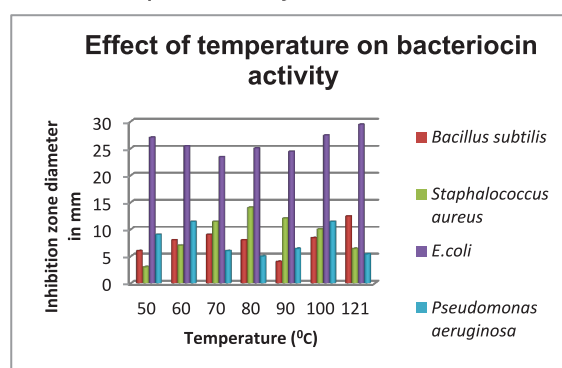
Laboratory strains of haloalkaliphilic isolates were screened for bacteriocin production against *Bacillus subtilis* and *Staphylococcus aureus*, (Gram positive bacteria) and *E. coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* (Gram negative bacteria). The result of this study is presented in Table-1.

TABLE-1 – Bacteriocin activity of the

laboratory isolates of extreme haloalkaliphilic archaea. Inhibition of the test culture is indicated as ‘+’ no effect as ‘-’. Medium used for the test was nutrient agar.

Strain Name	Bacillus Subtilis	Staphylococcus Aureus	E.coli	Pseudomonas aeruginosa	Proteus Vulgaris
Natrinema sp.- SSBJUP -1	+	+	+	+	-
Natrialba chahannaensis SSBJUP-2	-	-	-	-	-
Natrialbachahannaensis JUPSSB -3	-	-	-	-	-
Natronobacterium innermongoliae SSBJUP -4	-	-	-	+	-
Natrialbawudunaensis SSBJUP -5	-	-	-	-	-
Natrinema sp.	-	-	-	+	-

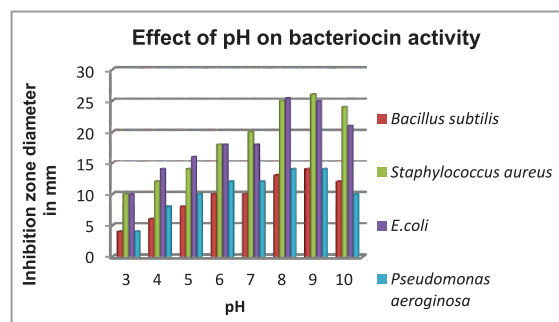
Table 1 showed that Natrinema sp.SSBJUP-1 produces bacteriocin against four of the bacteria – B. subtilis, Staphylococcus aureus, E. coli and Pseudomonas aeruginosa while bacteriocin of Natronobacterium innermongoliae (SSBJUP -4) and Natrinema sp. are seen effective only against Pseudomonas aeruginosa. Proteus vulgaris was not affected by any bacteriocin. The other three isolates are not seen to produce bacteriocin. The results for bacteriocin activity against close relatives showed that all the haloalkaliphilic archaeal strain were resistant to bacteriocin produced by each others.



**Fig. 1:Effect of temperature on bacteriocin activity:-**

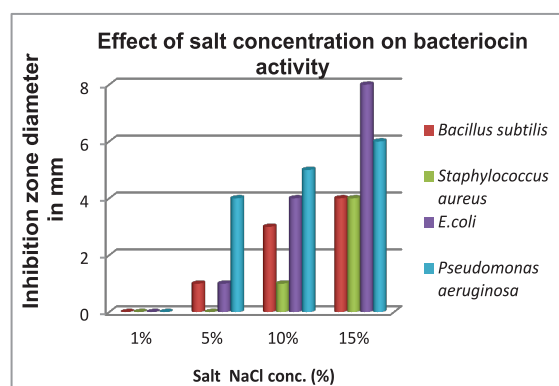
The heat sensitivity of the bacteriocin was determined by measuring its activity after incubation for 15 min at different temperatures. It was found that the bacteriocin was resistant

even to autoclaving temperature of 121°C although the sensitivity pattern of test bacteria differs with E. coli showing maximum inhibition to this bacteriocin.



**Fig. 2: Effect of pH on bacteriocin activity:-**

As bacteriocin produced by haloalkaliphilic organism it shows maximum residual activity against test organism at alkaline pH range. From pH 7 and below, its activity was found to reduce at acidic side.



### Fig 3: Effect of salt concentration on bacteriocin

Here again the fact of this bacteriocin being produced by haloalkaliphilic organism become evident. The maximum effectiveness of this bacteriocin was observed at 15% salt concentration while it was absent with 1% salt concentration and low to negligible at 5%. However, *S. aureus* was not affected sensitive at all the three higher salt concentration tested.

Discovery and identification of natural antimicrobial products from new sources like the extreme environments therefore plays an important role in the uncovering of novel drug leads and drug development process [12]. The broad spectrum of activity and stability to pH, salt and to heat observed with bacteriocin would be beneficial in possible development of drugs with this antimicrobial peptide. However, further studies are needed to identify the components responsible for the biological activity.

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