

A Novel Method of Optimized Bacteriosin Production from Marine Water and Testing its Efficiency as an Anti-Microbial Agent

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ABSTRACT: A strain of *Lacto bacillus* (LAB) was isolated from marine water in order to obtain a novel bacteriosin. Culture conditions for bacteriosin production were obtained in macconkey media, when incubated at 37°C for 48hrs in a rotary shaker at 100rpm. SDS-PAGE showed molecular mass of 4.5kDa, produced prominent inhibition zone in activity gel. Antibacterial efficacy of bacteriosin was proved against some food spoilage in sea foods which are caused by *Staphylococcus aureus*, *Vibrio cholera*.

KEYWORDS: Antibacterial; Bacteriosin; *Lacto bacillus*; Macconkey media;

I. INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram- positive, catalase-negative bacteria that produce various types of compounds such as bacteriosin, organic acid, diacetyl and hydrogen peroxide during lactic acidfermentation [10]. Among these compounds, bacteriosin is the most effective, as they can destroy or inhibit bacteria closely related to the producer strain. Bacteriosin are proteinaceous compounds that are inhibitory towards sensitive bacterial strains of their closely related members that are produced by both Gram-positive and Gram-negative bacteria [16]. Their efficacy has a predominant role in food preservation [1, 8, 12].

In the present study, in order to isolate a novel bacteriosin for efficient use as food preservative, we report a optimum conditions for the production from marine water source to isolate *Lacto bacillus* (LAB) in our laboratory [7,9].

II. BACTERIOSIN

Bacteriosin are proteins or short poly peptides, which are usually toxic only to bacteria that are closely related to the producer strains. A classic bacteriosin contains a toxin gene (bacteriosin), an immunity gene (which confers resistance to the fore mentioned toxin), and a lysis gene (which encodes a protein that aids in toxin release from the producing cell) [3]. These bacteriosin works by mechanism of destroying the cells by binding with surface receptors that are recognized by that specific bacteriosin [2, 3]. In a microbial community, cells can either be bacteriosin-o-genic (produce bacteriosin), sensitive, or resistant to each bacteriosin. When all three cell types are present, battle for limiting resources occurs between them. This interaction is observed in microbial communities that utilize bacteriosin. Only a small percentage of bacteriosin-o-genic cells will be induced to produce and release bacteriosin. Some sensitive cells are immediately destroyed by the bacteriosin, while other are mutated that confer resistance. These resistant cells rapidly displace the producer cells, due to the cost of bacteriosin production. However, the resistant cells grow more slowly than the insensitive counterparts, because resistance mutations often have a negative outcome on fitness. Bacteriosin produced by marine bacteria has generated a great deal of anticipation primarily due to their potential to serve as probiotics and antibiotics in the seafood industry [5, 6, 14]. A recent antimicrobial screen of 258 bacterial strains isolated from sediment water in the Yucatan peninsula revealed that 46 strains belonging to the genera *Aeromonas*, *Bacillus*, *Burkholderia*, *Photobacterium*, *Pseudomonas*, *Serratia* and *Stenotrophomonas* possessed antimicrobial activity. Approximately fifty percent (50%) of this antimicrobial activity was due to bacteriosin or BLIS (a novel bacteriosin like substance) [4]. Further, it's been proved that 10% surface-attached

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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bacteria isolated from Sydney Harbor (Australia), possess antibacterial activity. Proteinase K treatment showed that this inhibitory activity was associated with proteinaceous substances such as bacteriosin or BLIS [18]. Given the fact that bacteriosin and BLIS have been characterized in most bacterial species, it is appealing to speculate about the diversity of new substances. The marine environment will reveal.

III. APPLICATIONS AND IMPLICATIONS OF MARINE BACTERIA

The international sea food industry is one of the world's most profitable merchandise, worth more than \$75 billion per year (Food and Agriculture Organization of the United Nations [FAO], 2006). Fish and seafood are major proteins in some areas of the world. In 2006, of the 143 million tons of total fishery production (including fish, crustacean, and mollusks); 110 million tons was for direct human consumption [14]. To meet this demand, we had witnessed a noticeable rise in aquaculture (the farming of aquatic plants and animals) in the last decade (FAO, 2006). Recently, there have been spectacular changes in the seafood industry due to technological advances, consumer habits, and globalization of the food market [5]. In particular, there has been an increase in consumer preference for foods that are modestly processed or preserved (especially those that claim health-promoting benefits). Consumers are also demanding that these foods be fresh tasting and ready-to-eat [5]. The demands on the industry to provide fresh, modestly preserved products in the ever growing globalized food market is requiring a longer and more complex food-chain and increasing the risk of microbial contamination and spoilage [6].

MATERIALS AND METHODS

IV. BACTERIAL STRAINS AND MEDIA

A strain of *Lacto bacillus* LAB 5 was isolated from Marine Organisms, which was confirmed by morphological and biochemical studies. This was screened for bacteriosin production against two bacteriosin sensitive indicator strains *Staphylococcus aureus*, *Vibrio cholera*. Identification of the producer strain was done based on the cell morphology.

V. IDENTIFICATION OF *LACTO BACILLUS*

a) GRAM STAINING TEST:

The isolated bacteria were examined using gram staining kit and observed under light microscope with a magnification of 100x [17].

b) CATALASE TEST:

To perform this test, a single isolated colony was streaked on a glass slide and a drop of 3% hydrogen peroxide was added on to it. The effervescence of oxygen was not noticed which gives a negative response of the bacteria to catalase test [13].

VI. RESPONSE SURFACE METHODOLOGY (RSM CONTOUR PLOTS)

When a regression model is fitted using two or more continuous predictors, it is helpful to demonstrate a graphical visualization of the fitted surface [11]. A Response surface method (RSM) is an influential optimization tools in the arsenal of statistical design of experiments (DOE). Before employing RSM, process should take full advantage of a far simpler tool for DOE – two-level factorials, which can be very valuable for screening the few vital factors (including interactions) from the trivial many that have no significant impact. Assuming the potential for further financial gain, it's best to follow up screening studies by doing an in-depth investigation of the surviving factors via RSM. Then generate a response surface map and move the process to the optimum location.

1. The table was shaped from the subsequent second-order polynomial model, called a "quadratic," via least squares regression:

$$\hat{Y} = 808.77 - 250.45 X - 328.58 X^2$$

2. The curve was created by the following values obtained for mean and the standard deviation.

$$\text{Mean} = 255.71 + 23.69A - 49.06B - 35.14C - 25.54AC - 16.57B^2 + 27.75C^2 \quad (p < 0.0001, \text{ Adjusted } R^2 \text{ 0.84})$$

$$\text{Log10 Std Dev} = 1.82 - 0.077B + 0.012C + 0.18C^2 \quad (p < 0.0001, \text{ Adjusted } R^2 \text{ 0.76})$$

(Obtained from "design expert 9")

International Journal of Innovative Research in Science, Engineering and Technology

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VII. PRODUCTION OF BACTERIOSIN

Bacteriosin producer strain *Pediococcus acidilactici* LAB 5 and indicator LAB strains were cultured in *Lacto bacillus* macconkey agar and maintained at 4°C. The colonies are isolated from the marine water is inoculated in MRS media and incubated at 37°C for 24 hrs in a rotary shaker at 100 rpm. Then the culture was sonicated to break open the cells and produce the secondary metabolites (Figure 4).

VIII. ANTI-MICROBIAL ACTIVITY

The bacteriosin produced were examined by the disc diffusion method [11]. *S. aureus*, *V.cholera* were used for antibacterial test. The test microorganisms were seeded into relevant medium by spread plate method 10 µl (10⁶ cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the bacteriosin of 20 µl were sited on test organism-seeded plates. Streptomycin (10 µg ml⁻¹) used as positive control and MRS media (100 µg ml⁻¹) used as negative control. The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm.

IX. SDS-PAGE

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) is probably the world’s most broadly used biochemical method to detect the molecular weight of a compound [15]. To estimate the molecular weight of this bacteriosin, 15% SDS-PAGE gel is prepared. Protein marker is added to the first lane of this gel. Now the sample is mixed with the buffer and heated for 1-2 mins. Now 20 µl is added to the gel. Gel electrophoresis is carried out at 100v for 1 hr.

X. RESULTS AND DISCUSSION

- The above method shows how bacteriosin can be produced from marine water in an easy and efficient way, which could be scaled up for large scale production. Thus the produced bacteriosin was confirmed with a morphological and biochemical test. The catalase test (Figure 1) showed a negative result which proved the LAB from marine water is suitable for the production of bacteriosin.



FIGURE 1: CATALASE TEST

- Response surface methodology contour plot (Figure 3) was obtained from “Design expert 9”, in order to produce optimum condition for the production of bacteriosin. So, the table (Table 1), the RSM plot (Figure 2) gives us the most optimized production and the curve, which was also confirmed by practical evidences.

TABLE 1: OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY

RUN	PH	TEMPERATURE (°C)	PREDICTED RESULT (gm)	RESULTS OBTAINED (gm)
1	8	35	0.9807	0.9915
2	8	25	0.8706	0.96
3	8	35	0.9785	0.9944
4	4	45	0.8712	1.014

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5	4	35	0.8558	0.9215
6	8	35	1.023	1.019
7	8	45	1.027	1.094
8	8	35	0.8709	1.009
9	8	35	0.8894	1.002
10	8	35	0.8536	0.937
11	8	35	0.9824	0.8893
12	8	35	0.8494	0.985
13	4	25	1.024	1.044
14	12	35	1.011	1.156
15	12	25	0.9225	1.182
16	8	35	1.007	1.031
17	8	35	0.975	1.051
18	8	35	0.9922	1.025
19	12	45	1.157	1.34

Experimental design for bacteriosin production of *lab* by RSM comparing actual vs. predicted values

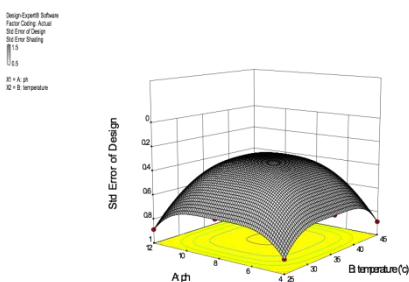


FIGURE 2: RSM PLOT

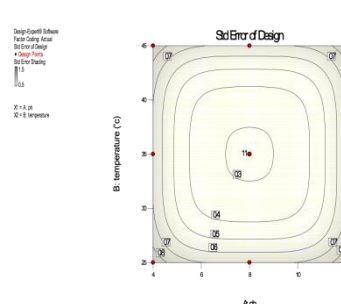


FIGURE 3: CONTOUR PLOT

- Bacteriosin produced was found to be of class II and also they belong to the category of Nisin. This was confirmed from molecular weight of the peptide obtained in the SDS-PAGE which was about 4.5 KDa. (Figure 4)

International Journal of Innovative Research in Science, Engineering and Technology

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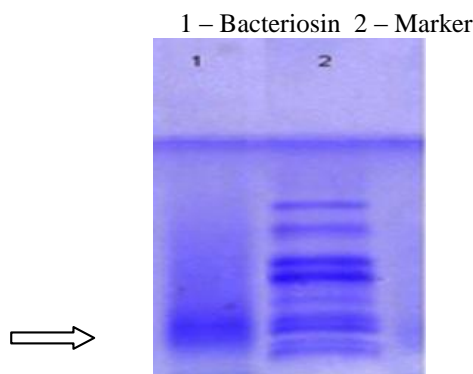


FIGURE 4: SDS – PAGE

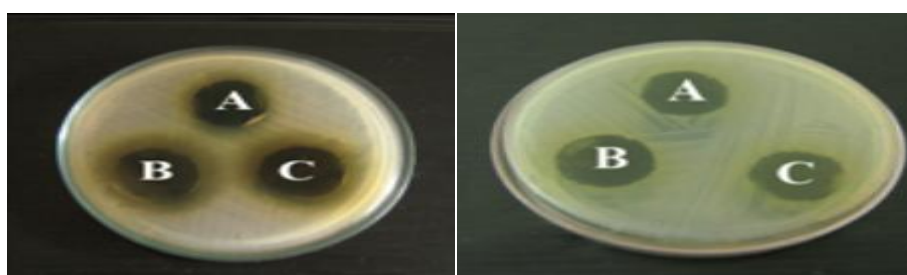
4. The bacteriosin also showed an effective anti-bacterial property against the common pathogens namely *Staphylococcus aureus*, *Vibrio cholera* which are most predominant in the sea food industry. (Figure 5) (Table 2)

TABLE 2: ANTI-MICROBIAL ACTIVITY

Micro-organism	Positive Control (Streptomycin)	Negative Control	TEST
<i>Vibrio cholera</i>	5.0 cm	0	5.5 cm
<i>Staphylococcus aureus</i>	6.0 cm	0	7.0 cm

Test sample (Bacteriosin) has higher potential against the test microorganisms than the positive control (streptomycin).

FIGURE 5: ANTIMICROBIAL ACTIVITY



Staphylococcus aureus

Vibrio cholera

A- Negative control; B- Positive control; C- Test

So this bacteriosin which is produced from the marine water could be used in sea food industry in packaging and transportation of the food across the country. This would save a huge amount of money that goes waste due to spoilage of food. The bacteriosin produced, is sensitive to temperature, pH and other hurdles that are mentioned in Hurdle technology of food preservation. But this could overcome, by combining bacteriosin with other food preservative for better efficiency.

XI. FUTURE RESEARCH

The bacteriosin produced from marine microorganisms are under study relative to their terrestrial counterparts. However, the profusion and range of bacteriosin in marine microorganisms remains to be fully explored. We predict that a wealth of novel bacteriosin proteins can be easily identified as the screening efforts proceed. Further, there is increased curiosity in the use of bacteriosin as alternative to classical antibiotics in aquaculture. Bacteriosin are highly

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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persuasive against marine pathogens and environmentally safe, due to the fact that they do not create intensive selection pressures for antibiotic resistance. Clearly, bacteriosin could prove extremely beneficial to the sea food industry and more research should be dedicated to exploring their potential applications as probiotics and therapeutics. Given the fact that all species of bacteria have the potential to produce bacteriosin, and only a handful has thus far been identified from marine microorganisms. We are confident that existing studies have exposed only the tip of the iceberg, in terms of bacteriosin diversity and potential use in the seafood industry.

XII.ACKNOWLEDGEMENT

We wish to express our sincere gratitude to Dr. Johanna Rajkumar, professor and Dean, Department of Biotechnology, Rajalakshmi Engineering College for providing us an opportunity to do our project work.

We sincerely thank Dr. V. Gayathri for her guidance and encouragement in carrying out this project work. We also wish to express our gratitude to our Head of the department and other faculty members who rendered their help during the period of our project work.

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International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 12, December 2015

BIOGRAPHY



Dr. V. Gayathri was born in Salem, Tamil nadu. She got her doctoral degree in the field of biochemistry-zoology from Presidency College, University of Madras. She has extensive passion and interest towards biochemistry and marine biotechnology. She currently holds the assistant professor position in Department of Biotechnology, Rajalakshmi engineering college. For the past decade, she has been guiding more than hundreds of students with their thesis work. Her extensive passion towards research let her publish a couple of books and papers . She always advises students to carry out product oriented research as they would have a great value to the society. In order to enhance the value of her Bio-products developed she has applied patents for three of her research work.



Aravind Jagannathan was born and brought up in Chennai, Tamil Nadu. He did his schooling at Velammal group of schools. He believes that almost every soul on this world is so much excited about the promises made by life science and the technology involved in to cure disease and to relieve suffering. Due to extensive passion towards the field of Life science, he pursues his undergrad in the field of Biotechnology from Rajalakshmi Engineering College, Chennai. His interest towards the subject was not definite at the beginning; but got the momentum from his second year. His dedication towards research and the subject fetched him great credits in and around the place including Indian Institute of Technology. He started excelling in academics as well as research, published papers and presented them in various international conferences. Though academics have conquered his major time he also proved his excellence in the field of photography. And he also holds the PRO post in BT society. The fire in him for research and his social motto has driven him to do his doctoral program in cancer biology.



Barath Muralidharan was born and brought up in Chennai, Tamil Nadu. He got his primary education from Modern senior secondary school, Chennai. The interest he showed towards biology made him to take up Biotechnology as his future study at Rajalakshmi Engineering College. The passion and interest in him made him to excel in academics and research. Owing to such interest he has done a couple of project and has published the same. His excellence in the area of life science was recognized by various universities and has given him awards for his research work. Management being another area of his interest he holds various positions in the core members of society. Combing his reach and Management skills he aspires to pursue his carrier in the field of life science management (MBS).