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Research Article

Bioactive compounds from marine Microbes

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Abstract

Natural compounds isolated from marine organisms have been found to be a very rich source of bioactive molecules. Reported biological effects of these compounds include anti-tumor, anti inflammatory and anti-viral activities as well as immunomodulatory and analgesic properties. Pharmaceutical market is growing rapidly and continuously. But, still the demand for new drug discovery is encouraged. The reason behind this motivation can be the growing number of drug–resistant infectious diseases and more and more upcoming disorders. Pharmaceutical market is growing rapidly and continuously. But, still the demand for new drug discovery is encouraged. The reason behind this motivation can be the growing number of drug–resistant infectious diseases and more and more upcoming disorders.

Keywords: *Bacillus cereus,* Lyophilization, SDS-PAGE.

Introduction

Natural compounds isolated from marine organisms have been found to be a very rich source of bioactive molecules. Reported biological effects of these compounds include anti-tumor, anti inflammatory and anti-viral activities as well as immunomodulatory and analgesic properties **(**Cipres *et al*., 2010). Since the late 1980s, more than 5000 natural products have been discovered from marine microorganisms. More than 15,000 marine organisms with biological activity were identified among which, 8000 had antibiotic and antitumor activities (Blunt *et al*., 2007 and Villarrean-Gomez *et al*., 2010).

Pharmaceutical market is growing rapidly and continuously. But, still the demand for new drug discovery is encouraged. The reason behind this motivation can be the growing number of drug–resistant infectious diseases and more and more upcoming disorders. The terrestrial resources have been greatly explored and thus academic and industry researchers are striving to get lead molecules from the inner space of oceans.

Current studies concentrate on the role of bacteria and fungi producing antibiotic substances in association with sponges. However, the strong increase of resistant bacteria causes severe problems in medical treatment and reinforces new investigations to search for compounds that are active even against multiresistant pathogens.

Sponges are considered as one of the most important sources of natural substances with

antibiotic, antitumoral or antiviral activities. This makes sponges a potentially important source of new products for medical treatment. In recent years, there has been a growing awareness on the bioactive potential of marine microbes. Hence the present study.

Materials and Methods

Collection of sample

Water and sediment samples were collected from Vellar estuary, Parangipettai.

Isolation of Bacteria

The samples were collected from marine environment and were serially diluted. Add 0.1ml of samples on Zobell marine agar plates and incubated at 37^0C for 48 hrs.

Culture filtrate activity

The culture filtrate activity of marine bacterial isolates was tested against human pathogenic bacteria (*Bacillus cereus)*. Results were recorded.

Optimization

Effect of pH , salinity, temperature ,Substrate on growth was estimated by various techniques.

Biomass production of isolate in shake flask

The optimized condition, temperature 30^0C, salinity 0.5%, Glucose 1.0%, beef extract 0.5% and pH 7 were maintained in the medium. Four such flasks were kept for incubation at 30 C in a shaker at 150 rpm for 42 hrs.

Ammonium sulphate precipitation and dialysis

The shake flasks kept for mass scale production were taken after 24 hrs and centrifuged at 15,000 rpm for 10min. To the supernatant, the amount of ammonium sulphate required to give 60% saturation was added slowly with stirring. Dialysis was followed in a tubular cellulose membrane against phosphate buffer for 24 hrs at 4°C.

Lyophilization

The partially purified protein was lyophilized in a Vertis lyophilizer and kept for further analysis .

Assay after separation of protein

The lyophilized powder of protein was dissolved in distilled water and well assay method was followed for inhibitory activity testing.

Protein Estimation

The protein concentration of the sample was determined by Lowry's method (Lowry et al., 1951) using bovine serum albumin as standard.

Protein Separation- SDS-PAGE-(Laemmli, 1973)

The proteins were separated in SDS-PAGE and size of polypeptide chains of given protein was determined by comparing its electrophoretic mobility in SDS-PAGE gel with mobility marker proteins of known molecular weight.

Results and Discussion

In soil samples $1.3x10^7$ CFU/g bacteria could be isolated where as in water samples the counts were only $1.0x10^5$ CFU/ml.The isolate was identified using of biochemical tests as per Bergey's Manual of Systemic Bacteriology. The name of the strain was designated as *B.cereus* SBS501.

Surprisingly all bacterial pathogens were inhibited by the potential strain *Bacillus cereus*. In bacterial pathogens most inhibited was *Staphylococcus aureus* (10mm) followed by *Vibrio cholerae* (8mm), *Salmonella paratyphi* (7mm), *Klebsiella pneumoniae* (6mm), *Salmonella typhi* (6mm), *Proteus mirabilis* (6mm), *Klebsiella oxytoca* (5mm), *E.coli* (4 mm), *Pseudomonas aeruginosa* (4mm) and *Lactobacillus bulgaricus* (4mm) .

Parameters like pH (7) ,temperature (30 C) , salinity (0.5%), carbon sources (glucose) and nitrogen sources (beef extract) were found to the optimum for growth. Chantharasophon et al.,

2011 found optimum pH and incubation temperature for *Bacillus sp* UBRU4 were 6.5 and 37°C.

Plate assay was done against most inhibited pathogen *Staphylococcus aureus* to decide the activity of inhibitory protein . Surprisingly it coincided with the maximum biomass obtained at 24hr. The inhibitory protein was found to be maximum at 60% ammonium sulphate precipitation.

The protein profile of the inhibitory protein showed 7 fractions with molecular weight of 31, 43, 51, 66, 69, 88 kda and 95 kda . Further purification is needed to decide the inhibitory activity of different fractions. The study also proved that the strain studied inhibited all the ten pathogens tested though the range of inhibition varied. Marine bacteria isolated from water and sediment samples as well as associated forms isolated from marine invertebrates especially sponges, corals, ascidians, tunicates have shown to produce a high percentage of antimicrobial metabolites (Barja *et al.,* 1989; Holmstrom and Kjelleberg, 1999 and Anand *et al*., 2006 and Jonathan *et al*., 2009).

As the incubation period was found to be only 24hrs this strain seems to be ideal for large scale production of bioactive substance it produces.

Though few less pathogenic forms like *E.coli*, *Pseudomonas aeruginosa* and *Lactobacillus bulgaricus* were tested the strain was inhibitory to important pathogens compared to these forms. *Staphylococcus aureus* is the pathogen that is developing resistance to Methicillin. At this situation the present study deserves credit as there is a possibility of developing lead molecules. The work done by Jamal *et al*., 2006 on *B. licheniformis* endorses the results obtained in the present study.

Sanders *et al*., 2003 reported that dozens of different peptide antibiotics exhibiting antagonism against a broad spectrum of microbes identified from the *Bacillus* genus. Berditsch *et al.,* 2007 also reported an antibiotic substance from *B.brevis* destroyed pathogens. The present study along with other studies on different *Bacillus* spp. proved that peptides of these microbes exerted a wide range of both antimicrobial effects on Gram+ve and Gram-ve bacteria.

As the cell free extract and the ammonium sulphate precipitation showed inhibitory activity it was found to be protein. Non-proteinaceous substance did not show any activity .To obtain the inhibitory protein alone, ammonium sulphate precipitation of cell free extract was done with progressively increasing it from 10% to 80%. The resultant protein was checked for its activity against *Staphylococcus aureus.* Accordingly 60% ammonium sulphate was decided as optimum to precipitate the inhibitory protein.

Mass scale was done in shake flasks to get enough inhibitory protein. It was dialysed and lyophilized and the partially purified protein was again checked for inhibitory activity. It showed maximum activity to *Staphylococcus aureus* (15mm), *Vibrio cholerae* (11mm) and *Salmonella para1typhi* (10mm).

The SDS-PAGE study revealed that the inhibitory protein had 7 protein fractions of varying molecular weight (i.e.) 31, 43, 51, 66, 69, 88 kda and 95 kda. Further purification is needed to confirm or correlate a particular protein to the inhibition of a particular pathogen. Jamal *et al*., 2006 recovered a 30.7KDa protein from *Bacillus licheniformis* which showed inhibitory activity against *Staphylococcus aureus.* The same protein inhibited *Enterococcus* and *Listeria monocytogenes* also. In the present study also 31KDa protein was observed. Apart from that as 6 different proteins obtained more than one lead protein molecule can be obtained.

The highlight of the present work is that the *B.cereus* SBS 501 strain is producing inhibitory proteins to 3 important human pathogen (i.e.) *Staphylococcus aureus, Vibrio cholerae* and *Salmonella paratyphi.* Further purification of this inhibitory protein could end up in the identification of lead molecules required for new drug development against these deadly pathogens.

Mass scale production in shake flask

Partially purification of protein using Dialysis membrane

Antimicrobial activity of *Bacillus cereus*

Staphylococcus aureus

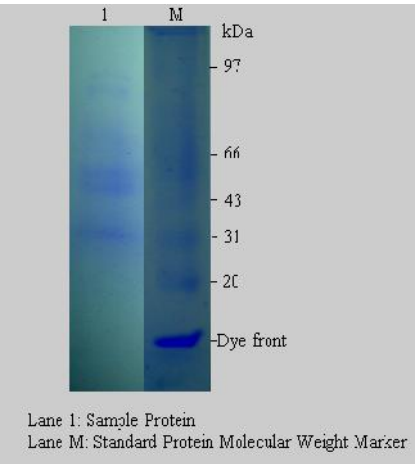
Vibrio cholerae

Salmonella paratyphi

Klebsiella pneumoniae

Salmonella typhi

Protein profile of inhibitory substance by SDS – PAGE



Summary and Conclusion

The highlight of the present work is that the *B.cereus* SBS 501 strain is producing inhibitory proteins to 3 important human pathogen (i.e.) *Staphylococcus aureus, Vibrio cholerae* and *Salmonella paratyphi.* Further purification of this inhibitory protein could end up in the identification of lead molecules required for new drug development against these deadly pathogens.

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