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ANTIMICROBIAL ACTIVITY OF MARINE BACTERIA ISOLATED FROM THE MANGALORE COAST, WEST COAST OF INDIA

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Abstract

Antibacterial substances from sea sediment were isolated from marine environment of Mangalore, west coast of India and characterized. Out of the 21 isolates subjected to secondary screening, 10 isolates were active against *Bacillus subtilis,* 12 against *Staphylococcus aureus*, 6 against *Escherichia coli*, 3 against *Proteus vulgaris* and 4 against *Salmonella typhi*. Metabolites in the extract of broth of overnight grown *Pseudomonas spp.* No.MN05 proved to have antimicrobial activity.

Keywords: Marine Bacteria, Antimicrobial activity, Minimum inhibitory concentration, *Pseudomonas* spp.

Introduction

The Ocean, which is called the 'Mother of origin of life', is also the source of structurally unique natural products that are mainly accumulated in living organisms. Several of these compound show pharmacological activities and are helpful for the invention and discovery of bioactive compound, primarily for deadly diseases like cancer, acquire immuno-deficiency syndrome, etc. The lives saving drugs are mainly found abundantly in microorganisms, algae and invertebrates and vertebrates. Modern technologies have opened vast areas of research for the extraction of biomedical compounds from ocean and seas to treat the deadly diseases.

The number of natural products isolated from marine organisms increases rapidly, and now exceeds 18,000, with hundreds of new compounds being discovered every year. A large proportion of these natural compounds have been extracted from marine invertebrates, especially sponges, ascidians, bryozoans and molluscs, and some of them are currently in clinical trials. Most marine invertebrates are sessile soft bodies that inhabit benthic rock environments. In the sea, rock substrate is limited, and benthic organisms have to compete for the space to live and develop. Invertebrate organisms have evolved defence strategies based on the synthesis of cytotoxic compounds in order to avoid predation and epibiosis. Many invertebrate animals, like sponges, tunicates, bryozoans, molluscs and oligochaetes are symbiotically associated with microorganisms belonging to the *Bacteria* and *Archaea* domains. In some cases, the source of the cytotoxic compounds isolated from marine invertebrates are the symbiotic bacteria. For instance, the tunicate *Lissoclinum patella* is simbiotically associated with the cyanobacteria *Prochloron* sp., which produces the cytotoxic compounds patellamides A and C, each with clinical potential. Davidson *et al*. provided evidence in the bryozoan *Bugula neritina* that its symbiont "*Candidatus* Endobugula sertula" is the source of bryostatins, which show excellent potential as therapeutic agents against leukemias, lymphomas, melanomas and solid tumors.

Screening of marine bacteria isolated from the surface of marine algae and invertebrates has shown that a high percentage produce Bioactive compounds. In addition, bacteria in biofilms formed on the surface of marine organisms have been documented to contain a high proportion of antibiotic producing bacteria than some other marine environment. The aim of this study was to investigate the antimicrobial activity of Pseudomonas species isolates from marine environment of Mangalore coast, West coast of India.

Materials and Methods

Sample collection

The sea sediment and water sample were collected from the Mangalore region, west coast of India (Lat. 12^{o} 52'N. Long. 074^{o} 53'E). The samples were brought to the laboratory in aseptic condition. Than the microorganisms were cultivated on Zobell Marine Agar 2216, than it was sub cultured on Modified Nutrient agar (MNA).

Screening of isolates for antimicrobial activity

In preliminary screening, determination of the antimicrobial activity of pure isolates was done by zone of inhibition method on Nutrient agar (NA) using *Salmonella typhi* as pathogen. Further screening was

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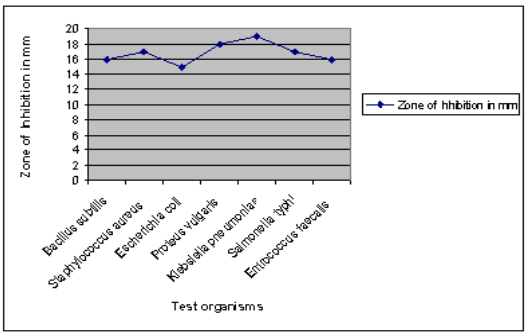
performed by zone of inhibition method against the standard test organisms. Active microbes were cultured for the screening of antibiotic substances in nutrient agar at 28°C for overnight. The test organisms used were: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris , Klebsiella spp., S. typh*i and *Enterococcus faecalis*

Figure 1: 25 µl of overnight grown culture of *Pseudomonas* spp. No.MN05 shows antimicrobial activity against all pathogenic strains



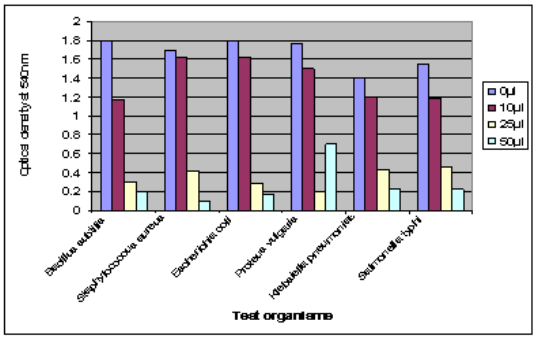
Characterization of isolates

The potent isolates selected from the secondary screening were characterized by morphological and biochemical methods. The results of microscopic examination were compared with Bergey's manual of Determinative Bacteriology, Ninth edition (2000) and the organism was identified. Various biochemical tests were performed for the identification of the potent isolates are as follows; Fermentation of sugars, Hydrolysis of starch, Indole production, Methyl red, Vogues Prauskauer, Citrate utilization, Urease test, 2%Peptone water, Nitrate reduction test, Gelatin liquefaction, Catalase test, Oxidase test.

Isolation and purification of antibacterial metabolites

Samples from *Pseudomonas* Culture No.MN 05grown in 1 L nutrient broth at 28°C and 220 rpm were taken and extraction was carried out using ethyl acetate solvent extraction [ethyl acetate : filtrate 1:1 (v/v)] on shaker at 220 rpm for 1 hr. The ethyl acetate phase that contains antibiotic was separated from the aqueous phase and by evaporation in water bath at 80-90°C the residue was obtained and weighed. The compound thus obtained was used to determine antimicrobial activity and minimum inhibitory concentration. The antimicrobial activity was determined by spectrophotometric method. The residue obtained was dissolved in 1 ml 0.2 M phosphate buffer (pH 7.0). 100 µl of preparation was loaded into a well in the 24 well plates, containing pathogenic cultures and were incubated at 37°C for 18 - 48 hr and optical densities measured at 540 nm using ELISA plate reader (Figure 1). For thin layer chromatography (Becker and Lechavier, 1964), silica gel plates (Merck) 5 X 20 cm, 1 mm thick were used. 10 µl of the ethyl acetate fractions and reference antibiotics were spotted on the plates and the chromatogram was developed using ethylacetate : iso-propanol : acetonitrile (1:4:5) as solvent system.

Figure 2: Effect of extract of overnight grown culture of *Pseudomonas* spp. No.MN05 on various pathogenic cultures



Results and Discussion

Out of 38 isolates were subjected for primary screening process, only 21 isolates showed activity against test organisms. Of which, only 3 were active against gram negative organism, 10 against gram positive organisms and 12 against both gram positive and gram negative organisms. Among these isolates, 29 of them showed positive inhibitory effect against *B. subtilis*, 27 against *S. aureus*, 17 against *E. coli*, 15 against *S. typhi* and 14 against *Proteus* species. Out of the 21 isolates those were subjected for the secondary screening, 10 isolates were active against *B. subtilis,* 12 against *S. aureus*, 6 against *E. coli*, 3 against *P. vulgaris* and 4 against *S. typhi*. It can be seen that the Pseudomonas culture No.MN05 shows maximum zone of inhibition against pathogenic culture. Culture no 7 which showed activity against all pathogenic strains (Figure 1). Figure 2 extrapolates the effect of extract of 48 h grown culture of *Pseudomonas* spp. colony 7 on various pathogenic cultures in the form of optical densities obtained. Identification of the potent antibiotic producing strains reveals that most of the specimens belong to the genus *Pseudomonas*. The minimum inhibitory concentration of the extract from *Pseudomonas* spp. No.MN 05was 0.73 mg.mL-1. Also the results from thin layer chromatography showed the spot given by the extract of *Pseudomonas* spp. was with Rf value 0.78.

Although the antimicrobial agents obtained in this study can't be declared as new bioactive compound, there is the probability of finding new bioactive compound in Mangalore because of its wide biodiversity. For proper identification of the extracts of bioactive compound, it is necessary to obtain in pure form, which requires a series of purification process and different chemical analysis. As we know the land of

Mangalore is virgin in this field, so lots of works should be done to explore the new bioactive compound because any new bioactive compound and its producing organism have been a great demand.

References

Berdy J (2005). bioactive microbial metabolites. J. Antibiotechnol. (Tokyo), 58: 1-26.

Baltz RH (2006). combinatorial biosynthesis of novel antibiotics and other secondary metabolites in actinomycetes. SIM News 56: 148-158.

Blunt JW, Prinsep MR (2006). Marine natural products. Nat. Prod. Rep. 23: 26-78.

Becker BM, Lechavier HA (1964). Rapid differentiation between Nocardia and Streptomyces by paper chromatography of whole cell hydrolysates. Appl. Microbiol. 12: 421-423.

Bergey's manual of determinative bacteriology (2000). Actinomycetales, 9th edition. Bull AT, Stach JEM (2005). Marine actinobacteria: perspectives, challenges, future directions. Antonie Van Leeuwenhoek, 87: 65-79.

Imada C, Okami Y (1998). A novel marine Bacillus with multiple amino acid analog resistance and selenomethionine-dependent antibiotic productivity. J. Mar. Biotechnol. 6: 189-192.

Kim TK, Garson MJ (2005). Marine actinomycetes related to the 'Salinospora' group from the Great Barrier Reef sponge *Pseudoceratina clavata*. Environ. Microbiol. 7: 509-518.

Lam KS (2006). Discovery of novel metabolites from marine actinomycetes. Curr. Opin. Microbiol. 9: 245-251.

Mann J (2001). Natural products as immunosuppressive agents. Nat. Prod. Rep. 18: 417-430.

Masayuki H, Hideo N (1987). Humic Acid-Vitamin Agar, a New Medium for the Selective Isolation of Soil Actinomycetes. J. Fermentation Technol. 65: 501-509.

Strohl WR (2004). Antimicrobials In Microbial Diversity and Bioprospecting. Edited by Bull AT. ASM Press: 336-355.

Shiomi KT, Takeuchi (1990). New antibiotic produced by *Micromonospora globosa*. J. Antibiotechnol. 43: 1000-1005.