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Antibacterial Activity of Cyanobacterial Species from Adirampattinam Coast, Southeast Coast of Palk Bay

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Abstract: The 12 marine cyanobacterial species were isolated from Adirampattinam coast, and cultured in ASN III media. Based on their growth characteristic, three species namely *Oscillatoria* sp., *Phormidium* sp. and *Lyngbya majuscule* were selected for antibacterial assay against the human pathogenic bacteria such as *Streptococcus mutants*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae*. The experiments for the inhibition activity on human pathogenic bacterial strains were carried out at different concentration levels (250, 500 and 750 μg mL^{-1}) of crude extract of 3 species of Cyanobacteria. Among the 3 cyanobacterial species extracts, *Oscillatoria* sp. showed the maximum inhibition (20 nm) against pathogen strain compared to other species and the minimum inhibition activity (7.2 nm) was observed in the extract of *L. majuscule*. The species *Oscillatoria* showed maximum zone of inhibition against pathogenic bacterial active revealed the potential of cyanobacterial extract.

Key words: Adirampattinam coast, antibacterial activity and marine cyanobacteria

INTRODUCTION

Cyanobacteria are an assemblage of gram-negative eubacteria. They are structurally diverse and widely distributed through out the world and are later known as blue green algae. Cyanobacteria are characterized by their capacity to perform biological nitrogen fixation and oxygenic photosynthesis. As cyanobacteria are very resistant to extreme environmental conditions. They are assuming increasing importance in frontier areas of biotechnology. The typical anabiosis and rapid restoration of activity under favorable conditions are characteristic of them (Pankratova, 1987). The several classes of marine micro- and macro-algae have been identified over the last few decades and their chemical constitution and pharmacological activity have been studied in detail (Umemura *et al*., 2003; Takamatsu *et al*., 2003; Mayer and Gustafson, 2003). These have proven their potential in several fields, particularly as new therapeutic agents for a variety of diseases (Harada *et al*., 2002; Romanos *et al*., 2002). Secondary metabolites from cyanobacteria are associated with toxic, hormonal, antineoplastic and antimicrobial effects (Carmichael, 1992; Patterson *et al.,* 1994). The antimicrobial substances involved may target various kinds of microorganisms, prokaryotes as well as eukaryotes. The properties of secondary metabolites in nature are not completely understood (Metting and Pyne, 1986; Inderjit and Dakshini, 1994). Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogeneticaly important. Antimicrobial effects from Cyanobacterial aqueous and organic solvent extracts are visualized in bioassays using selected microorganisms as test organisms (Kellam *et al*., 1988; Frankmolle *et al*., 1992; Falch *et al*., 1995). Methods commonly applied are based on the agar diffusion principle using pour plate or spread-plate techniques. Antimicrobial effects are shown as visible zones of growth inhibition. Bacterial bioassays comprise different test bacteria. *Micrococcus luteus*, *Bacillus subtilis*, *B. cereus* and *Escherichia coli* are commonly used to detect antibiotic residues in food (McGill and Hardy, 1992). Therefore the present study was undertaken to evaluate antimicrobial activity of cyanobacteria.

MATERIALS AND METHODS

In order to study the antibacterial efficiency of marine Cyanobacteria, samples were collected from various sites of Adirampattinam, Southeast coast of Palk Bay during September 2006. Samples were isolated and identified by standard microbiological methods by using ASN III medium (Waterbury and Stainer, 1981). The antibacterial activity of cyanobacterial species such as *Oscillatoria* sp., *Phormidium* sp. and *Lyngbya majuscula* were tested against the pure cultures of *Streptococcus mutants*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae* obtained from Raja Muthiah Medical College, Annamalai University, maintained in Nutrient Agar Medium. Aqueous cyanobacterial extracts of the species were prepared at a concentration of 100 mg mL^{-1} (Kreitlow *et al*., 1999). The extracted sample was taken for antibacterial assay. Microbial assay was carried out by well method in Petri dishes in ranges of 250, 500 and 750

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μg mL^{-1} with slight modification of Patra *et al*., (2008). Overnight cultures of each bacterial strain were swabbed with sterile cotton on the surface of Muller Hinton Agar plates. The plates were incubated for 24 h at 37°C; solvent control was performed in each case. After 24 h, areas of inhibition of bacterial growth were observed as clear zone around the well and diameter of zone of inhibition was measured and activity was expressed in nanometers.

RESULTS AND DISCUSSION

Totally 12 species of cyanobacteria were isolated, based on their growth characteristic 3 species *Oscillatoria* sp., *Phormidium* sp. and *Lyngbya majuscule* were selected for antibacterial assay and the antibacterial activity of these three species were given in Fig. 1-3. The inhibition zones of extracts at different concentration against the specific test organisms were measured. The extract restricted the growth of pathogen on the media around the well. The experiments for the inhibition of human pathogen strain were carried out at different concentration of 3 samples, namely 250, 500 and 750 μg mL^{-1}. And all the 3 cyanobacterial species against pathogen strain while the *Oscillatoria* sp. showed the maximum inhibition (20 nm) when compared to other test organisms and the minimum inhibition zone (7.2 nm) was observed the extract of *L. majuscule* (Fig. 1-3). These results indicate that the extracts contained different antibacterial substances and reflect the variety of environmental stress (Schwartz *et al*., 1990; Patterson *et al*., 1994). In *Oscillatoria* sp. showed maximum inhibition zone at important factors affecting the size of the inhibition zone are the chemical and physical properties of the growth medium and the size and ionic charge of the antibiotic molecule (Crosby, 1991).

Dose response curves drawn from the width of the inhibition zones related to the logarithm of extract concentration showed a linear relationship. This observation is in agreement with dose response characteristics of antibiotics (Crosby, 1991). The MIC values based on disc dilution of the extracts varied from 38 to 1875 mg freeze-dried cyanobacteria in the different bioassays. *Aeromonas hydrophila* seemed to be the most sensitive bioassay. Frankmolle *et al.,* (1992) showed that the antifungal MIC of laxaphycins in a broth dilution method varied from 16 to 64 mg mL^{-1}. In this present study the zone of inhibition was more when the higher concentration of cyanobacterial extracts inoculated in the well against the bacterial strains.

Campbell *et al*. (1994) reported that the toxic effects of cyanobacterial extracts on luminescent bacteria did not correlate with the concentration of microcystin-LR, but appeared to be due to other compounds present in the cyanobacteria. Present results are in agreement with these observations. Among the extracts, extract from effluent grown cyanobacteria was less effective in entering the bacteria tested. This might be due to the toxic effect of effluent on the cyanobacterial, biochemistry and resulted in lowing of antibacterial substances.

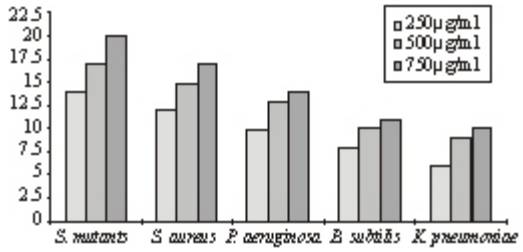

Fig. 1: Antibacterial activity of *Oscillatoria* sp.

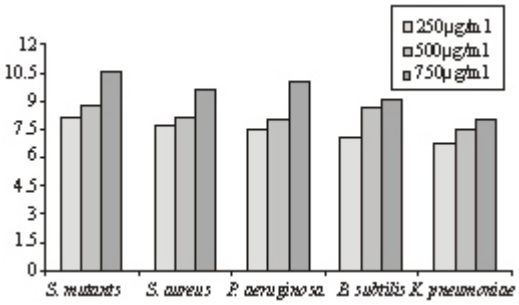

Fig. 2: Antibacterial activity of *Phormidium* sp.

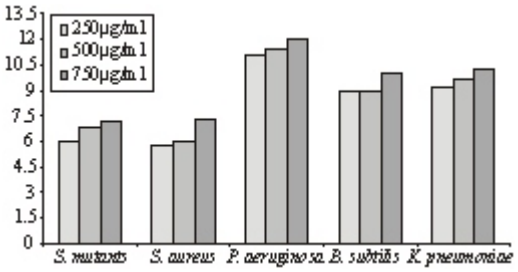

Fig. 3: Antibacterial activity of *L. majuscule* sp.

The results obtained in the present investigation were only based on crude extracts and did not indicate any defined antibacterial substance. However, suitable bacterial bioassays have been established to recognize and quantify antibacterial effects of cyanobacterial extracts. Further studies have to be made on fractionation and separation of crude extracts in order to find out the principle antibacterial compound.

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