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Research Article

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Antibacterial activity of marine bacteria isolated from sponge *Spirastrella inconstans*

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ABSTRACT

Sponge associated marine microbes recognised as potential candidate for screening and isolation of bioactive compounds for range of disease. In this study totally 14 heterotrophic and morphologically different bacterial colonies were isolated from marine sponge Spirastrella inconstans. The isolated strains were cultured in marine broth and extracted with ethyl acetate for screening of antimicrobial activity against a panel of human pathogens. The pigmented strains showed moderate activity of which strain MB13 showed inhibitory activity against all the tested pathogens was identified as Pseudoalteromonas piscicida using 16s rRNA sequencing. Two of non-pigmented strains also showed week antimicrobial activity.

Keywords: Antimicrobial, *Spirastrella inconstans*, Pseudoalteromonas, pathogens, sponge.

INTRODUCTION

Marine microbiome has rich genetic diversity to synthesize various unique molecules which is not produced by their counterparts in terrestrial. The organisms living in marine environment have been evaluated to adapt stress conditions also they exert range of chemical substances to protect itself from predators. Specifically the invertebrate associated microorganisms in high competition for their resident and nutrition which makes them as a suitable candidate for exploring pharmacologically promising molecules. Sponges are gold mine for isolation of wide variety of clinically important active compound for rang of disease from antimicrobial to antitumour. Sometimes the associated microbes are the original producer of active substances extracted from sponge tissue. Sponges are well known host for large community of microorganisms from bacteria to fungi. *S. inconstans* sponge was documented for its anti Methicillin Resistance *Staphylococcus aureus* (MRSA) [1]. A polyketide 14,15-secocurvularin isolated from fungi of *Spirastrella* sp. was described as antibiotic against *Bacillus subtilis* when compared to tetracycline [2]. Crude extract of *S. inconstans* was reported for its potential antioxidant activity as well as growth inhibitory activity in HeLa cells [3].

The emergence of infectious diseases and drug resistance mechanism developed by infectious microorganisms makes the natural product scientist to find effective molecules from marine environment to treat the disease accurately. The pathogens developed drug resistance mechanism progressively to the exposed therapeutic agents and caused remerging of infection [4]. *Pseudomonas aeruginosa* was a kind of opportunistic pathogen and one of the most common causes of nosocomial infections by the intrinsic resistance to many antimicrobial agents [5]. Considering above mentioned strategies, the present work was aimed to isolate a antimicrobial capacity possessing bacteria from tissue a *S. inconstans.*

EXPERIMENTAL SECTION

Isolation and culturing of microorganisms

The sponge specimen *Spirastrella inconstans* (Dendy) was collected from the intertidal zone of Palk Bay, Rameshwaram during low tide. Initially, the sponge sample was washed with jets of filtered and autoclaved seawater until they were visibly free of debris. Then the sponge surface was sterilized with rapid swab of 70% ethanol followed by the specimen immersed in autoclaved seawater and then aspirated. One gram of central core of sponge tissue was homogenized with 99 ml of sterilized seawater. The homogenate was serially diluted upto 10^{-5} and spread on the entire surface of 1:10 Marine Agar (peptone, 0.5 g; yeast extract, 0.1 g; FePO4, 0.1 g; agar, 15 g; dissolved in 1 litre of seawater; pH 7.2–7.6) [6]. The plates were incubated at room temperature (approx 30-37ºC) for 5 days and isolation of bacteria with different colony characteristic was carried out from third day onwards till fifth day. On fifth day counts were used for calculation of colony forming unit (CFU). Different types of colonies were isolated and were repeatedly streaked to obtain pure cultures and stored in marine agar slant at 4^{o} C for further studies.

For the production of secondary metabolites, the purified marine bacteria as above were cultured in 300 ml of Marine broth (peptone 5 g, yeast extract 1 g and FePO4 0.1 g, dissolved in 1 L of seawater, pH 7.2) in 500 ml Erlenmeyer flasks [6]. Flasks were then incubated on a shaker at 220 rev min^{-1} for 7 days at 27±3^{o} C. On 7th day the broth was centrifuged at 5000 g for 30 min to remove the cell and the supernatant extracted with ethyl acetate (EtoAc) (100 ml x 3). EtoAc was removed under reduced pressure at 37^{o} C. Extracts were dissolved in methanol to make a stock solution for screening of antimicrobial activity.

Assay for Anti-Pathogenic Activity

A panel of human pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Proteus vulgaris, Enterococcus faecalis, Klebsiella pneumoniae, Vibrio cholerae, salmonella typhi* and *Salmonella paratyphi* were obtained from Raja Muthiah Medical College Hospital, Annamalai University, Chidambaram. Bacterial strains were maintained on nutrient agar slants (Hi-media, Bangalore, India).

Assay for the antimicrobial activity of bacterial extracts were done by agar-well diffusion method using Muller Hinton Agar 12. 0.1ml of test organism was taken from the stock (broth) and swabbed on the agar medium. The methanol extract of above mentioned bacterial broth were dissolved in dimethyl sulphoxide (DMSO) (50mg/10ml) were added into the wells. The diameter of the zone of inhibition (mm) around the well was measured after incubation at 24-28° C after 48hours. Kanamycin was used as positive control and DMSO as negative control.

Identification of potential bacterium

The potential strain MB13 which showed significant anti-pathogenic activity was identified by 16s rRNA sequencing. The genomic DNA extracted from the marine sponge associated bacterium MB13 was PCR amplified for 16s rRNA genes using the universal bacterial primers Eubac 27F (5'- AGA GTT TGA TCG TGG CTG AG- 3') and 1492R (5'- GGT TAC CTT GTT ACG ACT T- 3'). The partial 16s rRNA gene sequencing was done using Perkin Elmer Applied Biosystems and ABI prism software was used to align the sequence and compared sequence were retrieved by the queries generated by BLAST of Genbank Database. Phylogenetic analysis was performed with MEGA 4.0 [7]. The tree topologies were inferred using the neighbour-joining method and submitted to NCBI Genbank.

RESULTS AND DISCUSSION

Ocean has under-explored and endless source of microbial diversity with rich genetic diversity. The sponge specimen *S. inconstans* was collected from intertidal zone of Palk Bay region and 14 different bacterial colonies were purified. The isolated colonies were marked as MB1 to MB14. Five out of 14 strains were pigmented like yellow, brown, pale yellow in colour. All the pigmented strains showed atleast one zone of clearance on lawn of pathogen spread plate. Of which the strain MB13 showed inhibitory activity on all the test pathogens. It showed maximum of 16mm clearance on *E. coli* spread plate. Four non-pigmented colonies also showed week anti-pathogenic activity. Our result was in concordance with statement of Bowman (2007) [8] that pigmented strains produce a variety of bioactive compounds with antimicrobial, antifouling, and algicidal activities, whereas non-pigmented strains often lack these traits. The antimicrobial activity of isolated strain's metabolite were given in table:1. The potent strain showed antibacterial activity against all the tested pathogen was identified as *Pseudoalteromonas piscicida* using 16s rRNA partial sequencing. The 16S rRNA gene, consisting of 1542 bases, is highly conserved among microorganisms and is therefore an excellent tool for studying phylogenetic relationships [9]. The sequence data was analysed comparatively with already available database and performing a phylogeny

search. Fig.1 clearly indicated that strain MB13 was close to the member of *P. piscicida*. This sequence was submitted to Genbank (Accession number: KF113883).

Table 1: Anti-pathogenic activity of different bacterial metabolites

Pathogens / Strains	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. cereus*	*P. vulgaris*	*E. faecalis*	*K. pneumoniae*	*V. cholerae*	*S. typhi*	*S. paratyphi*
MB1	-	-	-	-	-	-	-	-	-	-
MB2	+	-	-	+	-	-	-	-	+	-
MB3	-	-	-	-	-	-	-	-	-	-
MB4	-	-	-	-	-	-	-	-	-	-
MB5	-	-	-	+	-	-	-	-	-	-
MB6	-	-	-	-	-	-	-	-	-	-
MB7	-	-	-	-	-	-	-	-	-	-
MB8	+	+	+	-	-	+	-	+	+	-
MB9	-	-	-	-	-	-	-	-	-	-
MB10	+	-	+	-	-	-	-	-	-	-
MB11	+	-	-	-	-	-	-	-	-	-
MB12	-	-	-	-	-	-	-	-	-	-
MB13	++	+	+	+	+	+	+	+	+	+
MB14	+	-	-	-	-	-	-	-	-	-

(+ showing activity, ++ showing strong inhibition, - Nil activity)

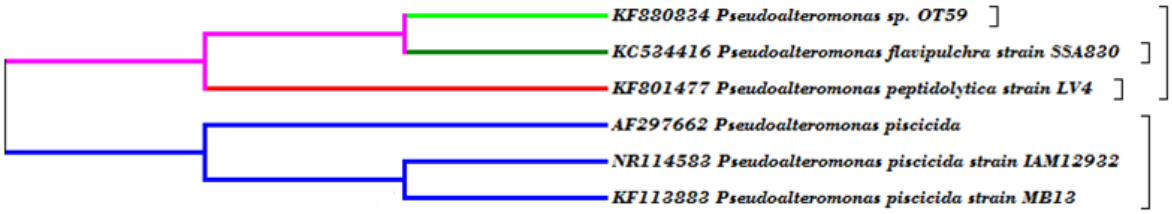

Fig.1. Phylogenetic tree constructed with MEGA 5 software using neighbour joining method

Pseudoalteromonas genus is a group of marine bacteria belonging to the class γ-*proteobacteria* within the order of *Alteromonadales* (uniprot taxonomy). These rod-shape Gram negative bacteria colonizing non-geothermal marine habitats are motile and they are strictly aerobic showing a chemoheterotrophic metabolism. *Pseudoalteromonas* genus displays a particular preference for living in association with eukaryotic organisms surfaces [10-11].

Pseudoalteromonas has been reported for different secondary metabolome includes polyanionic exopolymers, alkaloids, cyclic peptides, and different bromine substituted bioactive compounds [8]. Andersen *et al.* (1974) [12] identified violacein and pentabromopseudilin as bioactive compounds from *P. luteoviolacea*. Biologically active components of the marine *P. tunicata* have been identified as alkaloid tambjamines, the chemical structures of which are partly similar to prodiginines well known for its anticancer property [13]. Screening of the biological activities of some tambjamines against certain human cancer cell lines (HL-60, Breast carcinoma) has revealed that they possess moderate antitumour activity compared to the doxorubicin control [14].

The study concluded that complex bacterial community of sponge tissue can act as promising source of clinically important drug molecules. We can consider sponge associated microbes as candidate for discovering active compounds.

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