

Antimicrobial activity of *Serratia* sp isolated from the coralline red algae *Amphiroa anceps*

P. Karthick^{1*}, R. Mohanraju¹, Kada Narayana Murthy¹, CH. Ramesh¹, C. Mohandass², R. Rajasabapathy² & S. Vellai Kumar³

¹Department of Ocean Studies and Marine Biology, Pondicherry University, Brookshabad Campus, Port Blair, 744112, Andaman and Nicobar Islands.

²Biological Oceanography Division, CSIR-National Institute of Oceanography, Dona Paula, Goa-403 004.

³Department of Plant Molecular Biology and Bioinformatics, Tamil Nadu Agricultural University, Coimbatore -641003.
Corresponding author* [E.Mail: karthickmicrobes@gmail.com]

Received 21 April 2014 ; revised 22 September 2014

Bacterial isolates (6 nos) were obtained from the coralline red algae *Amphiroa anceps*. Crude extract of these isolated were tested for antimicrobial activity against 20 different human pathogenic bacterial and fungal strains. Among this crude extract of a single red pigmented strain AA1 was found to show activity against (12) bacterial pathogens and (1) fungi. Higher range of antibacterial activity was observed against *Staphylococcus aureus* and antifungal activity against *Rhizopus* sp, while moderate antibacterial activity was observed with *Salmonella typhi*, Enteropathogenic *E.coli* and *Klebsiella pneumonia*. Identification based on morphological, biochemical and 16S rDNA sequencing showed that the strain AA1 was identified as *Serratia* sp (KC149511). Fractioned extract of the bacterial isolate was analyzed in a Gas Chromatography Mass Spectrometry (GC-MS). Results showed the presence of certain metabolites such as octadecanoic acid, phenol, 2, 4-bis (1, 1- dimethyl ethyl), nonanoic acid- 9 oxo methyl ester which could be responsible for the antifungal and antibacterial activities. Results showed that the pigmented *Serratia* sp is a potential source for bioactive substances and could contribute to the discovery of several novel compounds of pharmaceutical importance.

[**Keywords:** Antimicrobial, epiphytes, *Serratia*, macroalgae]

Introduction

Marine microorganisms provides potential novel bioactive metabolites and bio natural products of the pharmaceutical importance in applications. These microorganisms are found to be a better source of bioactive metabolites than higher organisms (Okami 1986). Macro algae are particularly known to produce valuable pharmaceutical components and are considered to be an important source for biologically valuable secondary metabolites (Gamal 2009). These algae are known to harbor several microbial communities exhibiting antimicrobial properties (Kanagasabhapathy *et al.*, 2008) and these epiphytic bacteria are found to release bioactive metabolites into the surrounding environment for their survival against the competition of colonization (Zheng *et al.*, 2005). Various secondary metabolites have been obtained from the marine microorganisms which are found to be different from that of the marine invertebrates

(Kelecom 2002). The epibionts associated with macro organisms are host specific (Goecke *et al.*, 2013), found to protect their hosts from fouling communities and play a significant role as ecological protective barrier to their hosts (Armstrong *et al* 2001; Kanagasabhapathy *et al.*, 2008; Wilson *et al.*, 2010), and paves a way for the development of novel drugs (Menezes *et al.*, 2010). The objective of this study was to screen the epiphytic bacteria associated with the coralline red algae *Amphiroa anceps* for antimicrobial properties against human pathogens. Crude bacterial extracts were analyzed by using GC-MS for the compounds. This potential strain was identified up to genus level by molecular techniques.

Materials and Methods

Red algae *Amphiroa anceps* was collected by hand picking with sterile gloves from the Burmanallah coast (South Andaman's lat 11° 33' N and long. 92° 43' E) and

transported to the laboratory in sterile plastic bag. Samples were rinsed with sterile seawater to remove loosely bounded epiphytic bacteria. Seaweed surface was scrapped with a sterile cotton swab and plated on to Zobell marine agar plate (Himedia, Mumbai) (Lemos *et al.*, 1985) and were incubated for 18 to 24 hours at 32°C. After 24 hrs each isolated single colony was picked and restreaked and tested for purity. These were further sub cultured and maintained on agar slants at 4°C. Stab culture were maintained on marine broth containing 0.8% agar and frozen cultures were maintained on marine broth containing 50% glycerol. Biochemical analysis for the identification of these isolates were carried out following Bergey's manual of systemic Bacteriology (Brenner et al 2005) and also using the ABIS online software.

Genomic DNA was prepared from the bacterial isolate by following the method as described by Mohandass et al (2012). PCR amplification of 16SrRNA gene was conducted in a final volume of 25µl with the bacterial consensus universal forward and reverse 16SrDNA primers 27F and 1492R (Lane, 1991). The reaction mixture contained 1x PCR buffer (Sigma, USA), 2.5 mM MgCl₂, 200 µM DNTP's, 1U of Taq DNA polymerase, 25 pmol of each forward and reverse oligonucleotide primers and approximately 20 ng of genomic DNA. Amplification profile consisted of an initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. This was followed by a final extension step of 72 °C for 5 min. The samples were held at 4 °C until further analysis. PCR products were sequenced by an automated Sequencer (Applied Biosystems, Foster City, USA) at the National Institute of Oceanography, Goa, India. The sequences were submitted to GenBank and accession numbers were assigned.

The partial 16SrRNA gene sequence of the isolate AA1 were compared with the existing database. Identification to the species level was determined by 16SrDNA sequence similarity of 99% with that of the prototype sequence available in Gen Bank. Sequence alignment and comparison was performed using the multiple

sequence alignment program Clustal X 1.81 (Thompson *et al.*, 1997). Sequences were edited manually to remove the gaps. Phylogenetic tree was constructed by Neighbor-joining method by using MEGA4 software (Tamura *et al.*, 2007) and the maximum likelihood method was adopted for calculating the evolutionary distance (Tamura *et al.*, 2004).

Epiphytic bacterial isolates were subjected to cross streak method for the antagonistic activity against the human pathogens on solid media (Lemos *et al.*, 1985). Isolate showing antagonistic properties were selected picked and subjected to the extraction of secondary metabolites in 30ml screw cap tubes containing 10ml of Zobell's marine broth and Luria broth (Himedia, Mumbai) respectively and incubated at 32°C for 2 days in a water bath shaker. After incubation cultured broth was centrifuged at 10000 rpm for 10 min, cell free supernatant was extracted with equal volume of ethyl acetate and final crude extract obtained was screened for antimicrobial activity against bacterial and fungal pathogens.

Culture AA1 was mass cultured for the extraction of secondary metabolites in a minimal medium (inorganic salt) composed of (NH₄)₂SO₄ (2.64), KH₂PO₄ (2.38), MgSO₄·7H₂O (1.00), CuSO₄·5H₂O (0.0064), FeSO₄·7H₂O (0.0011), MnCl₂·4H₂O (0.0079), ZnSO₄·7H₂O (0.0015) in (g/liter) with pH adjusted to 7 (Pridham and Gottlieb, 1948).

The bacterial strain AA1 was cultivated in 30 ml minimal medium supplemented with 3% NaCl, 1% glucose and 1% yeast extract as carbon and nitrogen sources respectively and incubated at 31°C for 24 hrs in an incubator shaker. One milliliter of this growth culture was used as inoculum for mass culture (Jafarzade *et al.*, 2013).

One milliliter of the inoculum (of the isolate AA1 grown in minimal media) were supplemented with different concentration of glucose 1%, 2%, 3% and yeast extract of 1%, 2%, 3% along with 0.75 % of NaCl for the production of antimicrobial compounds with various pH 6,7 and 8 at a constant temperature of 31°C incubated for 24hrs (Jafarzade *et al.*, 2013).

Twenty milliliter of minimal medium was dispensed into 100 ml Erlenmeyer flasks and sterilized. To this filter sterilized yeast extract (1%) and glucose (1%) were added as nitrogen and carbon sources just prior to inoculation. One milliliter of the inoculum was transferred into the Erlenmeyer flask and incubated for 24 hrs. The effect of salinity for the production of antimicrobial properties was studied at various concentration of sodium chloride ranging from 1%, 2% and 3%.

The active strain AA1 were subjected to mass culture in 250ml Erlenmeyer flask containing different media viz., Zobell's marine broth, Luria broth (HiMedia, Mumbai) and minimal media supplemented with sodium chloride, glucose, yeast extract at different concentration incubated at 32°C for 5 days in a water bath shaker. After growth cultured broth was centrifuged at 10000 rpm for 10 min and the cell free supernatant was extracted with equal volume of ethyl acetate and the medium polar organic extracts obtained were concentrated under vacuum in a rotary evaporator (Buchi) at 40°C. The concentrated extracts were dissolved in dimethyl sulfoxide (DMSO) and were analyzed for antimicrobial properties by both disc and well diffusion assay.

Bacterial crude extracts of strain AA1 obtained by mass culture were separated with column fraction 4:4:2 ratio of methanol: ethyl acetate: chloroform. Different fractions were collected and concentrated in a rotary evaporator and were screened for antimicrobial assay. Active fraction was collected and analyzed in a GC-MS for the identification of secondary metabolites present in the crude extract.

Column purified ethyl acetate fraction was analyzed in a Gas Chromatography and Mass spectrometry (Perkin Elmer Clarus 500 USA), to quantify the secondary metabolites present in the extract which had shown good antimicrobial activity. 1 μ l of purified crude extract was loaded into the DB-5 Column with Helium as a carrier gas at a flow rate of 1 ml/min. Split Injection mode of the ratio of 1:20 was adopted. Temperature programming was

from 75°C for 2 min further increased to 175°C with 15°C/min and then increased up to 280°C at the rate of 5°C/min. Sample run time was up to 29.67 min. The peaks representing mass to charge ratio characteristic of the antimicrobial fractions were compared with mass spectrum of NIST library for identifying corresponding organic compounds.

Seventeen human bacterial pathogens *Escherichia coli* MTCC 443, *Klebsiella pneumoniae* MTCC 109, *Salmonella typhi* MTCC 733, *Shigella flexneri* MTCC 1457, *Staphylococcus aureus* MTCC 96, *Shigella boydii* type 1 NK2379, *Shigella sonnei* NK4010, *Shigella dysenteriae* type 5 NK2440, Enterotoxigenic *Escherichia coli* serotype 0115, Enteropathogenic *Escherichia coli* serotype 0114, Shiga toxin producing *E.coli* serotype O157:H7 VT3, *Vibrio fluvialis* IDH 02036, *Vibrio parahaemolyticus* serovar O3: K6 K5030, *Vibrio cholera* O139, *Vibrio cholera* 01, Ogawa Eltor, *Aeromonas hydrophilla* IDH1585, *Salmonella enterica* serovar typhi C6953 and three fungal strains *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* sp were used for studying the antibacterial and antifungal property. 18-24 hrs old bacterial broth cultures were seeded on Muller Hinton Agar plates (Hi Media, Mumbai) while the fungal cultures were seeded on Potato Dextrose Agar plates (Hi Media, Mumbai) by using a sterile cotton swab.

Antibacterial and antifungal activity was determined by the disc and well diffusion assay following the techniques as described by (Bauer et al 1966). Gentamicin and Clotrimazole were used as a positive control and dimethyl sulfoxide (DMSO) used as negative control. Plates were incubated at 37°C for 24 hrs for bacterial and 48 hrs for antifungal activities. Inhibition zones around each discs and wells were measured in (mm). All assays were performed in triplicates.

Results

In the present study 6 strains were isolated from the red algae *Amphiroa anceps* and among these five strains did not showed any antibacterial or antifungal activities (Table 1 & 2). Only one red pigmented strain (AA1)

showed antifungal and antibacterial activities and showed pigmentation in both marine agar plates and broth. This isolate was morphologically and biochemically identified. The isolate was found to be a Gram negative, motile, rod shaped bacteria, methyl red, citrate, catalase, starch hydrolysis, proteolysis activity showed positive reaction, while Indole, Voges Proskauer, oxidase, urease, lipase activity and gelatin liquefaction tests were negative. Comparative 16S rRNA gene sequence analysis showed that the strain AA1 needed to be

positioned within the genus *Serratia*, being closely related to *Serratia marcescens subsp. sakuensis* (AB061685) and *Serratia nematodiphila* (EU036987). Phylogenetic tree (Fig.2) shows that the strain AA1 is closely related to *Serratia marcescens subsp. sakuensis* (AB061685). The 16S rRNA gene sequence has been uploaded in NCBI database and the accession number assigned as KC149511 and identified as *Serratia* sp with similarity of 99% and did not match with any other species in the existing library, suggesting to be a novel isolate.

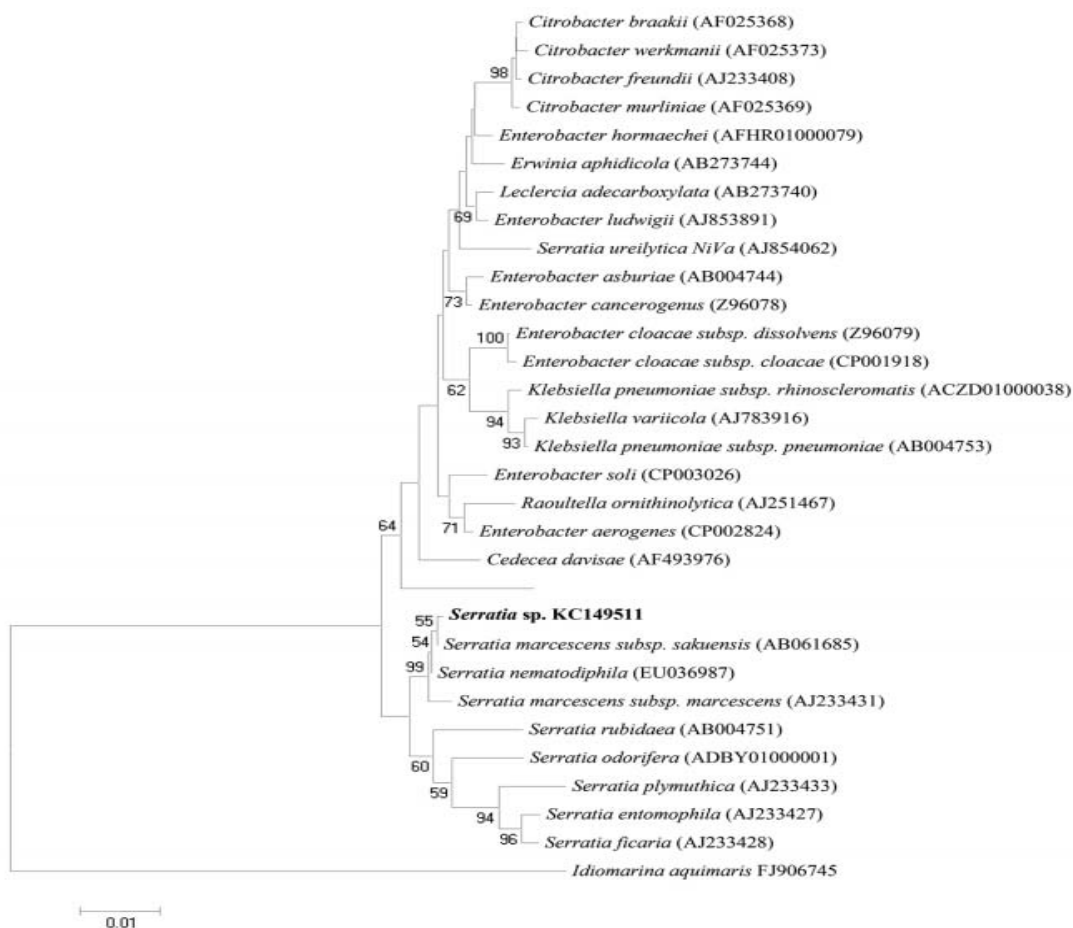


Fig 2. Neighbour-joining phylogenetic tree based on 16SrRNA gene sequences showing the relationship of KC149511 with its closest neighbours. Bootstrap values (50 %) are shown at branch points. *Idiomarina aquimaris* (FJ906745) used as out-group.

Table 1: Morphological and biochemical characterization of the isolated strains from *Amphiroa anceps*.

Identification Tests	<i>Serratia</i> sp AA1	<i>Bacillus</i> <i>pumilus</i> AA2	<i>Paenibacillus</i> <i>timonensis</i> AA3	<i>Bacillus subtilis</i> AA4	<i>Bacillus</i> sp AA5	<i>Paenibacillus</i> sp AA6
Morphological tests						
Grams staining	-ve	+ve	+ve	+ve	+ve	+ve
Motility	+	+	+	+	+	+
shape	Rod	Short Rod	Rod	Short Rod	Short Rod	Short Rod
Biochemical tests						
Indole	-	-	-	-	-	-
Methyl red	+	-	-	-	-	-
Voges proskauer test	-	-	-	+	-	-
Citrate utilization	+	+	+	+	-	-
Catalase	+	+	+	+	-	-
Oxidase	-	+	+	+	+	+
Urease	-	-	+	-	+	-
Starch hydrolysis	+	-	+	+	+	+
Protease hydrolysis	+	+	-	+	-	-
Lipase activity	-	-	-	+	-	-
Gelatinase liquefaction	-	-	+	-	-	-

The crude ethyl acetate extract of the isolate AA1 was assayed for antibacterial and antifungal properties by both disc and well diffusion assay against seventeen bacterial and three fungal strains at different concentration. Marine broth extract exhibited maximum activity against *Staphylococcus aureus* (18 mm), Enteropathogenic *Escherichia coli* (16mm), *Klebsiella pneumoniae* (16mm), *Salmonella typhi* (16mm) and *Rhizopus* sp (20mm). Moderate activity against *Shigella boydii*, *Shigella sonnei*, *Vibrio parahaemolyticus* (15mm) respectively. The extracts did not show any zone of activity against *Escherichia coli*, Enterotoxigenic *E.coli*, *Shigella flexneri*, *Aspergillus niger*, *Aspergillus flavus*, *Vibrio cholerae* O139, *Vibrio cholerae* Eltor, *Aeromonas hydrophilla* (Table 3).

Whereas Luria broth extract exhibited only few activity with *S. aureus* (12 mm), *K.pneumoniae* (13 mm) and Ep. *E.coli* (12 mm) (Table 3).

The bacterial isolate AA1 cultured in minimal media showed antibacterial activity with a pH range of 6-8. Maximum zone of activity was observed against *S.aureus* (16mm) *S. boydii*, *V.parahaemolyticus* (15mm) at an optimum pH of 7. Less activity was observed against Enteropathogenic *E.coli* (12mm) at pH of 6. Antibacterial production was observed to be more in 2% NaCl concentration showing maximum activity against *S.typhi* (19 mm), Enteropathogenic *E.coli* (17mm) and activity was less observed in 2% of NaCl against *S. flexneri* (Table 3).

Table 2. Sugar fermentation test for bacterial isolates from *Amphiroa anceps*

Bacterial Isolates	<i>Serratia</i> sp AA1	<i>Bacillus</i> <i>pumilus</i> AA2	<i>Paenibacillus</i> <i>timonensis</i> AA3	<i>Bacillus subtilis</i> AA4	<i>Bacillus</i> sp AA5	<i>Paenibacillus</i> sp AA6
Sucrose	+	+	-	+	-	+
Galactose	-	+	-	-	-	+
Arabinose	-	+	+	+	-	+
Mannose	-	+	+	+	-	+
Mannitol	+	+	-	+	-	+
Lactose	+	-	+	-	-	+
Maltose	-	+	+	+	-	+
Melibiose	-	-	+	-	-	+
Trehalose	+	+	+	+	-	+
Rhamnose	-	-	-	-	-	-

Antibacterial compound production was more or less similar with 1 and 2% glucose and higher activity at 3% glucose against *K.pneumoniae* (18mm) and *S.aureus* (17 mm) and did not show inhibition activity towards any of the fungal and other bacterial pathogens (Table 3).

Production of antimicrobial compound in yeast extract exhibited maximum activity (18mm) with *K.pneumoniae* at a range of 3% yeast extract and very less was found to be *S.aureus* (11mm). 1 and 2% yeast extracts did not show any activity against other bacterial and pathogens tested (Table 3).

GC-MS analysis of the fractioned ethyl acetate extract of the bacterial isolate AA1 showed the presence of antibacterial compounds phenol, 2, 4-bis (1, 1- dimethyl ethyl), antifungal and antibacterial compound such as nonanoic acid- 9 oxo methyl ester and octadecanoic acid, insecticidal compounds such as chlorpyrifos and antioxidants like heptadecanoic acid. Besides these, other compounds like, dimethyl silanediol, Beta 1, 5 dibenzoylribofuranose, tetradecanol, decanoic acid, 4-dimethyl amino benzoic acid methyl ester, benzene acetic acid, 1-ethoxy, 2,2,2-bicyclo octane and docosanolid were also found to be present (Fig 1).

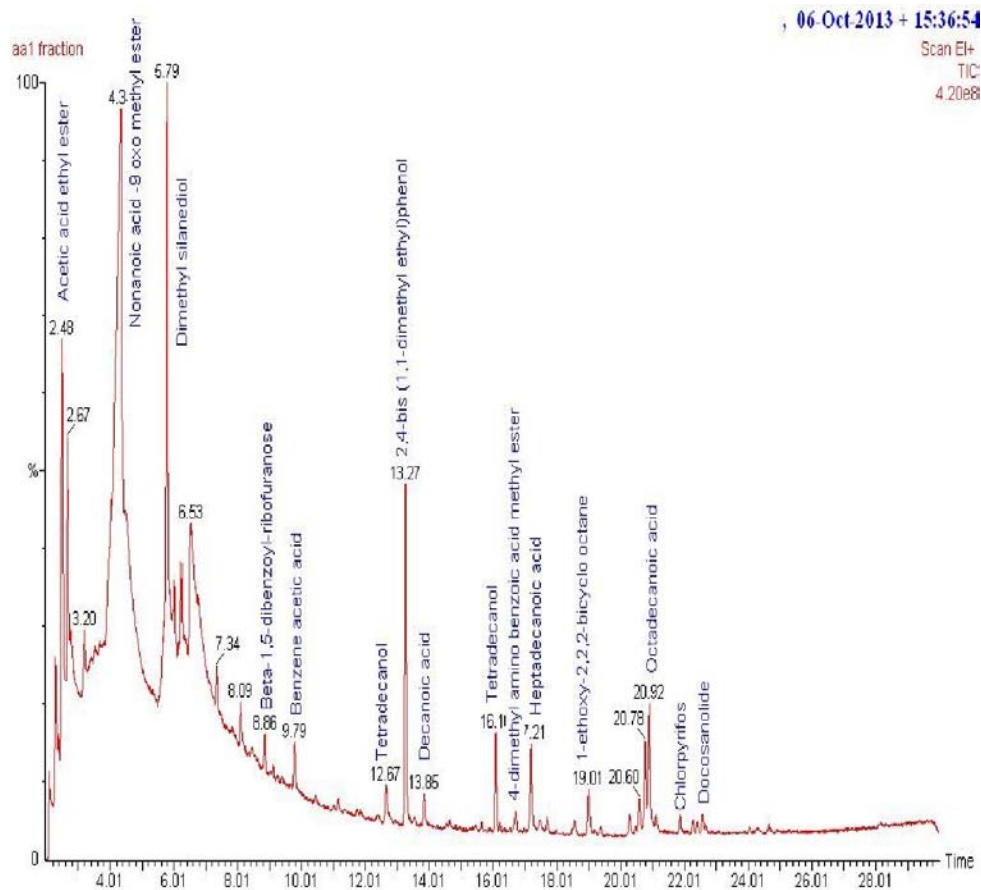


Fig 1. GC-MS spectrum represents the list of compounds present in the ethyl acetate fractioned crude extract of *Serratia* sp AA1.

Table 3: Results of antibacterial and antifungal activities produced by *Serratia* sp **KC149511** against human pathogens

S. No	Strain	Diameter of zone of inhibition (mm)																		
		Minimal media supplemented with						Glucose						Yeast extract			Marine broth pH 7	Luria broth pH 7	Gentamicin 10 mcg	Clotrimazole 10 mcg
		pH		NaCl		Glucose		Yeast extract												
		6	7	8	1%	2%	3%	1%	2%	3%	1%	2%	3%	1%	2%	3%				
1.	<i>S.aureus</i>	10	16	14	14	14	12	14	11	17	11	11	11	18	12	22	-			
2.	<i>S.flexneri</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	-			
3.	<i>S.boydii</i>	-	15	12	-	-	-	-	14	11	-	-	-	15	-	29	-			
4.	<i>S.sonnei</i>	-	-	-	-	-	-	-	-	-	-	-	-	15	-	28	-			
5.	<i>S.dysenteriae</i>	-	14	12	-	10	-	12	-	-	-	-	-	14	-	24	-			
6.	<i>K.pneumoniae</i>	16	15	15	16	15	12	15	15	18	12	13	18	16	13	24	-			
7.	<i>E.coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19	-			
8.	<i>Ep.E.coli</i>	12	13	-	15	17	15	-	12	-	-	-	-	16	12	21	-			
9.	<i>Et.E.coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23	-			
10.	<i>St.E.coli</i>	-	13	13	-	13	-	-	-	-	-	-	-	13	-	26	-			
11.	<i>Y.cholerae Eltor</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26	-			
12.	<i>Y.cholerae</i> 0139	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21	-			
13.	<i>Y.fluvialis</i>	-	13	-	-	13	13	-	12	-	-	-	-	13	-	23	-			
14.	<i>Y.parahaemolyticus</i>	13	15	15	-	13	10	-	-	-	-	-	-	15	-	18	-			
15.	<i>Aeromonas hydrophilla</i>	-	-	-	-	-	-	14	14	11	13	-	-	16	-	21	-			
16.	<i>S.typhi</i>	13	16	-	15	19	13	15	15	12	-	-	-	-	-	18	-			
17.	<i>S.enterica</i> serovar <i>typhi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-			
18.	<i>Rhizopus</i> sp	-	15	13	13	-	-	-	13	13	-	-	-	20	-	-	15			
19.	<i>A.niger</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17			
20.	<i>A.flavus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15			

(-) denotes no activity

Discussion

Marine bacteria are known to play a dynamic role in the production of natural compounds and their association with several marine plants and animals have revealed them as potential source of natural compounds with bioactivity. In the present study six isolates were obtained from seaweed *Amphiroa anceps* among them one pigmented strain AA1 showed antifungal and antibacterial activities. Earlier studies have reported the production of various antibacterial metabolites by marine pigmented bacteria (Lemos *et al.*, 1985). Jayanth *et al* (2002) had also reported the antagonistic nature of pigmented bacteria isolated from various marine samples which inhibited at least one of the pathogens. This study show that pigmented bacteria isolated from coralline algae possess antimicrobial activities. Surface associated marine bacteria are known to exhibit higher proportion of antimicrobial activity than the free living marine bacteria (Burgess *et al.*, 1999). Due to the presence of nutritious surface seaweed epiphytic bacteria produce significant amount of antibiotic metabolites which helps in preventing the fouling of other organisms or colonization of other microbial communities paving a way to explore novel compounds for pharmaceutical industry based applications (Brenner *et al.*, 2005). Marine bacteria are able to release antimicrobial metabolites even under limited nutrient factor and stress (Barry and Wainwright 1997). Recent Study by Ali *et al* (2012) reported on bacteria associated with coralline algae *Jania rubens* are known to produce antibacterial and antifungal compounds against certain pathogens. The present study also proves that the pigmented epiphytic isolate AA1 identified as *Serratia* sp obtained from coralline red algae exhibited both antibacterial and antifungal activity against selected human pathogens. Present investigation also support recent studies on *Serratia* sp isolated from Malaysian marine environment (Jafarzade *et al.*, 2013).

Optimization of pigmented *Serratia* sp have been reported to produce strong antibacterial and antifungal compounds at different pH, glucose and yeast concentration with an optimum pH of 7 (Jafarzade *et al.*, 2013). In the present study

Serratia sp KC149511 isolated from the marine algae *Amphiroa anceps* were also subjected to optimization for the antimicrobial compounds production with a different range of salinity, pH, glucose and yeast as carbon and nitrogen source respectively for the growth. Crude extracts obtained were subjected to antimicrobial assay which showed a broad spectrum of antifungal and antibacterial activities at a pH of 7 with 1 and 2% glucose, 2% yeast extract and 2 % of NaCl along with minimal media at a pH of 7 and also with marine broth, whereas it showed less activity with Luria broth (Table 3). Based on 16SrRNA antimicrobial compounds produced by *Serratia* sp KC149511 showed similarity with *Serratia marcescens* subsp. *sakuensis* (AB061685) and *Serratia nematodiphila* (EU036987) (Fig 2) based on the existing database in the library. *Serratia* sp KC149511 could be a novel as well as a potential candidate for pharmaceutical products.

The GC-MS analysis of crude ethyl acetate extract of isolate *Serratia* sp (AA1) revealed the presence of antimicrobial metabolites such as phenol, 2, 4-bis (1, 1- dimethyl ethyl), nonanoic acid- 9 oxo methyl ester and octadecanoic acid. These metabolites have also been reported from various microorganisms and plants of terrestrial sources. Abdullah *et al* (2011) reported the presence of an antibacterial compounds phenol, 2, 4-bis (1, 1- dimethyl ethyl) from Malaysian mango *Mangifera indica*. The same compound used as antioxidants a reported of Ajayi *et al* (2011). Yogeswari *et al* (2012) found these compound from fungi *Monochaetia kansensis* showing both antimicrobial and antioxidant properties. The other compounds Nonanoic acid- 9 oxo methyl ester and octadecanoic acid were found in the extract of neem leaves showing antifungal and antibacterial properties (Akpuaka *et al.*, 2013).

Studies by (Kanagasabapathy *et al* 2006) found that 20% of epiphytic bacteria were able to produce antibacterial metabolites against pathogenic bacteria. 9.6% of epiphytic bacterial communities released antimicrobial compounds against target pathogens (Wilson *et al* 2010). Seaweed associated epibionts are capable of producing novel antimicrobial compounds which

could be useful for novel drug discovery (Ali *et al.*, 2012; Ravisankar *et al.*, 2013; Zheng *et al.*, 2005). Outcome of the present investigation showed the presence of various antifungal, antibacterial, antioxidant and other compounds produced by the strain *Serratia* sp KC149511. A potential strain for various pharmaceutical and industrial applications. Earlier reports the Prodigiosin have been extracted from marine *Serratia* sp and other bacteria have been found to possess antifungal, antibacterial, antimalarial, immunosuppressive and anticancer activities (D'Alessio *et al.*, 2000; Han *et al.*, 1998; Montaner *et al.*, 2000; Montane *et al.*, 2003; Perez-Tomas *et al.*, 2003). Current observation suggests that the epiphytic bacterial communities of marine macro algae are found to be potential candidates for many antibacterial and antifungal compounds.

Acknowledgement

Authors wish to thank Dr. N. Ramaiah, National Institute of Oceanography, Goa for his help and support for sequencing, Dr. T. Ramamurthy, Deputy Director, National Institute of Cholera and Enteric Diseases, Kolkata for providing the pathogenic bacterial strains for this study and the University Authorities for providing the facilities.

References

1. Abdullah, A.S.H., Mirghani, M.E.S. and Jamal, P., Antibacterial activity of Malaysian mango. Kernel. *African J. Biotech.*, 10(2011): 18739-18748.
2. Ajayi, G.O., Olagunju, J.A., Ademuyiwa, O. and Martins, O.C., Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of *Plumbago zeylanica*, Linn. *J. Med. Plants. Res.*, 5 (2011): 1756-1761.
3. Akpuaka, A., Ekwenchi, M.M., Dasha, D.A. and Dildar, A., Biological Activities of Characterized Isolates of n-Hexane Extract of *Azadirachta Indica* A. Juss (Neem) Leaves. *Nature and Science.*, 11(5) (2013): 141-147.
4. Ali, A.I.B., Bour, M.E., Ktari, L., Bolhuis, H., Ahmed, M., Boudabbous, A. and Stal, L.J., *Jania rubens*-associated bacteria: molecular identification and antimicrobial activity. *J. Appl. Phycol.*, 24 (2012): 525-534.
5. Armstrong, E., Yan, L., Boyd, K.G., Wright, P.C. and Burgees, J.G., The symbiotic role of marine microbes on living surfaces. *Hydrobiologia.*, 461 (2001): 37-40.
6. Barry, K.J. and Wainwright, N.R., Biosynthetic Induction of a Secondary Metabolite by a Marine Bacterium under Nutritional Stress: Potential Role of the Incomplete Oxidation of an Organic Acid. *Biol. Bull.*, 193 (1997): 274-275.
7. Bauer, A.W., Kirby, W.M.M., Sherris, J.S. and Turk, M., Antibiotic susceptibility by standardized single disc method. *American. J. Clinical. Pathol.*, 45(1966): 493-496.
8. Brenner, D.J., Krieg, N.R. and Staley, J.T., *Bergeys manual of Systematic Bacteriology*, In: Brenner, D. Krieg, N.R. and Staley, J.T, (Eds.) (Springer, New York), (2005), 1388.pp
9. Burgess, J.G., Jordan, E.M., Bregu, M., Spragg, A.M. and Boyd, K.G., Microbial antagonism: a neglected avenue of natural products research. *J. Biotech.*, 70 (1999): 27-32.
10. D'Alessio, R., Bargiotti, A., Carlini, O., Colotta, F., Ferrari, M., Gnocchi, P., Isetta, A., Mongelli, N., Motta, P., and Rossi, A., Synthesis and immunosuppressive activity of novel prodigiosin derivatives. *J. Med. Chem.*, 43 (2000): 2557-2565.
11. Gamal, A.A.E., Biological importance of marine algae. *Saudi Pharm. Journal.*, 18(2009): 1-25.
12. Goecke, F., Thiel, V., Wiese, J., Labes, A. and Imhoff, J.F., Algae as an important environment for bacteria phylogenetic relationships among new bacterial species isolated from algae. *Phycologia.*, 52 (1) (2013), 14-24.
13. Han, S.B., Kim, H.M., Kim, Y.H., Lee, C.W., Jang, E.S., Son, K.H., Kim, S.U. and Kim, Y. K. T- cell specific immune suppression by prodigiosin isolated from *Serratia marcescens*. *Int. J. Immuno. Pharmacol.*, 1998, 20 (1998): 1-13.
14. Jafarzade, F., Yahya, N.A., Shayesteh, F., Usup, G. and Ahmad, A., Influence of Culture Conditions and Medium Composition on the Production of Antibacterial Compounds by Marine *Serratia* sp.WPRA3. *J. Microbiol.*, 51 (3) (2013): 373-379.
15. Jafarzade, M., Yahya, N.A., Mohamad, S., Usup, G. and Ahmad, A., Isolation and characterization of pigmented bacteria showing antimicrobial activity from Malaysian marine environment. *Malaysian J. Microbiol.*, 9 (2) (2012): 152-160.
16. Jayanth, K., Jeyasekarav, G. and Shakila, R.J., Isolation of marine bacteria, antagonistic to human pathogen. *Indian J. Mar. Sci.*, 31(1) (2002): 39-44.
17. Kanagasabapathy, M., Sasaki, H., Haldar, S., Yamasaki, S. and Nagata, S., Marine Microorganisms as a Source of Bioactive Agents. *Ann. Microbiol.*, 56(2) (2006): 167-173.
18. Kanagasabapathy, M., Sasaki, H. and Nagata, S., Phylogentic identification of epibiotic bacteria possessing antimicrobial activities isolated from red algal species of Japan. *World J. Microbiol. Biotechnol.*, 24(2008): 2315-2321.
19. Kelecom, A., Secondary metabolites from marine microorganisms. *Ann. Acad. Bras. Cienc.*, 74(1) (2002): 151-170.

20. Lemos, M.L., Torenzo, A.E. and Barja, J.L., Antibiotic activity of epiphytic bacteria isolate from the intertidal seaweeds. *Microb. Ecol.*, 11 (1985): 149–163.
21. Menezes, C.B.A., Santos, R.C.B., Miqueoletto, P.B., Passarini, M.R.Z., Silva, C.H.D., Justo, M.R., Leal, R.R., Garboggini, F.F., Oliveira, V.M., Berlinck, R.G.S. and Sette, L.D., Microbial diversity associated with algae, ascidians and sponges from the north coast of Sao Paulo state. *Brazil. Microbiol. Res.*, 165 (2010): 466-482.
22. Mohandass, C., Rajasabapathy, R., Ravindran, C., Colaco, A., Santos, R.S. and Meena, R.M., Bacterial diversity and their adaptations in the shallow water hydrothermal vent at D. Joao de Castro Seamount (DJCS), Azores, Portugal. *Cah. Biol. Mar.*, 53 (2012): 65-76.
23. Montaner, B., Navarro, S., Pique, M., Vilaseca, M., Martinell, M., Giral, E., Gil, J. and Perez-Tomas, R., Prodigiosin from the supernatant of *Serratia marcescens* induces apoptosis in haematopoietic cancer cell lines. *Bra. J. Pharmacol.*, 131 (2000): 585–593.
24. Montaner, B. and Perez-Tomas, R. The prodigiosins: a new family of anticancer drugs. *Curr. Cancer. Drug. Targets.*, 3 (2003): 57–65.
25. Okami, Y., Marine Microorganisms as a Source of Bioactive Agents. *Microb. Ecol.*, 12 (1986): 65-78.
26. Perez-Tomas, R. and Montaner, B., Effects of the proapoptotic drug prodigiosin on cell cycle-related proteins in Jurkat T cells. *Histo. Histopathol.*, 18 (2003): 379-385.
27. Pridham, T.G. and Gottlieb, D., The utilization of carbon compounds by some Actinomycetales as an aid for species determination. *J. Bacteriol.*, 56 (1948): 107–114.
28. Ravisankar, A., Gnanambal, M.E.K. and Sundaram, L.R., A Newly Isolated *Pseudomonas* sp., Epibiotic on the Seaweed, *Padina tetrastromatica*, off Southeastern Coast of India, Reveals Antibacterial Action. *Appl. Biochem. Biotech.* (2013) DOI: 10.1007/s12010-013-0473-y.
29. Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D G., The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, 24(1997), 4876–4882.
30. Tamura, K., Nei, M. and Kumar, S., Prospects for inferring very large phylogenies by using the neighbor-joining method. *Pro. Nat. Acad. Sci. USA.*, 101(2004): 11030-11035.
31. Tamura, K., Dudley, J., Nei, M. and Kumar, S., MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, 24 (2007): 1596-1599.
32. Wilson, G.S, Raftos, D.A., Corrigan, S.L. and Nair, S.V., Diversity and antimicrobial activities of surface-attached marine bacteria from Sydney Harbour, Australia. *Microbiol. Res.*, 165 (2010): 300-311.
33. Yogeswari, S., Ramalakshmi, S., Neelavathy, R., Muthumary, J., Identification and Comparative Studies of Different Volatile Fractions from *Monochaetia kansensis* by GCMS. *Global. J.Pharm.*, 6 (2) (2012): 65-71.
34. Zheng, L., Han, X., Chen, H., Lin, W. and Yan, X., Marine bacteria associated with marine macroorganisms: the potential antimicrobial resources. *Ann. Microbiol.*, 55 (2) (2005): 119-124.