Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 45, Issue 1, March 2016
pages (11-19)

ISSN 1730-413X
eISSN 1897-3191

Antibiofilm and antifouling activities of extracellular polymeric substances isolated from the bacteria associated with marine gastropod *Turbo* sp.

by

Nadarajan Viju[1,*],
Sathyaneson Satheesh[2],
Stanislaus Mary Josephine Punitha[1]

DOI: **10.1515/ohs-2016-0002**
Category: **Original research paper**
Received: **April 12, 2015**
Accepted: **August 17, 2015**

[1]*Centre for Marine Science and Technology, Manonmanium Sundaranar University, Rajakkamangalam, Kanyakumari, Tamilnadu, India*

[2]*Department of Marine Biology, Faculty of Marine Sciences, King Abdulazuz University, Jeddah, Saudi Arabia*

* Corresponding author: *vijuuu333@gmail.com*

Abstract

The extracellular polymeric substances (EPS) produced by the bacteria associated with the gastropod *Turbo* sp. were isolated and screened for antibacterial activity against biofilm-forming bacteria. EPS of five out of 13 strains showed inhibitory activities in the antibacterial assay. Furthermore, the antibiofilm and antifouling activity of the most active EPS isolated from the strain KT1 was studied using various *in vitro* and *in vivo* bioassays. Results revealed that EPS significantly inhibited the growth and biofilm formation of bacteria. Furthermore, the antifouling coating developed with bacterial EPS considerably reduced the recruitment of fouling organisms on coated surfaces submerged in the seawater. The functional groups present in EPS, characterized by strong activity,were analyzed using FT-IR and the spectrum showed the presence of alcohol, amines, carboxylic acid and esters. The bacterium responsible for the production of bioactive EPS was identified as *Pseudomonas aeruginosa*, using the 16S rRNA gene. Since the findings of this study revealed the antibiofilm and antifouling activities of EPS, further long term field tests and characterization of the bioactive compound of the EPS could lead to the development of eco-friendly antifouling coating.

Key words: biofouling, surface associated bacteria, extracellular polymeric substance, antimicrobial activity, antibiofilm activity, antifouling activity

DE GRUYTER

The *Oceanological and Hydrobiological Studies* is online at oandhs.ocean.ug.edu.pl

Introduction

Extracellular polymeric substances (EPS) are metabolic products that accumulate on the cell surface and are composed of organic substances such as polysaccharides, proteins, lipids and nucleic acids (Frolund et al. 1996). Most of the microbial cells produce EPS for the purpose of protection (Sutherland 1997; Wozniak et al. 2003). However, production of extracellular polymeric substances by bacteria is important (Ferris, Beveridge 1985) and due to their unique physical and chemical properties they are widely used in the food, pharmaceutical, biomedical, bioremediation, waste water treatment and bioleaching fields. Previous investigations showed the antimicrobial activity (Gauthier, Flatau 1976; Shankar et al. 2010), antibiofilm activity (Fletcher, Floodgate 1973; Sayem et al. 2011) and antifouling activity (Rajasree et al. 2012) of the EPS produced by marine bacteria.

Settlement of organisms on the surface of man-made structures submerged in the marine environment commonly known as biofouling, has severe economic impact through increasing the use of manpower for the periodic cleaning and maintenance of the structures (Clare 1998; Armstrong et al. 2000). There are various antifouling methods available to solve this problem, among them antifouling coatings containing organotin and copper compounds were used as biocides to prevent fouling (Evans 2001; Yebra et al. 2004). These chemical antifouling agents were not only toxic to fouling organisms but also toxic to non-target organisms (Fingerman 1998). Due to the negative effect of the toxic biocides, a global ban was imposed by the International Maritime Organization (IMO) on the toxic organotin biocides used for biofouling control (Champ 2000), which necessitated the research focused on environment friendly biocides (LimnaMol et al. 2009).

Since most of thenatural products are biodegradable and nontoxic or less toxic than synthetic compounds toward other organisms, compounds of natural origin are suggested as the best alternative of toxic biocides (Omae 2003; Fusetani 2004). Marine environment is considered as an underexplored source of novel bioactive natural products (Gulder, Moore 2009; Imhoff 2011). Many bioactive compounds have been extracted from various marine animals like ascidians, sponges, soft corals, sea hares, nudibranchs, bryozoans, sea slugs and marine microorganisms (Donia, Hamann 2003; Haefner 2003). Especially, marine bacteria isolated from the surface of marine algae and invertebrates produce a higher percentage of antimicrobial metabolites (Lemos et al. 1986; Burgess et al. 1999; Zhang et al. 2005).Since the previous investigations were mainly focused on the microbes associated with sponges and ascidians, an attempt has been made in this study to investigate the bioactivity of EPS isolated from bacteria associated with a gastropod species. Results obtained in this study will further advance our knowledge on the bacteria associated with marine invertebrates and their application as a potential source for natural antifoulants.

Materials and methods

Collection of the gastropod and isolation of the associated bacteria

The gastropod (*Turbo* sp.) was collected from the Kanyakumari coast (West Coast of India), kept in a sterile container covered with an icebox and brought to the laboratory. The sample was gently washed with sterile seawater (Millipore filtered and autoclaved) in order to remove loosely attached organisms and the remaining bacteria attached to the shell were scraped off and mixed in 1 ml of filter-sterilized seawater. Subsequently, the suspension was serially diluted and plated onto Zobell marine agar (HIMEDIA, India) and incubated at room temperature (24 h) for the development of colonies. The developed colonies were isolated and purified by repeated streaking on Zobell marine agar plates. The isolated colonies were maintained on Zobell marine agar slants at 4°C for further analysis.

Isolation of an extracellular polymeric substance and testing the antimicrobial activity.

A loop full of bacterial culture was inoculated into the flask containing 100 ml Zobell marine broth and incubated for 72 h in a shaker. Following the incubation, the culture broth was centrifuged at 10000 × *g* for 15 minutes at 4°C and the collected supernatant was added with an equal amount of cold absolute ethanol. After one day of incubation at room temperature, the precipitated extracellular polymeric substances (EPS) were dissolved in

double distilled water and mixed with 95% ethanol (1:4 ratio, aqueous solution: ethanol). The resulting precipitate was filtered through a membrane filter and the clear final solution was dialyzed using 8 kDa membrane for 3 days. The resulting EPS fraction was used for antibacterial activity tests. The agar disc diffusion method was used to assess the antimicrobial activity: 50 µl of EPS was loaded on a sterile filter paper disc (6 mm, Himedia, India) and placed onto Mueller-Hinton agar (Himedia) plates, swabbed with biofilm-forming bacteria (*Alteromonas* sp. and *Pseudomonas* sp.) isolated from acrylic panels submerged in coastal waters (Satheesh et al. 2012). At the same time, the control disc was loaded with distilled water and maintained for cross reference. All plates were incubated at 37°C for 48 h and the zone of inhibition around the discs were measured after 24 h of incubation.

Preparation of bacterial cell suspension for laboratory bioassays

The overnight grown culture broth of biofilm-forming bacteria (*Alteromonas* sp. and *Pseudomonas* sp.) was centrifuged at 10000 × *g* (15 min) and the obtained cell pellets were re-suspended in PBS buffer to obtain OD_{540} = 0.2 and kept at 4°C for further studies.

Growth inhibition effect of EPS on biofilm bacteria

The bacterial EPS (50 µl) was collected in a test tube containing 5 ml of Zobell marine broth (ZMA) along with 500 µl of biofilm-forming bacterial (*Alteromonas* sp. and *Pseudomonas* sp.) cell suspension; control tubes were also prepared without the addition of EPS to compare the inhibitory effect. The tubes were incubated for 24 h at 37°C after taking the initial OD value of the culture medium in a spectrophotometer (UV-Vis.119, SYSTRONICS) at 600 nm. Following the incubation, the final OD values of the culture medium were measured at 600 nm and the growth inhibitory effect of EPS was calculated using the formula given below:

$$Growth\ Rate\ (\%) = \frac{Final\ OD\ value\ -\ Initial\ OD\ value}{Initial\ OD\ value} \times 100$$

Antibiofilm effect of EPS – Microtiter plate assay

The bacterial EPS (20 µl) was transferred into a well of a polystyrene microtiter plate (Cat No. 911296) containing 200 µl of the bacterial cell suspension and the reference was prepared by adding 20 µl of saline in place of bacterial EPS. The plate was incubated at 37°C for 24 h. Following the incubation, the content of each well was gently removed by tapping the plates and washed with 0.2 ml of phosphate buffer saline (PBS pH 7.4) to remove bacteria. The biofilm formed by adherent bacteria was fixed with sodium acetate (2%) and stained with crystal violet (0.1% wv^{-1}). The excess stain was rinsed off with deionized water and the plates were kept for drying. Finally, the optical density (OD) of stained bacterial cells attached to the plates was determined with a micro ELISA auto reader at a wavelength of 570 nm.

Antifouling effect of coatings in the natural seawater (Biomass method)

To prepare antifouling coating, the epoxy primer (3%) was added with epoxy resin (1%), hardener (1%) and bacterial EPS (1%). The control coat was prepared by adding saline (1%) in place of EPS. After proper mixing, the antifouling and reference coatings were coated on the fiber plates (18 × 12 cm) and kept in a sterile chamber to dry for a week. The dried plates were firmly fitted on a frame (iron) and submerged into the sea (Colachel coast, the west coast of India) about 2 meters from the mean sea level for a period of 50 days (3/8/2014 to 21/9/2014). After submersion, the plates were retrieved at 10 day intervals and brought to the laboratory. The fouling community recruited on the plates was scraped off and dried overnight in an oven at 60°C. The biomass of fouling on the reference and plates coated with EPS was calculated by using the following formula:

$$Growth\ Rate\ \text{of the fouling community}\ (\%) = \left(\left(\frac{FWFC}{IWFC} \right)^{\frac{1}{N}} - 1 \right) \times 100$$

where, IWFC–the initial weight of the fouling community; FWFC–the final weight of the fouling community; N–the number of measurements taken.

Fourier Transform Infrared Analysis

The analysis of functional groups present in the bioactive EPS was performed by FT-IR (2000, SHIMADZU) analysis. A small quantity of bioactive EPS was placed on the face of a highly polished KBr salt plate, and another KBr plate was positioned on the top to spread the compound in a thin layer.

Identification of marine bacteria associated with the gastropod

The bacterial strain was cultured in marine broth at 37°C, and the total genomic DNA of the strain was extracted using the phenol chloroform method. The 16S rRNA was amplified by polymerase chain reaction (PCR) using primers 16S (5`-AGAGTRTGATCMTYGCTWAC-3`) and 16S (5`-CGYTAMCTTWTTACGRCT-3`). The PCR product was sequenced using the same PCR primers and other internal primers to confirm the sequence. The obtained 16S rRNA sequence of the bacterial strain was analyzed using the Basic Local Alignment Search Tool (BLAST) and the phylogenetic tree was constructed using the 16SrRNA sequence of others obtained from the NCBI Gen Bank.

Statistical analysis

Student's *T*-test (*P* value <0.05 was considered as significant) was applied to analyze the difference between the reference and the treatments in antibiofilm and antifouling assays.

Results

Antibacterial activity of EPS

The EPS isolated from the marine bacteria associated with *Turbo* sp. was screened against biofilm-forming bacteria *Alteromonas* sp. and *Pseudomonas* sp. Five out of 13 strains showed inhibitory activity and the results are presented in Table 1. Among them, the EPS of strain KT1 showed strong antibacterial activity against both *Alteromonas* sp. and *Pseudomonas* sp. and the zone of inhibition were 12 and 13 cm, respectively.

Table 1

Antibacterial activity of EPS isolated from the marine bacteria associated with *Turbo* sp. against biofilm-forming bacteria

S. No.	Biofilm bacteria	EPS* (mm)				
		K1	K2	K7	K8	K9
1	*Alteromonas* sp.	12	9	9	12	10
2	*Pseudomonas* sp.	13	10	9	10	7

* - EPS of surface associated marine bacteria and their zone of inhibition against test bacteria

Growth inhibition effect of EPS on biofilm bacteria

The growth rate of biofilm-forming bacteria on the culture medium treated with bioactive EPS was much smaller than the control medium. The growth rate of *Alteromonas* sp. and *Pseudomonas* sp. in the control medium was 36.4% and 40%, respectively. Whereas, the bioactive EPS-treated medium significantly reduced the growth of *Alteromonas* sp. to 29.2% (Student's *T*-test, t stat=16.5, df=2, *P*<0.001) and *Pseudomonas* sp. to 30.6% (Student's *T*-test, t stat=5.1, df=2, *P*<0.05) (Fig. 1).

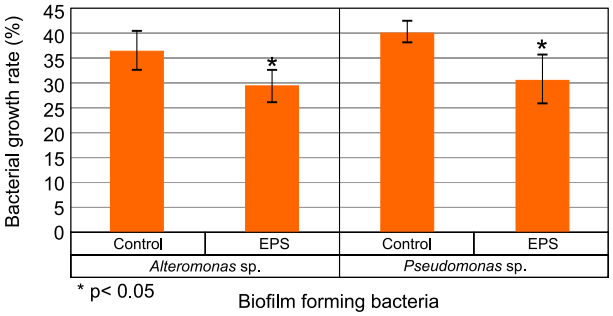

Figure 1

The growth inhibition effect of EPS on the growth of biofilm-forming bacteria *Alteromonas* sp. and *Pseudomonas* sp.

Antibiofilm effect of EPS - Microtitre plate assay

The biofilm formation of the bacterium *Alteromonas* sp. on the microtiter plate control well was 0.350±0.015, and it was significantly reduced, i.e. to 0.286±0.007 (Student's *T*-test, t stat=6.41, df=4, *P*<0.001) in the well treated with bioactive EPS. Similarly, the biofilm formation

of *Pseudomonas* sp. on the control well was 0.305±0.011, but it was significantly reduced to 0.269±0.017 (Student's *T*-test, t stat = 2.9, df = 4, *P*<0.05) in the well treated with bioactive EPS (Fig. 2).

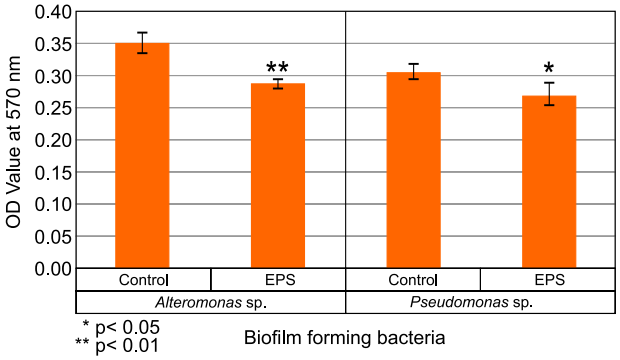

Figure 2

The antibiofilm effect of EPS against biofilm-forming bacteria using the microtiter plate assay

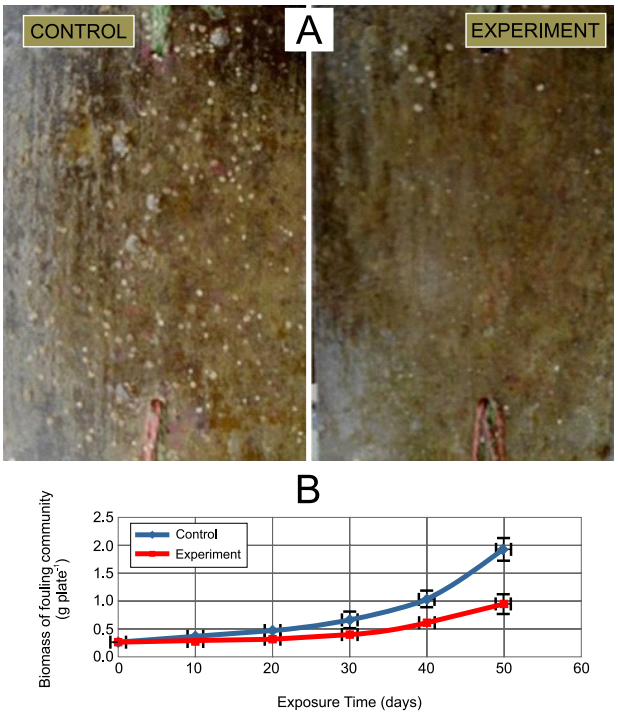

Figure 3

The antifouling effect of antifouling coating developed by incorporating the bacterial EPS into epoxy primer along with epoxy resin and hardener. (A) Recruitment of marine foulers on the reference and plates coated with bacterial EPS submerged in the marine environment for a period of 50 days. (B) The plot shows the inhibiting effect of antifouling coatings.

Antifouling effect of coating in the natural seawater (Biomass method)

The test panel coated with antifouling coating significantly (Student's *T*-test, t stat=2.2, df=5, *P*<0.05) reduced the recruitment of the fouling community. The recruitment of the fouling community on the control panel was 40.4% and 24.51% on the panel coated with antifouling coating prepared by incorporating the bacterial EPS (Fig. 3).

Fourier transform infrared analysis

The FT-IR spectrum of EPS showed the presence of alcohol, alkanes, alkynes, amines, carboxylic acid and ethers. The broad O-H stretch between 3400-3300 and the C-O-H bending between 1550-1220 and the C-O stretch between 1260-1000 cm^{-1} indicate the presence of alcohol. Similarly, the C-H stretch between 3000-2840, the CH_2 bending mode near 1450 and the CH_3 bending absorption near 1375 cm^{-1} indicate the presence of alkanes. Likewise, the C-H stretching frequency near 3300 and the C-C stretch near 2150 cm^{-1} indicate the presence of alkynes. Furthermore, the N-H stretch between 3500-3300, the N-H bending between 1640-1560 and the C-N stretch 1350-1000 cm^{-1} indicate the presence of an amine. Moreover, the O-H broad peak between 3400-2400, the C=O stretch at 1730-1700 and the C-O stretch between 1320-1200 cm^{-1} indicates the carboxylic acid group. The C=O stretch at 1300-1000 and the C-O-C asymmetric stretch near 1250 and the symmetric stretch near 1040 cm^{-1} revealed the presence of ethers (Fig. 4).

Identification and phylogenetic analysis of bacteria

A total of 1,158 consensus nucleotides representing the 16S rRNA genes were obtained from the amplified DNA fragments of the good active strain KT1, and the phylogenetic tree constructed showed 99% similarity with *Pseudomonas aeruginosa* (available sequences in the NCBI). The nucleotide sequence data have been deposited at GenBank (Fig. 5).

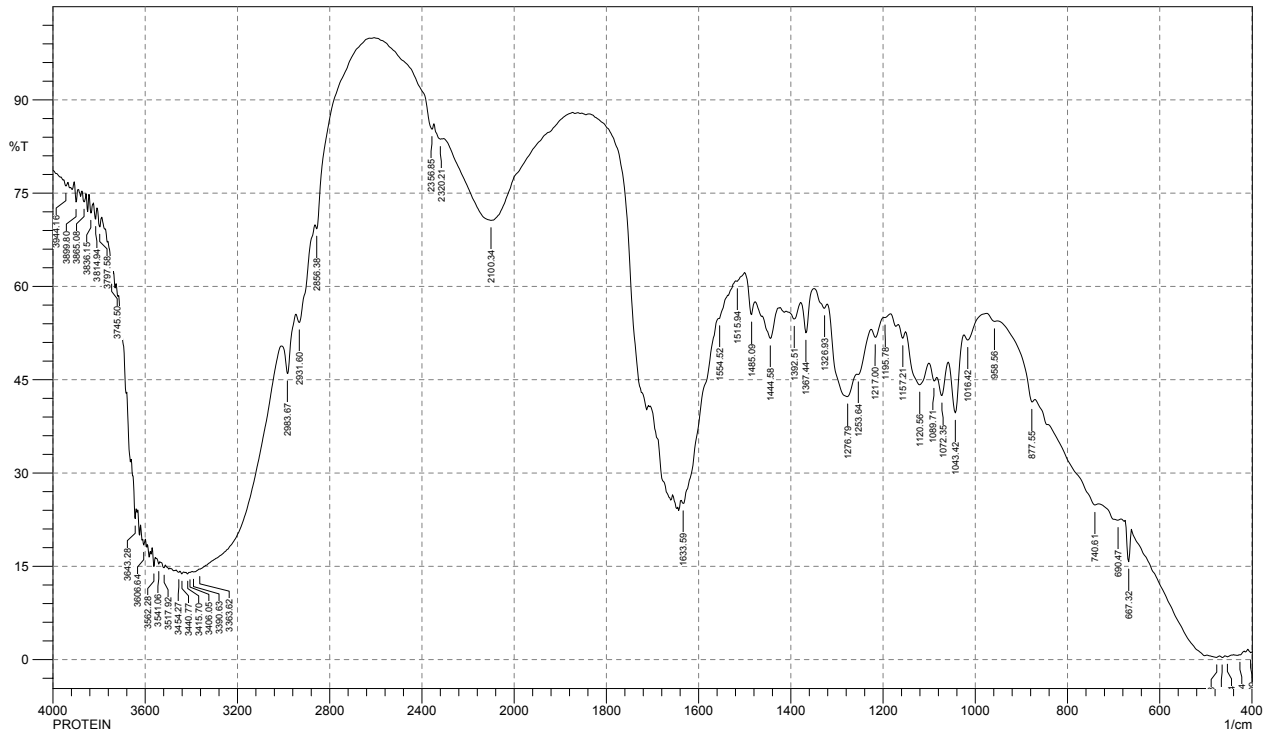

Figure 4

FT-IR spectrum of bioactive EPS isolated from the bacterial strain KT1

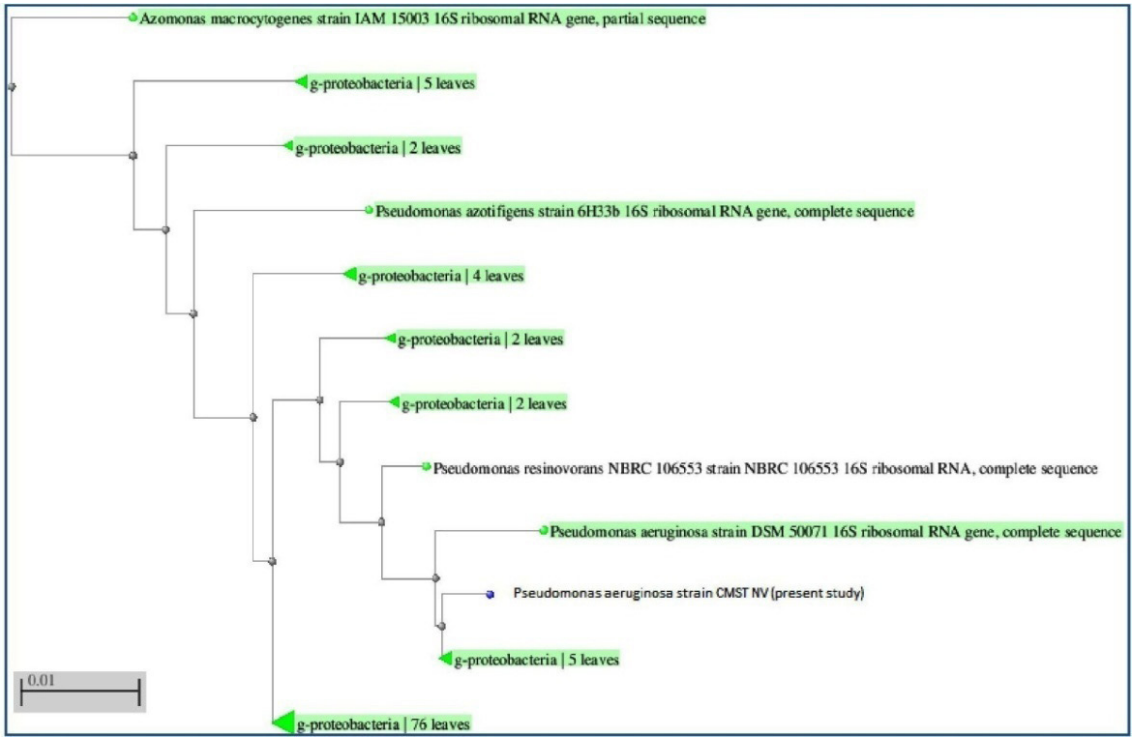

Figure 5

Identification of the strain using the 16S rRNA gene sequencing. The 16S rRNA was aligned and used to construct the neighbor-joining phylogenetic tree and it was identified as *Pseudomonas aeruginosa*.

Discussion

Production of bioactive metabolites by marine bacteria is a unique biological property (Fenical 1993). It has also been reported that bacteria associated with the surface of marine organisms are the higher antimicrobial and antifouling compound producers (Isnansetyo, Kamei 2003; Uzairet al. 2006; Satheeshet al. 2012). Moreover, bacteria can produce bioactive compounds including EPSs which have fascinating industrial applications (Querellou 2003). Some previous studies reported the antibacterial and antifouling activities of EPS isolated from marine bacteria (Balamurugan, Prakash 2012; Rajasreeet al. 2014). In this study, a total of 13 strains were isolated from the surface of *Turbo* sp., in which the EPS of 5 strains inhibited the growth of biofilm bacteria. Similarly, the previous study by Punitha et al. (2014) reported the antibacterial activity of EPS isolated from the bacteria associated with the gastropod. Moreover, the bioactive EPS significantly inhibited the growth rate of biofilm-forming bacteria in the spectrophotometric growth inhibition assay. This method is also used to assess the antibacterial activity of bioactive compounds (Satheeshet al. 2012). Similarly, Viju et al. (2013) assessed the antibacterial activity of the coconut husk extract using the spectrophotometric growth inhibition assay.

At the beginning of adhesion, bacteria colonize the surface and build up a biofilm (Kirchman et al. 1982) which leads to biofouling. Biofilm formation is a multistep process, including bacterial adhesion as the first step. Since bacterial adhesion is the beginning of biofilm/biofouling formation, there are many methods for screening of antibiofilm/antifouling compounds (Deighton et al. 2001; Arciola et al. 2002; Harraghy et al. 2006). In this study, the antibiofilm activity of EPS was assessed by the microtiter plate (directed) method modified after Ghaima et al. (2013). The results revealed that the EPS significantly reduced the biofilm formation of *Alteromonas* sp. and *Pseudomonas* sp. on the microtiter plate wells. The anti-adhesion and antibiofilm activity of exopolysaccharides isolated from the bacteria have already been documented (Velle et al. 2006; Rajasree et al. 2012; Karwacki et al. 2013).

The development of antifouling coatings by incorporating natural product as biocides into the binder has been done by many researchers (Armstrong et al. 2000; Peppiatt et al. 2000). For example, Satheesh et al. (2012) incorporated the sponge associated bacterial extract into a paint and studied the activity against microalgal settlement. In this study, we prepared in the same way the antifouling coat by incorporating the bacterial EPS into epoxy primer along with epoxy resin and amine hardener. The results revealed that the fiber plates coated with the antifouling coating considerably reduced the biofouling for a period of 50 days. Similarly, Bazes et al. (2009) incorporated the dichloromethane extract of the seaweed *Sargassum muticum* into the paint containing copper and that coating was more active against the marine biofoulers than the paint containing only copper over a period of 60 days (2 months) of submersion.

EPS is a complex mixture consisting of polysaccharides, proteins, nucleic acids, lipids and humic substances. Hence, it's necessary to isolate and characterize the bioactive molecule present in the crude EPS. But in the present study, we have only analyzed the functional groups present in the EPS using FT-IR. Based on the obtained FT-IR spectrum, the bioactive EPS may contain six functional groups such as alcohol, alkanes, alkynes, amines, carboxylic acid and ethers. Likewise, Viju et al. (2014) reported the presence of alcohol, alkenes, carboxylic acid, esters and amines in the bioactive EPS produced by the surface associated bacterium *Pseudomonas taiwanensis*. The strain (KT1) responsible for the production of bioactive EPS was characterized by 16S rRNA analysis and identified as *Pseudomonas aeruginosa* with 99% homology. Previous reports are available about the bioactivity of *Pseudomonas* sp. (Thakur et al. 2001; Kodani et al. 2002). For example, Jayatilake et al. (1996) isolated an antibacterial compound cyclo- L-proline-L-methionine from the marine bacterium *Pseudomonas aeruginosa*, obtained from the Antarctic sponge *Isodictya setifera*.

In conclusion, the present study presents the antifouling potential of marine bacteria associated with the surface of gastropod *Turbo* sp. It has already been reported that bacteria associated with marine organisms produce bioactive compounds having antibacterial/antifouling compounds. Moreover, the bacterium *Pseudomonas aeruginosa* responsible for bioactivity belongs to an important group of bacteria among the bioactive compound producers. Some antimicrobial and antifouling compounds have already been isolated from *Pseudomonas* sp. Therefore, further antifouling assays, long-term field tests and characterization of

the bioactive compound present in the EPS would be useful for the development of a novel ecofriendly antifouling agent from the bacterium *Pseudomonas aeruginosa*.

Acknowledgement

The authors are grateful to UGC for providing the financial support through the major project scheme (Ref. F. No. 39-294/2010 SR).

Reference

Arciola, C.R., Campoccia, D., Gamberini, S., Cervellati, M., Donati, E. et al. (2002). Detection of slime production by means of an optimised Congo red agar plate test based on a colourimetric scale in *Staphylococcus epidermidis* clinical isolates genotyped for ica locus. *Biomaterials.* 23: 4233-4239.

Armstrong, E., Boyd, K.G. & Burgess, J.G. (2000). Prevention of marine biofouling using natural compounds from marine organisms. *Biotechnol. Ann. Rev.* 6: 221-241.

Balamurugan, G. & Prakash, S. (2012). Extraction, partial characterization and antibacterial efficacy of extra cellular polysaccharides from *Bacillus Licheniformis* and *Klebsiella Pneumoniae* isolated from root nodule of *Tephrosia Purpurea*. *Int. J. Pharma. Bio. Sci.* 3(3): 306-316.

Bazes, A., Alla, A., Philippe, D., Fabienne, F., Nelly, K. et al. (2009). Investigation of the antifouling constituents from the brown alga *Sargassum muticum* (Yendo) Fensholt. *J. Appl. Phycol.* 21: 395-403. DOI: 10.1007/s10811-9382-9.

Burgess, J.G., Jordan, E.M., Bregu, M., Mearns-Spragg, A. & Boyd, K.G. (1999). Microbial antagonism: a neglected avenue of natural products research. *J. Biotechnol.* 70: 27-32.

Champ, M.A. (2000). A review of organotin regulatory strategies, pending actions, related costs and benefits. *Sci. Total Envir.* 258: 21-71. DOI: 10.1016/30048-9697(00)00506-4.

Clare, A.S. (1998). Towards non-toxic antifouling. *J. Mar. Biotechnol.* 6: 3-6.

Deighton, M.A. Capstick, J. Domalewski, E. & Nguyen, T. (2001). Methods for studying biofilms produced by *Staphylococcus epidermidis*. *Methods Enzymol.* 336: 177-195.

Donia, M. & Hamann, M.T. (2003). Marine natural products and their potential applications as anti-infective agents. *Lancet. Infect. Dis.* 3: 338-348.

Evans, S.M. (2001). Anti-fouling materials. Newcastle University, Tyne and Wear, London, pp. 170-176.

Fenical, W. (1993). Chemical studies of bacteria: developing a new resource. *Chem. Rev.* 93: 1673-1683. DOI: 10.1021/cr00021a001.

Ferris, G.G. & Beveridge, T.J. (1985). Functions of bacterial cell surface structures. *Bio. Science.* 35: 172-177.

Fingerman, S.W. (1988). Environmental pollution by heavy metals from antifouling compounds. In: M.F. Thompson, R. Sarojini, R. Nagabhushanam (Eds.). *Marine Biodeterioration: Advanced techniques applicable to the Indian Ocean*. Oxford & IBH. New Delhi 781-789.

Fletcher, M. & Floodgate, G.D. (1973) An electron microscope demonstration of acidic polysaccharide involved in adhesion of a marine bacterium to solid surfaces. *J. Gen. Microbiol.* 74: 325-334.

Frolund, B., Palmgren, R. & Keiding, K. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water. Res.* 30(8): 1749-1758.

Fusetani, N. (2004). Biofouling and antifouling. *Nat. Prod. Rep.* 21: 94-104. DOI: 10.1039/b302231p.

Gauthier, M.J. & Flatau, G.N. (1976) Antibacterial activity of marine violet – pigmented *Alteromonas* with special reference to the production of brominated compounds. *Canad. J. Microbiol.* 22: 1612-1619.

Ghaima, K.K., Rasheed, S.F. & Ahmed, E.F. (2013). Antibiofilm, antibacterial and antioxidant activities of water extract of *Calendula officinalis* flowers. *Int. J. Biolo. Pharma. Res.* 4(7): 465-470.

Gulder, T.A.M. & Moore, B.S. (2009).Chasing the treasures of the sea – bacterial marine natural products. *Curr. Opin. Microbiol.* 12: 252-260. DOI: 10.1016/j.rnib.2009.05.002.

Haefner, B. (2003). Drugs from the deep: marine natural products as drug candidates. *Drug. Discov. Today.* 8: 536-544.

Harraghy, N., Seiler, S., Jacobs, K., Hannig, M., Menger, M.D. & Herrmann, M. (2006). Advances in in vitro and in vivo models for studying the staphylococcal factors involved in implant infections. *Int. J. Artific. Org.* 29: 368-378.

Imhoff, J.F., Labes, A. & Wiese, J. (2011). Bio-mining the microbial treasures of the ocean: New natural products. *Biotechnol. Adv.* 29: 468-82. DOI: 10.1016/j.biotechadv.2011.03.001.

Isnansetyo, A. & Kamei, Y. (2003). *Pseudoalteromonas phenolica* sp. a novel marine bacterium that produces phenolic anti-methicillinresistant substances against *Staphylococcus aureus*. *Int. J. Syst. Evol. Microbiol.* 53: 583-588.

Jayatilake, G.S., Thornton, M.P., Leonard, A.C., Grimusade,

J.E. & Baker, B. J. (1996). Metabolites from an antartic sponge associted bacterium *Pseudomonas aeruginosa*. *J. Nat. Prod.* 59: 292-296.

Karwacki, M.T., Kadouri, D.E., Bendaoud, M., Izano, E.A., Sampathkumar, V. et al. (2013). Antibiofilm Activity of Actinobacillus pleuropneumoniae Serotype 5 Capsular Polysaccharide. *PLoS ONE.* 8(5): e63844. DOI: 10.1371/journal.pone.0063844.

Kirchman, D.L., Ducklow, H.W. & Mitchell, R. (1982). Estimates of bacterial growth from changes in uptake rates and biomass. *Appl. Environ. Microbiol.* 44: 1296-1307.

Kodani, S., Imoto, A., Mitsutani, A. & Murakami, M. (2002). Isolation and identification of the anti algal compound, harmane (1-methyl-b-carboline), produced by the algicidal bacterium, *Pseudomonas* sp. K44–1, *J. Appl. Phycol.* 14: 109-114.

Lemos, M.L., Toranzo, A.E. & Barja, L.J. (1986). Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. *Microbial. Ecolo.* 11: 149-163.

Limna Mol, V.P., Raveendran, T.V.& Parameswaran, P. S. (2009). Antifouling activity exhibited by secondary metabolites of the marine sponge, *Haliclona exigua* (Kirkpatrick). *Int. Biodeter. Biodegr.* 63: 67-72. DOI: 10.1016/j.ibiod.2008.07.001.

Omae, I. (2003). General aspects of tin-free antifouling paints. *Chem. Rev.* 103: 3431- 3448. DOI:10.1002/aoc396.

Peppiatt, C.J., Armstrong, E., Pisacane, T. & Burguess, J.G. (2000). Antibacterial activity of resin based coatings containing marine microbial extracts. *Biofouling.* 16: 225-234. DOI: 10.1080/08927010009378447.

Punitha, S.M.J., Viju, N., Sharmin Vini, S., Sunjay Shanker, C.V., Michael Babu, M. et al. (2014) Antibacterial activity of bacteria associated with of gastropod. *Int. J. Pure. Appl. Microbiol.* 7(4): 2879-2884.

Querellou, J. (2003). Biotechnology of marine extremopiles. *J. Thromb. Haemost.* 1: 12- 18.

Rajasree, V., Satheesh, S. & Prakash Vincent, S.G. (2012). Antifouling activity of a marine epibiotic bacterium from the seaweed *sargassum wightii. Thalassas.* 28(2): 37-43.

Rajasree, V., Sunjaiy Shankar, C.V., Satheesh, S. & Punitha, S.M.J. (2014). Biofilm inhibitory activity of extracellular polymeric substance produced by *Exiguobacterium* sp. associated with the polychaete *Platynereis dumerilii. Thalassas.* 30(2): 13-19.

Satheesh, S., Soniamby, A.R., Sunjaiy Shankar, C.V. & Punitha, S.M.J. (2012). Antifouling Activities of Marine Bacteria Associated with Sponge (*Sigmadocia* sp.). *J. Ocean. Univ. China.* 11: 354-360. DOI: 10.1007/s11802-012-1927-5.

Sayem, S., Manzo, M. E., Ciavatta, L., Tramice, A., Cordone, A., De Felice, A. Z. M. & Varcamonti, M. (2011) Antibiofilm activity of an exopolysaccharide from a spongeassociated strain of *Bacillus licheniformis*. *Microb. Cell. Fact.* 10: 74. DOI: 10.1186/1475-2859-10-74.

Sunjaiy Shankar, C.V., Jeba Malar, A.H. & Mary Josephine Punitha, S. (2010). Antimicrobial activity of marine bacteria associated with Polychaetes. *Biores. Bull.* 1: 24-28.

Sutherland, I.W. (1997). Microbial exopolysaccharides-structural subtleties and their consequences. *Pure. Appl. Chem.* 69: 1911-1917.

Thakur, R.P., Rai, K.N., Rao, V.P. & Rao, A.S. (2001). Genetic resistance in pearl millet male-sterile lines to diverse pathotypes of *Sclerospora graminicola*. *Plant. Dis.* 85: 621-626.

Uzair, B., Ahmed, N., Ahmed, V. & Kousar, F. (2006). A new antibacterial compound produced by indigenous marine bacteria; fermentation, isolation and biological activity. *Nat. Proc. Res.* 20: 1326-133.

Valle, J., Da Re, S., Henry, N., Fontaine, T., Balestrino, D. et al. (2006). Broadspectrum biofilm inhibition by a secreted bacterial polysaccharide. *Proc. Natl. Acad. Sci. USA*. 103: 12558-12563. DOI: 10.1073/PNAS.0605399103.

Viju, N., Anitha, A., Sharmin Vini, S., Sunjaiy Shankar, C.V., Satheesh, S. et al. (2014). Antibiofilm activities of extracellular polymeric substances produced by bacterial symbionts of seaweeds. *Ind. J. Mar. Sci.* 43(11): 2136-2146.

Viju, N., Satheesh, S. & Vincent Prakash, S.G. (2013). Antibiofilm activity of coconut (*Cocos nucifera* Linn.) husk fibre extract. *Saudi. J. Biol. Sci.* 20: 85-91. DOI: 10.1016/j.sjbs.2012.11.002.

Wozniak, D.J., Wyckoff, T.J.O., Starkey, M., Keyser, R., Azadi, P. et al. (2003). Alginate is not a significant component of the extracellular polysaccharide matrix of PA14 and PAO1 *Pseudomonas aeruginosa* biofilms. *Proc. Natl. Acad. Sci. USA*. 100: 7907.

Yebra, D.M., Kiil, S. & Dam-Johansen, K. (2004). Review. Antifouling technology-past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Prog. Org. Coat.* 50: 75-104. DOI: 10.1016/j.porgcoat.2003.06.001.

Zhang, J., Shen, Y. Liu, J. & Wei. D. (2005). Antimetastatic effect of prodigiosin through inhibition of tumor invasion. *Biochem. Pharmacol.* 69 (3): 407-414. DOI: 10.1016/j.bcp.2004.08.037.