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Evaluation of Antifouling Potential of Staphylococcus Sp. Isolated from Marine Sea Water

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ABSTRACT

Background: Marine biofouling indicates the accumulation of marine microorganisms, algae and other organisms on the immersed surfaces of boats and ships. Biofouling causes severe problems to marine industries and shipping in worldwide which leads to huge economic loss and technical problems. To reduce these problems some synthetic organic compounds are used as antifouling coatings. But these antifoulants are found to be toxic and lethal to marine life forms and could pollute the marine environment.

Objective: Based on these perseptives it is necessary to identify an ecofriendly novel antifoulant from marine microbes.

Methods: To investigate the antifouling activity, the bacterial strains were isolated from the Vizhinjam harbor and the isolated strains were subjected to screening of antifouling activity using cross streak, cover slip and microtiter assays. The extracellular protein of bacterial strain which showed remarkable antifouling activity was purified using TLC and column chromatography and the compounds were identified using LC-MS.

Result: The antifouling activity was found to be higher in A7, *Staphylococcus* sp. among the selected strains. The bioactive compounds present in the strains were Phytone synthase, 1-3–diphosphoglycerate, Benzophenone 2-3 cyclooctane and 2, 2 diphenyl-1- picryldrazyl. The current study revealed that the A7, *Staphylococcus* sp. could produce potent antifoulant which can be used to control biofouling in marine environment.

Novelty: The present findings indicate that marine microorganisms could provide a promising source for antifouling compounds. With the improvement of cultivation and isolation methods novel antifoulants may be obtained which will provide more candidates for the marine coating industries.

Keywords: Antiouling, Benzophenone 2-3 cyclooctane, LC-MS, *Staphylococcus* sp.

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1. INTRODUCTION

Marine biofouling is a complex assemblage of micro and macro organisms on artificial structures comprising microcolonies that are attached to a surface and shielded themselves in an extracellular matrix of polysaccharide, protein and nucleic acids [1-2]. Marine biofouling is a serious worldwide problem affecting marine industries and manmade structure exposed to marine environment such as ships, pipelines, oil platforms, fish cages and fishing nets[3] increases the hydrodynamic drag and decreases the maneuverability in naval and merchant ships and cause more than $200 billion loss/annum.Inorder to overcome the biofouling problems antifouling paints for marine structures have been developed. Antifouling paints based on tributyltin and otherorganotin compounds as the active agents pose a seriousthreat to the marine environment[4]. Tributyltin hasbeen found to be harmful and toxic to non-target organisms and is not easily degraded in the environment[5].Consequently, because of the above-mentioned problems, International Maritime Organization banned theuse of tributyltin since September 2008[6].Many substitutes for TBT was introduced such as Irgarol1051 and Diuron, have been found to be harmful to many nontarget organisms. One of the organotin compound, Tributyltin oxide (TBTO) is still used as a biocide and has been used as reference or positive control for the evaluation of antifouling activity of new natural compounds. The other common biocides are 10-β-formamidokalihinol-A and kalihinol A, polymeric 3-alkylpyridinium salts, terpens and pyrrole-imidazole alkaloids, succinic acid, taurine acid substituted bromopyrrole alkaloids and dibromophakellin derivatives, 3-phenyl-2-propenoic acid, 2- hydroxymyristic acid and cis-9-oleic acid [7].

Inorder to overcome these drawbacks researchers now focused on natural antifouling compounds from the marine organisms. Diketopiperazines were identified from a deep-sea bacterium, *Streptomyces fungicidicus*, showed promising antifouling activity]Formamidokalihinol-A and kalihinol A, which are isolated and purified from the marine sponge *Acanthella cavernosa*, inhibit bacterial growth, suppress larval settlement, and exhibit antifouling properties.[7]In this context an attempt has been made to identify the potent antifoulant from marine microbes.

2. MATERIALS AND METHODS

2.1 Sample collection

The marine water samples were collected from boat harbouring area from VizhinjamHarbour, Thiruvananthapuram and brought to the laboratory in sterile polythene bags for further investigation.

2.2 Isolation of marine bacteria

The collected sample was subjected to vigorous vortexing for 5 min and serially diluted using sterilized seawater. A volume of 100 µl of the diluents was spread on sterile Zobell's Marine

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Agar 2216 (Himedia, Mumbai) and incubated at 37°C for 24-48 h. After incubation, the selected bacterial colonies were isolated and subcultured in Zobell Marine agar medium for further analysis [8].

2.3 Morphological and Biochemical characterization of bacteria

The isolated colonies were purified by repeated streaking on Zobell marine agar plates and it was maintained on Zobell marine agar slants at 4^0C for further test. The isolated colonies were identified through morphological and biochemical tests based on the Bergey's manual of Determinative Bacteriology, Ninth edition (2000) [9].

2.4 Screening of biofouling activity by coverslip method

All the bacterial isolates were tested for adherence property by inoculating into sterile nutrient broth containing glass cover slips in the test tubes. After 24 hours, the cover slips were removed and stained with 0.4% crystal violet to check the adherence of bacteria. Bacterial isolates which form a slimy layer on the cover slips were selected for further characterization [10].

2.4.1 Screening of biofouling activity by microtiter plate assay

The isolated bacterial aliquots (3µl) were inoculated in 96 well microtiter plates. Then the plate was incubated at 37^0 C for 72 hours. After the incubation the wells were rinsed with physiological saline to remove the detached bacterial cells and fixed with 2µl of 99.99% ethanol for 10 min. The attached bacterial strain was then stained by adding 2µl of crystal violet (2%) for 20 min. The plates were rinsed with tap water and amounts of attached cells were observed [11-12].

2.4.2 Screening of antifouling activity of A2 and A7 strain

The antifouling activity of antifouling strains were preliminarily screened by cross streaking method[13-14]. A single line streak of bacterial strains were made on the surface of the modified nutrient agar medium separately and incubated overnight. After observing ribbon like growth on the surface of the plates the biofouling bacterial strains were streaked at right angles to the original streak and incubated at 37°C for 24-48 h. Based on the presence and absence of inhibition zone, the antifoulant producing bacterial strains were selected.

2.5. Extraction of Extracellular polymeric substances (EPS)

For the extraction of EPS, 2 ml bacterial aliquot was added to sterile centrifuge tubes. The contents were homogenized for about 30 seconds and spun at 3500 x g for 5 minutes at 4°C. The supernatants were transferred to sterile centrifuge tubes and further spun at 9000 x g for 30 minutes at 4°C. Pellets were resuspended in 2 ml distilled water, freeze dried and dissolved in Phosphate and Tris-Maleate buffers. Dissolved pellets were filtered through 0.8/0.2 µm filters. Filtrates were assayed for EPS composition [15].

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2.5.1 Estimation of the carbohydrate concentration in the EPS

The carbohydrate concentration was determined according to Anthrone method. According to this method pellets were dissolved in 1 ml of phosphate and Tris-Maleate buffers. Freshly prepared Anthrone solution (1ml) was added in each test tube. The mixture was incubated in a water bath at 95^0 C for 15 min. After incubation, the mixture was allowed to cool to room temperature. Cooled aliquots (200µl) were transferred to microtitreplate wells and read at 620 nm using a plate reader [16].

2.5.2 Estimation of the protein concentration in the EPS

Protein concentration was determined by the modified method of Lowry's method. 10µl of extracellular polymeric substances (EPS) were added into wells of a microtiter plate. Control wells were added with phosphate buffer and 3µlof Coomassie reagent. The plate was incubated at room temperature for 10min. After incubation, absorbance was read at 595 nm using a plate reader[17].

2.6 Extraction of antifouling compounds

The antifoulant bacterial strain was grown at 28°C at150 rev/min for 5 days in Zobell marine broth. The culture was centrifuged at 8,000 rev/min at 4°C for 20 minutes. The supernatant was thoroughly extracted three times with an equal volume of chloroform. All the extracts were pooled, and the solvent was removed at low pressure at 45°C until the extract was completely dry[18].The extracted compounds were subjected to further analysis.

2.6.1 Purification and identification of antifouling compounds

The extracted compounds were purified using thin layer and column chromatography over silica gel and eluted with an ethyl acetate/methanol combination with increasing polarity (0-100%). Fractions were collected separately in different test tubes and numbered consecutively. The collected eluents were subjected to the identification of antifoulants[19].

2.6.2 LC- MS analysis

LC-MS analysis was carried out on a Restekstabilwax instrument. The column used was DB 35-MS Capillary standard non-polar column measuring 30 m × 0.25 mm with a film thickness of 0.25 µm composed of 50% aqueous acetonitrile. The carrier gas used was Helium at a flow rate of 35 cm/sec. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 100°C for 1 min, then an increase to 250°C at 10 ^{o} C/min and hold for 4.00 min. Total run time was 20 min. LC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the LC-MS library. Measurement

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of peak areas and data processing were carried out by NIST Quantitative Infrared Database search[20].

3. RESULTS

3.1 Isolation, Characterization and Identification of bacterial strains

The present study evaluates the isolation and identification of antifoulant from marine microbes collected from marine water samples, VizhinjamHarbour, Thiruvananthapuram, using Zobell Marine agar. Totally seven bacterial strains were isolated and named as A1, A2, A3, A4, A5, A6 and A7. The isolated strains were characterized morphologically and biochemically based on the Bergey's manual of Determinative Bacteriology, Ninth edition (2000) and identified as *Pseudomonas sp, Mycobacterium sp, Staphylococcus sp, Shigella sp, Neisseria sp*and *Lactobacillussp*.

3.2 Screening of Biofouling activity using assays

The isolated bacteriaial strains were subjected to preliminary screening to check the biofouling efficacy. During preliminary screening the bacterial strains A2 and A7 showed absence of adherence in the coverslip and microtitre plate and produce zone of inhibition and hence, A2 and A7 are considered as antifoulants. The bacterial strains A1, A3, A4, A5 and A6 showed attachment in the surface of coverslip, microtitre and fails to produce zone of inhibition and are considered as biofouling strains. Among the selected strains, A2 and A7 showed remarkable antifouling activity**.** Hence the A2 and A7 strains were subjected to secondary screening such as cross streak using biofouling strains A4 and A6.

3.3 Estimation of carbohydrate and protein concentration

The A2 and A7 strains were subjected to protein and carbohydrate estimation and the result showed that the A7 strain possess 0.45 µg/ml of protein and 0.43µg/ml of carbohydrate and A2 strain possess 0.29 µg/ml of protein and 0.35 µg/ml of carbohydrate respectively (Table 1).

3.4 Identification of Antifouling compound by LC- MS Analysis

The antifoulant compounds present in the A7 strain was detected using LC-MS analysis. Totally four compounds were identified which include Phytone synthase, 1-3 – diphosphoglycerate, Benzophenone 2-3 cyclooctane and 2,2 diphenyl-1- picryldrazyl and the most predominant one is Benzophenone 2-3cyclo octane (Table 2 and Fig 1). The result showed that the isolated compounds are responsible for antifouling activity which can be used as an antifouling agent in marine sector.

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4. DISCUSSION

Marine derived secondary metabolites may constitute one of the most promising alternatives to the toxic and harmful synthetic antifoulants. Previous reports suggested that the synthetic antifoulants may hazardous to marine life and cause lot of environmental pollutions. So there is a need for the extensive research for the continuous exploration of marine organisms for novel antifoulants is worthy due to the growing technological or economical concerns associated with fouling organisms. So the present study mainly focuses on isolation and identification of potential antifouling metabolites effective against marine biofouling organisms. Specifically this study reports the isolation, screening and identification of potent antifouling compound Benzophenone 2-3 cyclooctane from *Staphylococcus* sp. isolated from water samples. In this present study seven bacterial strains were isolated and among that A2 and A7 strains showed remarkable antifouling activity.

Diana *et al*., in the year 2016 isolated eight biofouling isolates showed the ability to form biofilm from the boat surfaces in Segara Ayu Beach, Indonesia[21]. Viju *et al*., in 2014 isolated 9 bacterial strain from the sea weed and screened for their antibacterial activity against three biofilm forming bacteria and all the 9 strains were inhibited the growth of biofilm forming bacteria and the best potent strains were selected for further in vitro and in vivo assays[22]. Dhanasekaran *et al*., in 2009 isolated 11 isolates from three ships from Royapuramharbour, Chennai, Tamil Nadu, India. Among the 11 isolates only DR4 showed maximum biofouling activity in the microtiter plate assay with a significant optical density of 0.596 [23].

Bhosale *et al.*, (2002) used the glass cover slip on Zobell marine broth contains the bacterial strain to check the adherence of bacteria to screening the biofouling strains. The biofouling strains were isolated by the staining of cover slip by crystal violet and the adherence of strains was checked by microscopic analysis[10]. In 1996 Clare isolated antifouling natural products from marine sources and identified a number of bioactive compounds with antifouling activities [24]. These are biodegradable and less toxic to the environment. Most of the bacteria present in the biofilms are embedded with extracellular polymeric substance[25]. The extracellular polymeric substance also serves many other functions such as adhesive foundations, structural integrity; inter cellular communication and bacterial protection.

Bhattarai *et al.,* (2006) reported that paints that contain antifouling agent was suitable to prevent both microfouling and macrofouling[26]. In 2019 Venugopal Gopikrishnan *et al*., reported 214 natural metabolites and 23 of synthetic derivatives with antifoulant activity [27]. Structural analyses of these natural or synthetic compounds revealed that 82 compounds were novel, which highlights the efficiency of marine organisms to produce antifouling metabolites. Marine microbes are being explored as potential sources for the production of environmental friendly antifouling metabolites.

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Similarly, Darya *et al* in 2020 evaluated the antifouling and antibacterial activities of bioactive extracts from different organs of sea cucumber, *Holothuria leucospilota*[28]. Doghri *et al* in 2019 determined the antibiofilm activity in the culture supernatant of a marine *Pseudomonas* sp. bacterium[30]. Venugopal *et al* in 2019 isolated taxifolin from mangrove-derived *Streptomycin samponii* PM33 and evaluated its antifouling potential[29]. Maria *et al* in 2019 evaluated the antifouling potential and ecotoxicity of secondary metabolites derived from red algae of the genus *Laurencia*[30].

5. CONCLUSION

Marine microbes are taxonomically diverse and unique, which makes them as potential source for discovery of novel bioactive molecules. The microbial association with living surfaces in the marine environment provides ample opportunities for bioprospecting natural products. The main mechanism of antibiofilm activities of microbial strains includes antibiotic activity affects the extracellular polymers production which is essential for biofilm formation. However, most of the studies were conducted under laboratory conditions and failed to test the compounds in natural water for commercial applications. In the present study, the metabolites which showed antifouling and antimicrobial activities were investigated through different antifouling assays using various target species. This form of crude extracts would have high application potential as a source of useful compounds for antifouling technology. The bacteria which were isolated from marine biofilms, were found to produce potential antifouling compound and it was identified by LC-MS analysis. These findings strengthen the applications of antifoulants isolated from *Staphylococcus* sp. From this work it is concluded that the microbes associated with marine macroorganisms are an untapped source for natural product antifouling compounds and many more novel compounds.

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7. REFERENCE

1. Qian PY, Chen L, Xu Y. Mini review: Molecular mechanisms of antifouling compounds. Biofouling. 2013;29(4):381-400. https://doi.org/10.1080/08927014.2013.776546.

2. Costerton JW, Lewandowski Z, Caldwell DE, Kerber KP, Lappin-Scott HM. Microbial biofilms. Annual Review of Microbiology.1995;49:711-745.https://doi.org/10.1146/annurev.mi.49.100195.003431.

3. Soliman YA, Ibrahim AM, Tadros HR, Abou-Taleb AE, Moustafa AH, Hamed MA. Antifouling and antibacterial activities of marine bioactive compounds extracted from some red sea cucumber. International Journal of Contemporary Applied Science. 2016;3(9):83–103.https://doi.org/10.12988/ijcas.

4. Iyapparaj P, Revathi P, Ramasubburayan R, Prakash S, Anantharaman P, Immanuel G, et al. Antifouling activity of the methanolic extract of Syringodium isoetifolium, and its toxicity relative to tributyltin on the ovarian development of brown mussel *Perna indica*. Ecotoxicology and Environmental Safety. 2013. 89:231–8.https://doi.org/10.1016/j.ecoenv.2012.12.001.

5. Soliman YAA, Brahim AM, Moustafa AH, Hamed MAF. Antifouling evaluation of extracts from Red Sea soft corals against primary bioflm andbiofouling. Asian Pacific Journal of Tropical Biomedicine. 2017;7(11):991–7. https://doi.org/10.1016/j.apjtb.2017.09.016.

6. Qi SH, Zhang SI, Yang LH, Qian PY. Antifouling and antibacterial compounds from the gorgoniana Subergorgia suberosa and Seripearia gracilis. Natural Product research. 2010;22 (2),154-166. https://doi.org/10.1080/1478641070164244.

7. Yang LH, Lee OO, Jin T, Li XC, Qian PY. Antifouling properties of 10β-formamidokalihinol-A and kalihinol A isolated from the marine sponge Acanthella cavernosa. Biofouling;The Journal of Bioadhesion and Biofilm Research. 2006;22:23-32. https://doi.org/10.1080/08927010500498623.

8. Saravanan P, Prabagaran SR, Venkata Nancharaiah Y, Krishnaveni M, Venugopalan VP, Jayachandran S. Isolation and characterization of Pseudoalteromonasruthenica(SBT033), an EPS producing biofilm bacterium from the seawater intake point of a tropical power station. World Journal of Microbiology and Biotechnology. 2008;24(4),509-515.https://link.springer.com/article/10.1007/s11274-007-9501-9.

9. David Hendricks. Bergey and John G Holt.Bergey's manual of determinative bacteriology. Ninth Edition 2000.

10. Bhosale SH, Nagle VL, Jagtap, TG. Antifouling potential of some marine organisms from India against species of Bacillus and Pseudomonas. Marine Biotechnology. 2002;4(2),111-118.https://doi.org/10.1007/s10126-001-0087-1.

11. Abdi-Ali A and Peeters. Bactericidal activity of various antibiotics against biofilm producing *Pseudomonas aeruginosa*.International journal of Antimicrobial agents. 2006; 27(3),196-200.https://doi.org/10.1016/j.ijantimicag.2005.10.007.

12. Peeters E, Nelis, HJ, Coeny T. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. Journal of Microbiological Method. 2008;72 (2),157-165. https://doi.org/10.1016/j.mimet.2007.11.010.

13. Subramani Ramesh and NarayanasamyMathivanan. Screening of marine actinomycetes isolated from the Bay of Bengal, India for antimicrobial activity and industrial enzymes. World journal of Microbiology and Biotechnology. 2009;(25),2103-2111.https://link.springer.com/article/10.1007/s11274-009-0113-4.

14. Mounyr Balouiri, Moulay Sadiki, Saad KoraichiIbnsouda. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016;71-79.https://doi.org/10.1016/j.jpha.2015.11.005.

15. Phyllis Molobela T, Eugene Cloete, Mervyn Beukes. Protease and amylase enzymes for biofilm removal and degradation of extracellular polymeric substances (EPS) produced by Pseudomonas fluoresces bacteria. African Journal of Microbiology Research. 2010. Vol. 4(14), pp. 1515-1524.Available online http://www.academicjournals.org/ajmr.

16. Thomas G, Ludwig, Hyman JV, Goldberg.The Anthrone Method for the Determination of Carbohydrates in Foods and in Oral Rinsing. Journal of Dental Research. 1956. https://doi.org/10.1177/00220345560350012301.

17. Lowry OH, Rose Brough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 1951;193(1):265-75. https://doi.org/10.1016/S0021-9258(19)52451-6.

18. Hari Datta Bhattarai, ViswanadhSarmaGanti, BabitaPaudel, Yoo Kyung Lee, Yong-Ki Hong, Hyun Woung Shin et al. Isolation of antifouling compounds from the marine bacterium, *Shewanellaoneidensis* SCH0402. World Journal of Microbiology and Biotechnology.2007;23,243-249.https://link.springer.com/article/10.1007/s11274-006-9220-7.

19. Wilfred LFArmarego. Common Physical Techniques Used in Purification. Purification of Laboratory Chemicals (Eighth Edition). 2017;1-70. https://doi.org/10.1016/B978-0-12-805457-4.50001-X.

20. Lynch KL. Mass Spectrometry for the Clinical Laboratory. Science Direct. 2017. Chapter 6 - Toxicology: Liquid chromatography mass spectrometry. Pages 109-130. https://doi.org/10.1016/B978-0-12-800871-3.00006-7.

21. Diana Elizabeth Waturangi, Jessen PurwaHariyanto, Windy Lois, Rory Anthony Hutagalung, Jae Kwan Hwang. Inhibition of marine biofouling by aquatic Actinobacteria and Coral associated marine bacteria. Malaysian journal of Microbiology. 2017;Vol 13(2),92-99.https://doi.org/10.21161/mjm.86016.

22. Viju N, Anitha A, SharminVini S, Sunjaiy Shankar CV, Satheesh S, Mary Josephine Punitha S. Antibiofilm activities of extracellular polymeric substances produced by bacterial symbionts of seaweeds. Indian Journal of Geo-Marine Sciences. 2014;Vol 43 (11).http://nopr.niscair.res.in/bitstream.

23. Dhanasekaran D, Thajuddin, N, Rashmi M, Deepika TL and Gunasekaran M.Screening of biofouling activity in marine bacterial isolate from ship hull. International Journal of EnvironmentalScience and Technology. 2009;6(2),197-202.https://link.springer.com/article/10.1007/BF03327622.

24. Clare AS. Marine natural product antifoulants: Status and potential. Biofouling. 1996;9:211-229.https://doi.org/10.1080/08927019609378304.

25. Lawrence JR, Korber DR, Hoyle BD, Costerton JW, Caldwell DE.Optical sectioning of microbial biofilms. Journal of Bacteriology. 1999;173,6558-6567.https://doi.org/10.1128/jb.173.20.6558-6567.1991.

26. Bhattari, HD, Lee YK, Cho KH, Shin HW. The study of antagonistic interactions among pelagic bacteria: a promising way to coin environmental friendly antifouling compounds. Hydrobiologia.2006;568:417-423.https://link.springer.com/article/10.1007/s10750-006-0220-2.

27. Venugopal Gopikrishnan, Manikkam Radhakrishnan, ThangavelShanmugasundaram, Meganathan P, Ramakodi, Ramasamy Balagurunathan. Isolation, characterization and identification of antibiofouling metabolite from mangrove derived *Streptomyces sampsonii* PM33. Scientific reports on nature research. 2019;(9):12975. https://www.nature.com/articles/s41598-019-49478-2#:

28. Darya, M., Sajjadi, M.M., Yousefzadi, M. Antifouling and antibacterial activities of bioactive extracts from different organs of the sea cucumber *Holothuria leucospilota*.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 6650 – 6661
Received 05 March 2021; Accepted 01 April 2021.

Helgoland Marine Research. 2020;74(4). Pages: 13. https://doi.org/10.1186/s10152-020-0536-8.

29. IbtissemDoghri, Florence Brian- Jaisson, Marianne Graber, Alexis Bazire, Alain Dufour, Marie- NoelleBellon- Fontaine, Jean- Marie Herry, Ana Caroline Ferro, Valerie Sopena, Isabelle Lanneluc and Sophie Sable. Antibiofilm activity in the culture supernatant of a marine *Pseudomonas* sp. Bacterium.Microbiology. 2020. https://doi.org/10.1099/mic.0.000878.

30. Maria Protopapa, Manto Kotsiri, Sofoklis Mouratidis, Vassilios Roussis, Efstathia Ioannou, Skarlatos G. Dedos. Evaluation of Antifouling Potential and Ecotoxicity of Secondary Metabolites Derived from Red Algae of the Genus *Laurencia*. Marine drugs. 2019;16;17(11):646. https://doi.org/10.3390/md17110646.

Table 1: Estimation of biomolecules in antifouling strain.

Serial No	Sample		Estimation of carbohydrates		Estimation of protein	
			OD at 620nm	Conc of carbohydrate (µg/ml)	OD at 595nm	Conc of Protein (µg/ml)
1	A2		0.172	0.35	0.117	0.29
2	A7		0.142	0.43	0.181	0.45

Table 2: Bioactive compound or Antifouling bacterial metabolite illustrated by LC-MS Analysis

Hit Analytes	Compound Name	Mol.Wt.	Formula
1	Phytoene Synthase	544.952	$C_{40}H_{64}$
2	1,3-diphosp hoglycerate	266.035	$C_3H_6O_{10}P_2$

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3	Benzophenone 2-3 Cyclo Octane	191.112	$C_8H_{15}Br$
4	2,2-diphenyl-1-picrylhydrazyl	394-32	$C_{18}H_{12}N_5O_6$

Fig 1: LC-MS of antifouling bacterial isolate