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Antifouling Activity of Epibiotic Bacteria Associated with Soft Coral *Sarcophyton* sp. Collected from the Central Red Sea

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Abstract. The microbial communities associated with marine invertebrates are considered as a prolific source of bioactive metabolites. In this study, the bacteria associated with the soft coral *Sarcophyton* sp. collected from the Red Sea was screened for antifouling activity to identify potential natural antifouling compounds. The extract of bacterial strain *Ruegeria lacuscaerulensis* KAU-MB3 associated with the soft coral showed strong antifouling activity in laboratory assays. The crude bacterial extract reduced the growth of biofilm-forming bacteria and inhibited the biofilm formation significantly. Barnacle larval settlement assay indicated a significant reduction in the settlement of larvae treated with the crude extract. The crude extract was analysed by GC-MS to understand the chemical composition. GC-MS results revealed the presence of compounds such as hexadecane, octatriacontyl pentafluoropropionate, cis-Z-à-bisabolene epoxide and geranyl isovalerate. In conclusion, this study indicated that the bacteria associated with the soft corals could be used as one of the potential sources for the natural product antifoulants.

Keywords: Soft corals; bacterial symbionts; biofouling; bioactive compounds; microbial ecology; Red Sea.

1. Introduction

Soft corals (Alcyonacea) are the main components of the benthic communities in the reef environment with great diversity in tropical regions of the Indian and Pacific oceans, Fabricius and Alderslade 2001), including the Red sea (Reinicke 1997). Previous studies showed that many microbial species are associated with coral species mainly in the mucus, tissues and surfaces (Ritchie and Smith, 1997; Rohwer *et al*., 2001; Koren and Rosenberg, 2006). The microbes associated with the corals may have many functions based on the relationships such as mutualistic and pathogenic (Harvell *et al*., 1999; Ben-Haim *et al.*, 2003). Generally, the microbial symbionts of corals provide protection to the hosts through the production of biologically active metabolites or antagonistic activity (Ritchie, 2006; Rosenberg *et al*., 2007). Besides, the microorganisms associated with the corals may have many ecological functions such as supply nitrogen and phosphorus to the host, participation in nutrient recycling *etc*. (Rosenfeld *et al*., 1999; Anthony and Fabricius, 2000).

In marine waters, almost all surfaces (both living and non-living) are colonized by microorganisms and macroorganisms. Some marine organisms have defence mechanism (antifouling defence) against unwanted colonization. The microorganisms associated with marine organisms are also reported to play important role in the antifouling defence of these organisms. These microorganisms could be a source for the isolation of novel antifouling metabolites to control the biofouling development on artificial materials

(Satheesh *et al*., 2016). Biofouling development on hard substrates is one of the serious issues for marine technology and maritime sectors due to the ecological and economical complications (Satheesh *et al*., 2016). The biofouling development process usually begins with the formation of biofilm (microbes and microalgae) on the substrates, attachment of larvae and algal spores and finally assemblage of macroorganisms (Kwon *et al*., 2002; Wesley and Satheesh, 2009; Satheesh and Wesley, 2010; Satheesh *et al*., 2016).

Many methods are used to control the biofouling development on technical objects (Qian *et al*., 2009). Tributyltin is one of the popular antifouling compounds used in coatings (Nehring, 2001). Though TBT based coatings are very effective to prevent biofouling development, the side effects to the environment and other non-target marine organisms warrant strict guidelines and complete prohibition from 2008 by International Maritime Organization (Satheesh *et al*., 2016). After the ban on TBT, many alternatives are suggested but most of those compounds are also reported to possess toxic effects on marine organisms (Nehring, 2001). The natural products from marine organisms and their microbial symbionts are reported to exhibits strong antifouling activity in laboratory assays (Qi *et al*., 2008; Qian *et al*. 2009; Viju *et al*., 2017; Salama *et al*., 2018).

Marine microbes attracted the attention of researchers due to their capability to produce novel metabolites (Milinski, 1993; Bernan *et al*., 1997; Fenical, 1997). These secondary metabolites serve as model systems in the discovery of new drugs as well as ecologically relevant compounds. Bacteria associated with sponges, corals and seaweeds have been studied for their antimicrobial activity (Burgess *et al*., 1999; Boyd *et al*., 1999; Thiel and Imhoff, 2003; Radjasa *et al*., 2007; Kennedy *et al*., 2009) and many bioactive compounds have been reported (Chandramohan, 1997; Anand *et al*., 2006; Devi *et al*., 2010). However, only a few works are available on the antifouling activity of bacteria associated with soft corals. Hence, in this study, the bacterial communities associated with the surface of the soft coral species collected from the Red Sea was analysed for their antifouling activities. The results obtained in this study will improve our understanding of the chemical defence role of bacteria associated with marine invertebrates.

2. Materials and Methods

2.1 Collection of Soft Corals and Isolation of Surface-Associated Bacteria

The soft coral *Sarcophyton* sp. was collected from the Obhur Creek, Jeddah coast of central Red Sea. The collected coral samples were immediately transferred to the laboratory in a sterile container that contained filtered and sterilized seawater. In the laboratory, soft coral samples were rinsed in filtered and sterilized seawater to remove the debris and other organisms. The surface of the soft coral samples was swabbed with cotton. After that, the cotton swab was kept in 2 ml filtered and sterilized seawater and vortexed. Following this, the suspension was diluted and spread on agar plates (Marine agar). The plates were kept at 30°C for 24-48h for the development of bacterial colonies. The bacterial colonies developed on the plates were separated based on colony morphology. The isolated colonies were purified by the streak plate method on marine agar plates. After that, the purified colonies were maintained in marine agar slants at 4^{o}C for further studies.

2.2 Preparation of Crude Extract of Coral-Associated Bacteria

The coral-associated bacterial isolates were cultured in marine broth in conical flasks (250 ml). The culture broth was kept at 30°C in a shaker (100 rpm) for 3 days. Following this, the culture broth was centrifuged at 10000 x *g* at 4^{o}C for 15 min. After centrifugation, the resulting pellet was collected and washed with 10 mM phosphate-buffered saline. The cell pellet was extracted with 2 ml methanol. The

extraction process was carried out for 24 h in a shaker. After that, the supernatant was collected and concentrated under reduced pressure in a rotary evaporator. The resulting crude extract was mixed with the required amount of methanol for further assays.

2.3 Screening of Antibacterial Activity of Coral-Associated Bacterial Extracts

The traditional disc diffusion assay was used to test the antibacterial activity of the crude extracts of the coral-associated bacteria. Two biofilm-forming bacteria, *Vibrio harveyi* and *Planomicrobium* sp. isolated from the artificial materials submerged in the Obhur Creek (Balqadi *et al*., 2018) were used as target bacteria for the anti-biofilm assays. The crude extract was mixed with methanol (100 µg in 1 ml) and used for the disc assay. Paper discs (6 mm) were prepared using Whatman filter paper. About 50 µl of the extract was loaded on each treatment disc. The control discs were loaded with 50 µl of methanol. The extracts and solvent loaded discs were kept in a chamber for 1 h. After that, the paper discs were placed on marine agar plates that were seeded with biofilm-forming bacteria. Following this, the plates were kept in an incubator at 30°C for 24-48h. The plates were checked for the inhibition zones around the discs. The zone of inhibition on the plates was measured using a scale. The coral-associated bacterial extract which showed strong activity in the disc diffusion assay was selected for further studies.

2.4 Identification of Coral-Associated Bacteria

Genomic DNA from the bacteria was extracted using a commercially available DNA extraction kit by following the protocol recommended in the kit instructions. The extracted DNA samples were subjected to PCR amplification using universal 16S rRNA primers for bacteria. The PCR conditions and sequencing protocols described previously by Balqadi *et al*. (2018) was used in this study. The sequences were aligned and the bacterial strain was identified using National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI-BLAST). The sequence was submitted to NCBI Genbank.

2.5 Biofilm-forming Bacterial Growth Inhibition Assay (Spectrophotometer Method)

Bacterial growth inhibition assay was conducted using the spectrophotometric method. About 3 ml of overnight grown biofilm-forming bacterial culture was taken in test tubes. To this, two different concentrations (25 and 50 µg ml^{-1}) of coral-associated bacteria were added and incubated for 5 h. Two types of control wells were maintained, one with 25 and 50 µl ml^{-1} methanol and the other control without extract or solvents. The OD (optical density) of the test and control samples will be read at 630 nm in UV-Vis spectrophotometer at 1 h interval for 5 h duration.

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

2.6 Antibiofilm Activity Assay (Microtitre Plate Method)

The microtitre plate biofilm assay described by O'Toole (2011) was used to test the antibiofilm activity. In brief, overnight grown biofilm-forming bacteria culture was taken into 96 well microtitre plates. Two different concentrations of the extracts (25 and 50 µg ml^{-1}) were added to the wells and incubated for 24-72 h at 30°C in an incubator. Two types of control wells were maintained, one with 25 and 50 µl ml^{-1} methanol and the other control without extract or solvents. After incubation, the culture along with the unattached bacterial cells was removed by gently turning the plates over and washed with phosphate buffer saline. Crystal violet (0.1%) was added to the wells to stain the attached bacterial cells. The plates were incubated for 15 minutes and after that washed with distilled water to remove excess stain. The attached biofilm cells were quantified by adding 30% acetic acid to the wells followed by incubation

for 15 minutes at 30°C. The crystal violet eluted from the wells was transferred to a new microtitre plate and the optical density was measured at 550 nm in a microtitre plate reader (Biotek). The experiment was conducted in replicates (n=6) with a different batch of biofilm-bacteria culture.

2.7 Barnacle Larval Settlement Assay

The barnacle *Amphibalanus amphitrite* adults were collected from the Obhur Creek and maintained in a glass tank in the laboratory. The barnacles were kept in the tank with moderate aeration and provided a mixed diet consisted of *Artemia* nauplii microalga (*Chaetoceros*). The nauplii released by the adults were collected and transferred to small tanks. The nauplii were reared up to cypris stage (settling stage) using a mixed algal diet according to the conditions outlined in Salama *et al*. (2018). The cypris larvae were used for settlement assays. The settlement assay was conducted in 6-well plates using 25 larvae in each well. The extracts in two different concentrations (25 and 50 µg ml^{-1}) were added to the wells. Two types of control wells were maintained, one with 25 and 50 µl ml^{-1} methanol and the other control without extract or solvents. The plates were kept in dark at room temperature and the number of cyprids settled was counted under a microscope after 24, 48 and 72 h. The percentage of settlement for each concentration was calculated after deducting the values observed in the methanol control. The experiment was conducted in replicates (n=3) using a different batch of barnacle larvae.

2.8 GC-MS Analysis of Coral-Associated Bacteria Extracts

The column purified extract of the soft-coral associated bacteria was carried out using Shimadzu GC-MS QP 2010 according to the protocol described previously (Viju *et al*., 2020). In brief, the sample was injected using the carrier gas helium at the flow rate 1ml/ minute. The temperature of the injection port was kept at 250ºC, while the temperature of the ion source was managed at 280ºC. The column oven temperature was held at 110ºC for 2 minutes then programmed at 10ºC / minute and the ending temperature was increased to an isothermal point at 280ºC/9 minute. Subsequently, the mass spectra of the fraction were taken at 2-minute scan interval and the compounds identification was made by comparing their mass spectra with the mass spectral library of NIST (National Institute of Standards and Technology).

2.9 Statistical Analysis

The data obtained from bacterial growth inhibition and antibiofilm activity were subjected to one-way ANOVA (analysis of variance) to find the variation between control and treatment samples. The barnacle larval settlement assay data was analysed by two-way ANOVA using extract concentration and exposure time as factors. The statistical analysis was conducted using MS-Excel ($P<0.05$ was considered significant).

3. Results

3.1 Antimicrobial Activity and Identification of Coral-Associated Bacteria

In this study, five coral-associated bacterial isolates were recovered from the surface of soft coral *Sarcophyton* sp. The crude extracts of all 5 isolates from soft coral were tested for their antibacterial activity against the two biofilm-forming bacteria. All the isolates showed antibacterial activity against the biofilm-forming bacteria (Fig. 1). However, the strain KAU-MB3 exhibited strong activity (inhibition zone size: 18 mm) than the other surface-associated bacterial strains. Hence, this bacterial strain was selected for further antifouling studies.

3.2 Identification of Bacterial Strain KAU-MB3

The 16S rRNA gene sequences obtained from the strain KAU-MB3 showed 99.91% similarity with the bacterium *Ruegeria lacuscaerulensis* in the NCBI database (GenBank). The phylogenetic analysis in

presented in Fig. 2. The sequence of the strain KAU-MB3 was submitted to NCBI GenBank (accession number: MW881524).

3.3 Growth Inhibitory Activity of the Coral-Associated Bacterial Extract Against Biofilm-Forming Bacteria

The results of the present study indicated that the extract of *R. lacuscaerulensis* inhibited the growth of both *V. harveyi* and *Planomicrobium* sp. (Fig. 3). The growth rate of *V. harveyi* was observed as 77.66% for a period of 5 h under laboratory conditions. The *V. harveyi* culture treated with 25 and 50 µg ml^{-1} of the coral-associated bacterial extract showed a growth of 57.33 and 37.33 % respectively. One-way ANOVA revealed a significant variation in the growth of *V. harveyi* treated with different concentrations of coral-associated bacterial extract ($F=46.33$, $df=2,6$; $P<0.001$). Further, post-hoc Tukey test results indicated significant variation in the growth of *V. harveyi* between the control and treatments (Table 1).

The *Planomicrobium* sp. control culture showed a growth rate of 81.33% during the 5h period under the laboratory conditions (Fig. 3). However, the *Planomicrobium* sp. cultures treated with 25 and 50 µg ml^{-1} of coral-associated bacterial extract recorded a reduction in growth. The *Planomicrobium* culture treated with 25 µg ml^{-1} recorded a growth of 47.66% and 50 µg ml^{-1} exhibited a growth percentage of 39. ANOVA results indicated a significant difference in the growth of *Planomicrobium* sp. treated with different concentrations of coral-associated bacterial extract ($F=162$, $df=2,6$; $P<0.001$). Moreover, the growth of *Planomicrobium* sp. differed significantly between control and extract treated cultures (Tukey test, Table 1).

3.4 Antibiofilm Assay

The antibiofilm assay indicated that the extract of coral-associated bacterial strain inhibited the settlement of biofilm-forming bacteria (Fig. 4). The settlement of *V. harveyi* ($F=93.09$, $df=2, 15$; $P<0.001$) and *Planomicrobium* sp. ($F=77.79$, $df=2, 15$; $P<0.001$) on microtitre plate was reduced significantly (Table 1) due to the treatment of coral-associated bacterial extract.

3.5 Barnacle Larval Settlement Assay

The results of the barnacle larval settlement assay are presented in Fig. 5. In the control well, an average of 84% (21 individuals) settled on the plates after 48 h. However, the settlement of barnacle larva was reduced considerably when treated with the coral-associated bacterial extract. The number of larvae settled on the wells treated with 25 µg ml^{-1} of the extract was 11 (44%) after 48 h of the experiment. Likewise, a low settlement was recorded from the wells treated with 50 µg ml^{-1} of extract (7 individuals, 28%) during the same period. Further, the settlement was very low in the treatment wells during the initial 24 h of the experiment. Two-way ANOVA results showed significant variation in the settlement of barnacle larvae in relation to extract concentration and observation time (Table 2).

3.6 GC-MS Analysis of the Coral-Associated Bacterial Extract

The GC-MS analysis of the coral-associated bacterial extract showed the presence of bioactive metabolites such as hexadecane, octatriacontyl pentafluoropropionate, cis-Z-à-bisabolene epoxide geranyl isovalerate, pentadecane- 7-methyl, tetradecane, 2-Ethylhexyl -2-ethylhexanoate, p-Toluic acid- 2-ethylhexyl ester, 1-hexacosene, 1-chloroeicosane and dibutyl phthalate (Fig. 6, Table 3). Among the compounds, p-Toluic acid- 2-ethylhexyl ester, dibutyl phthalate and Cis-Z-à-bisabolene epoxide were exhibited higher concentrations (based on peak area).

Table 1. Approximate Probabilities for Post Hoc Tukey HSD tests. P<0.05= significant.

Factor 1	Factor 2	Bacterial growth inhibition		Antibiofilm assay	
		V. harveyi	*Planomicrobium* sp.	*V. harveyi*	*Planomicrobium* sp.
25µg	50 µg	0.007	0.029	0.004	<001
	Control	0.006	<001	<001	<001
50 µg	control	<001	<001	<001	<001

Table 2. Two-way ANOVA results for the settlement of barnacle larvae treated with coral-associated bacterial extract. Extract concentration and experiment duration were considered as factors. P<0.05= significant.

	Degrees of Freedom	F	p
Concentration	2	244.884	0.000
Time	3	46.029	0.000
Concentration*Time	6	2.217	0.076
Error	24		
Total	35		

Table 3. The compounds identified from the extract of soft-coral associated bacterium *R. lacuscaerulensis*.

Compound name	**RT**	**Area (%)**
Heptane, 2,2,4,6,6-pentamethyl	4.67	0.16
Tetradecane, 2,6,10-trimethyl	10.95	0.11
Hexadecane	11.08	0.1
3-(2H)-Benzofuranone, 2-methyl	13.91	0.05
Pentacosane, 13-phenyl	14.00	0.06
ert-Hexadecanethiol	16.17	0.19
Octatriacontyl pentafluoropropionate	16.65	0.07
Geranyl isovalerate	18.12	0.30
Methoxyacetic acid, 2-tetradecyl ester	18.95	0.53
17-Pentatriacontene	20.27	0.35
Pentadecane, 7-methyl	21.78	4.57
Tetradecane	22.01	1.31
2-Ethylhexyl 2-ethylhexanoate	22.11	5.65
Benzoic acid, 2-ethylhexyl ester	22.52	3.26
1-Hexacosene	23.11	1.54
1-Chloroeicosane	23.56	0.66
1-Octadecanesulphonyl chloride	24.27	0.30
Heptadecane	24.59	1.50
Dodecane, 2,6,11trimethyl	24.72	1.60
2-Octyl benzoate	24.88	1.96
Cholestan-3-ol, 2-methylene,	25.32	0.39
Cis-1-Chloro-9-octadecene	25.81	0.56
Benzene, (1,3,3trimethylnonyl	26.40	2.23
p-Toluic acid, 2-ethylhexyl ester	27.47	20.61
Cis-13-Eicosenoic acid	28.53	0.13
1,2-Benzenedicarboxylic acid, bis-(2methylpropyl) ester	28.63	1.48
Ethaneperoxoic acid,	29.91	0.06
Ethyl isoallocholate	30.83	0.06
Dibutyl phthalate	31.24	14.08
Fenretinide	32.60	0.14
a-D-Mannofuranoside, farnesyl	35.84	0.08
Trans-Geranylgeraniol	35.98	0.38
6-epishyobunol	36.16	0.17
Cis-Z-à-Bisabolene epoxide	36.46	3.02
4,8,13-Cyclotetradecatriene1,3-diol,	38.73	1.08
Squalene	47.76	0.56

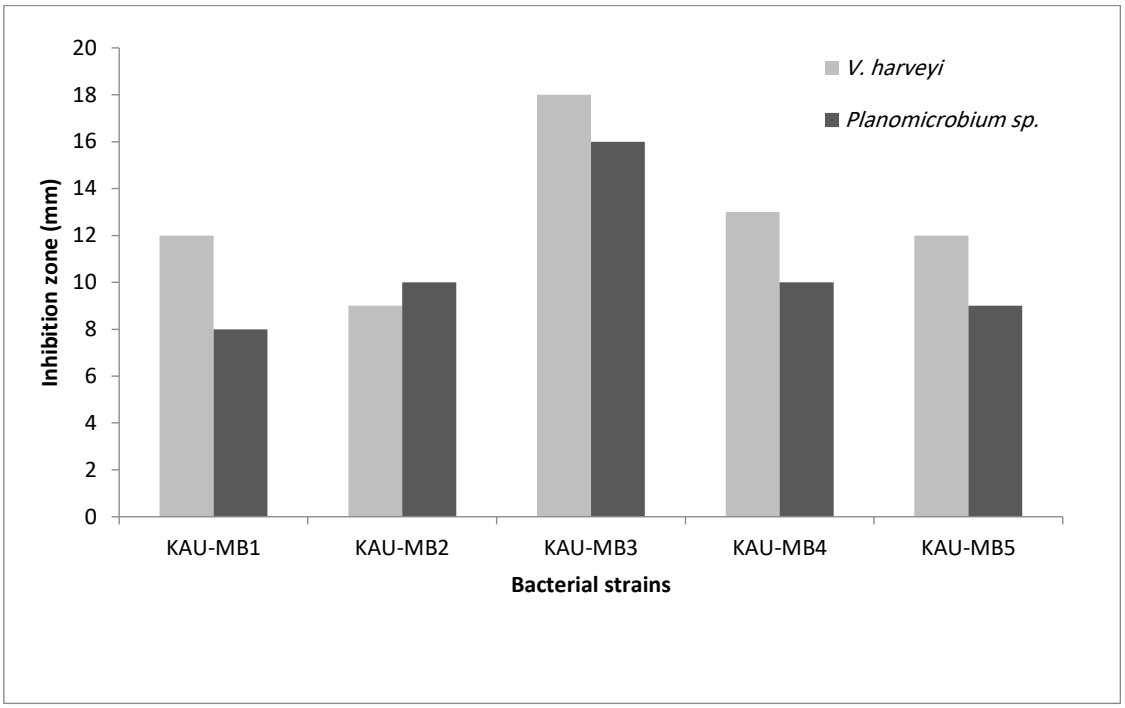

Fig. 1. Antiibacterial activity of coral-associated bacterial extract against biofilm-forming bacteria.

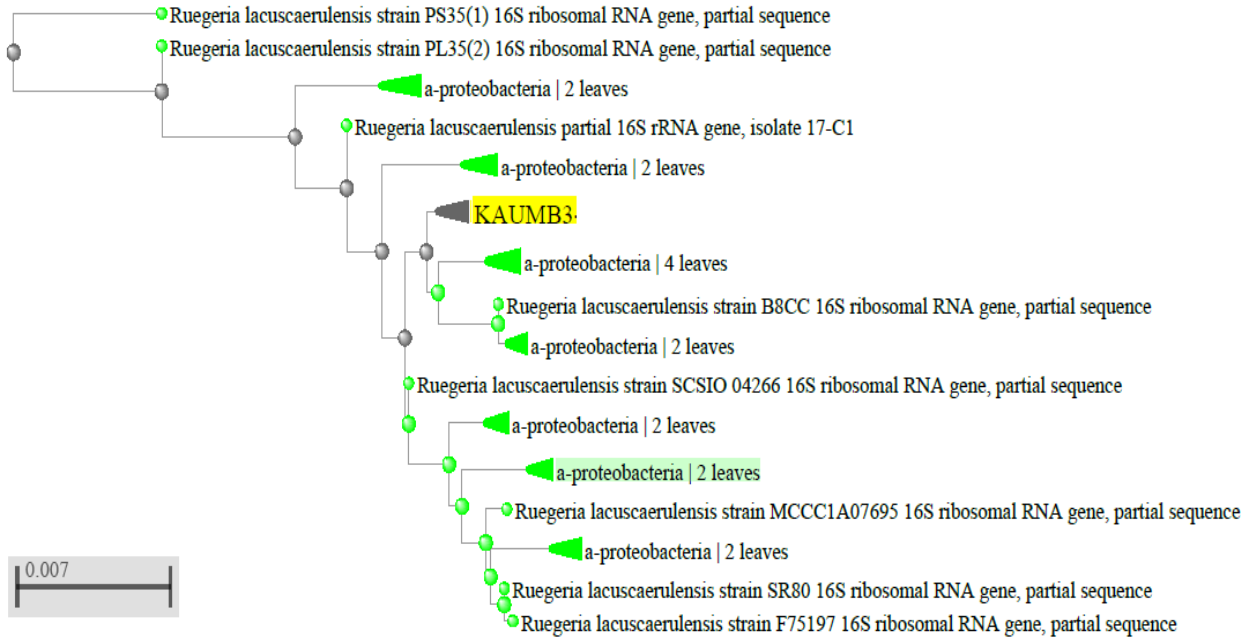

Fig. 2. Phylogenetic tree of coral-associated bacterial strain KAU-MB3. The strain was identified as *Ruegeria lacuscaerulensis.*

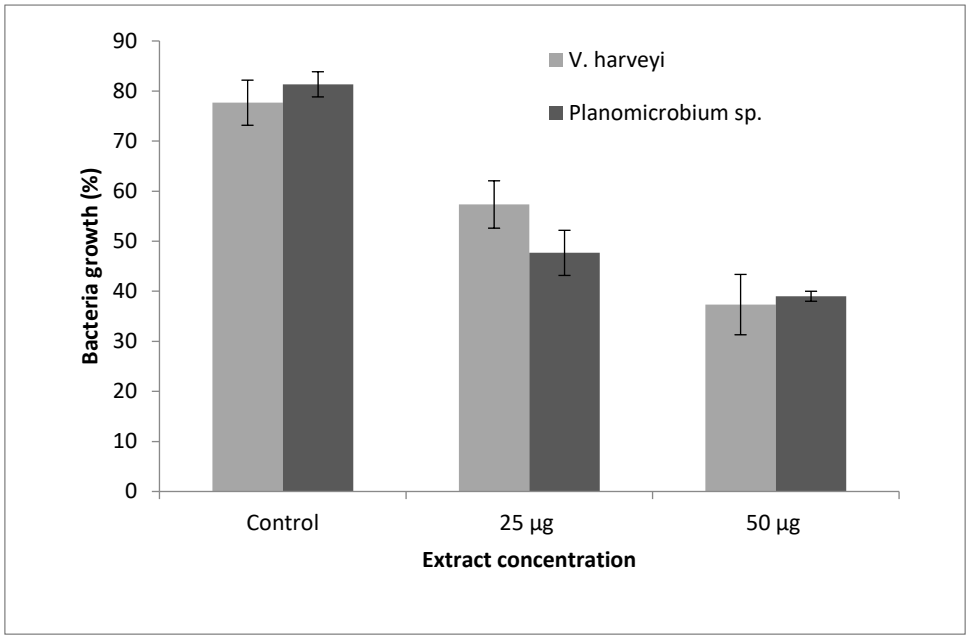

Fig. 3. Growth inhibitory activity of coral-associated bacterial extract against biofilm-forming bacteria.

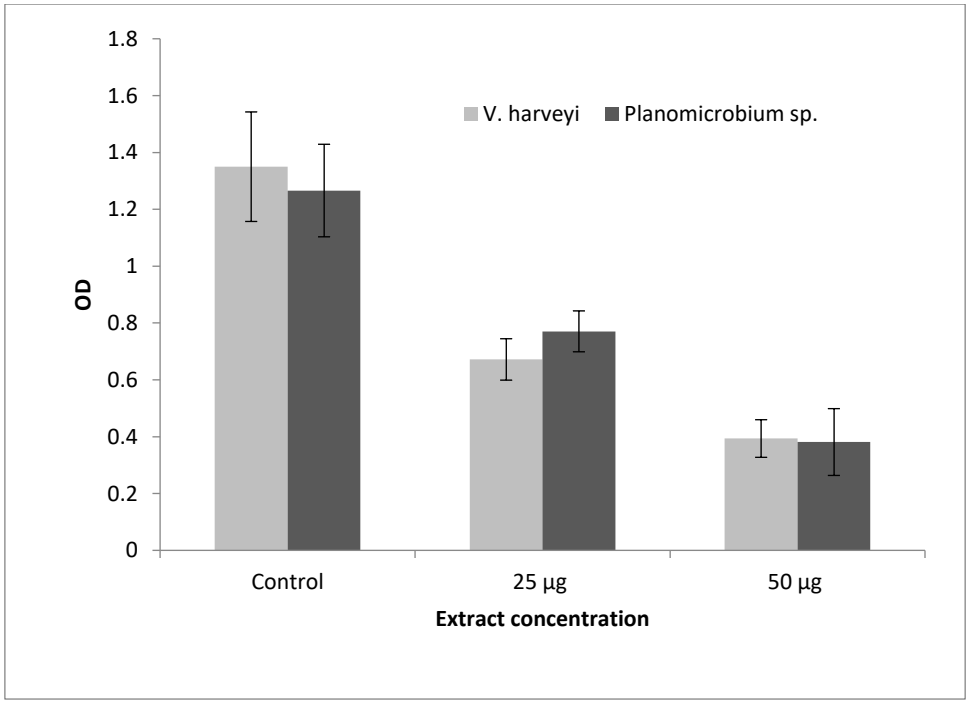

Fig. 4. Antibiofilm activity of coral-associated bacteria against two biofilm-forming bacteria.

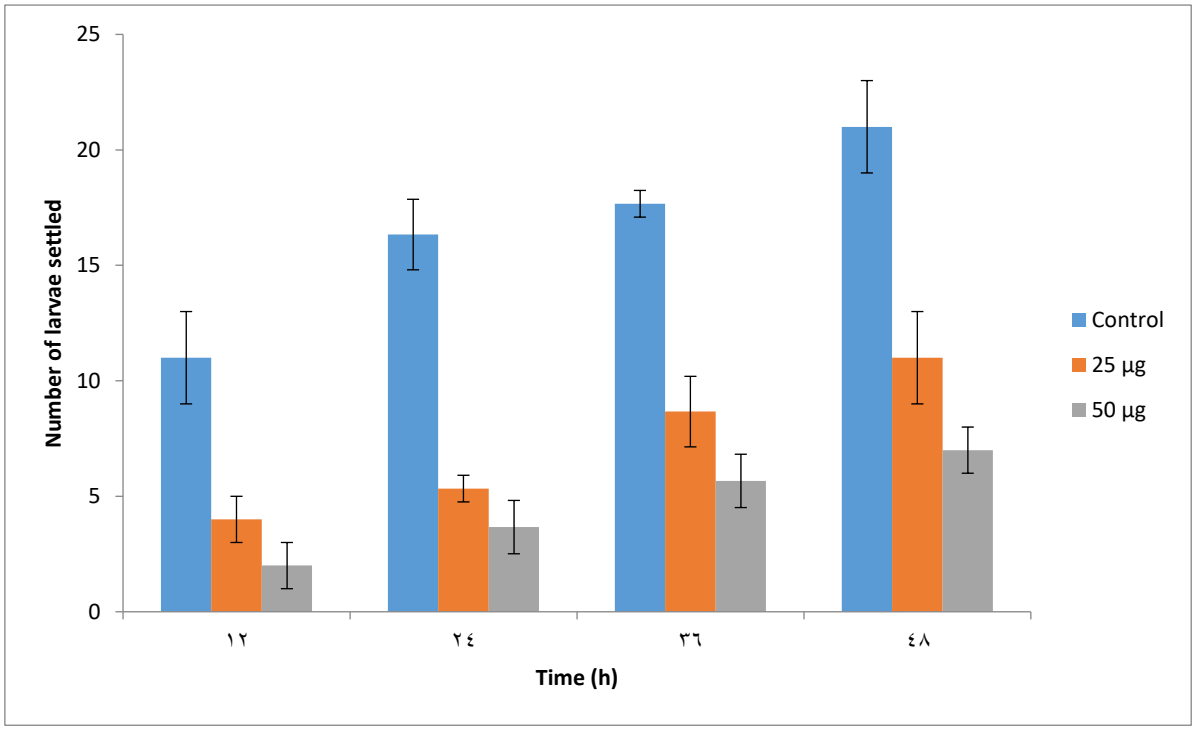

Fig. 5. Barnacle larval settlement inhibitory activity of coral-associated bacterial extract.

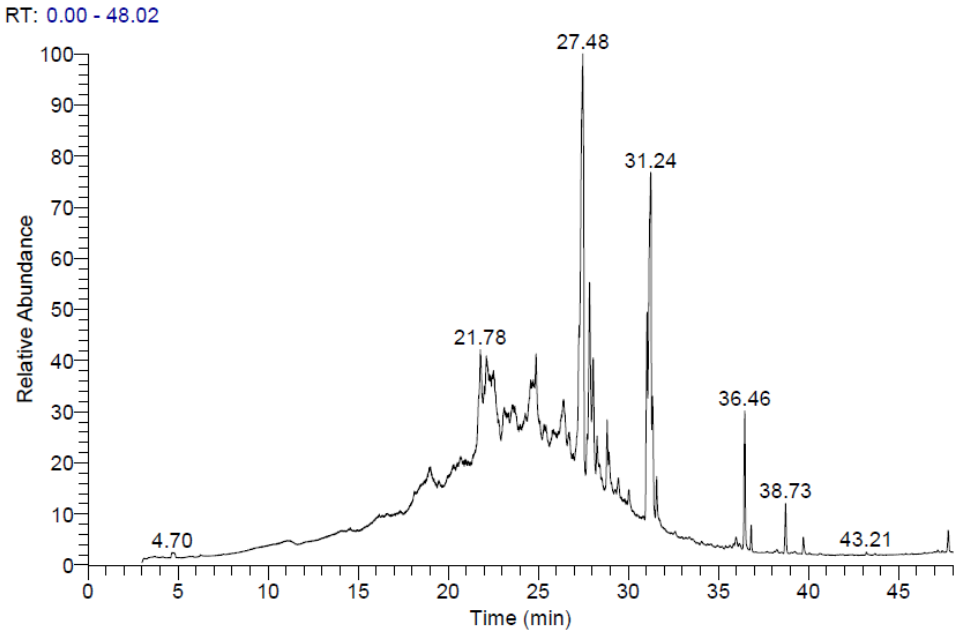

Fig. 6. GC-MS analysis of crude extract of coral-associated bacterium *Ruegeria lacuscaerulensis*.

4. Discussion

The results of the present study indicate that soft corals harbour many bacterial species which may provide protection to the host through the production of secondary metabolites. Many microorganisms including bacteria have a wide range of metabolic and physiological attributes which enable them to grow and survive in a diverse environment. A number of bioactive metabolites have been isolated from soft corals and their associated microbes (Sarma *et al*., 2009; Sang *et al*., 2019). The microbes associated with soft corals have also been reported to possess antifouling activity (Sabdono and Radjasa, 2006; Zhang *et al*. 2019). Previous studies also indicated that the bacteria associated with marine invertebrates could be a potential source for the biologically active compounds (Gil-Turnes and Fenical, 1992, Fenical, 1993). For instance, the bioactive compound Ubiquinone-8 that was isolated from the sponge-associated *Alteromonas* sp. showed strong antifouling activity against the barnacle larvae (Kon-ya *et al.*, 1995).

The genus *Ruegeria* (Roseobacter-clade) is a Gram-negative, aerobic rods mainly reported from the marine waters (Vandecandelaere *et al*., 2008). This genus includes the formerly known marine *Agrobacterium* species. Further, the association of bacteria *R. lacuscaerulensis* with tropical and subtropical coral reefs was previously reported by Gong *et al*., (2020). Previous studies reported the presence of *Pseudomonas Alteromonas, Flavobacterium* and *Vibrio* from the surface of marine invertebrates (Vacelet and Danady, 1997; Santavy and Colwell, 1990; Ward-Rainey *et al*., 1996; Chelossi *et al*., 2004). *Enterobacteriaceae*, *Aeromonas*, *Actinomyces* and *Streptomyces* were rarely reported from the invertebrates (Chelossi *et al*., 2004). *Cornybacteria, Actinomyces* and *Streptomyces* are widely distributed in the marine environment. They are considered as a source of bioactive agents and display competitive biosynthetic capabilities.

The results of this study indicated that bacteria associated with soft corals could be used as a potential source for the isolation of natural product antifouling compounds. Microbes as a source for the extraction of bioactive metabolites have more advantages than using marine invertebrates and algae. Mainly, for extraction of bioactive compounds from marine organisms requires the collection of a large amount of raw material from the natural sources (Satheesh *et al*., 2016). However, microorganisms are cultivable under laboratory conditions and using the modern fermentation methods, the required amount of metabolites can be extracted from the microbial sources (Satheesh *et al*., 2016).

In conclusion, the present study suggests that bacteria associated with the soft corals could serve as a potential source for the searching of new secondary metabolites. Since bacteria multiply quickly and produce large quantities of biomass within a short duration, the microorganisms producing biologically active compounds can be easily obtained on a biotechnological scale without the need for collecting the soft corals from the marine environment. Though laboratory assays of the extract obtained in this study showed strong antifouling activity, further field tests are essential to confirm the activity.

References

Anand, T.P., Bhat, A.W., Shouche, S.Y., Roy, U., Siddharth, J. and **Sarma, S.P.** (2006). Antimicrobial activity of marine bacteria associated with sponges from the waters off the coast of South East India. *Microbiological Research*, **161**: 252-262.

Anthony, K.R.N. and **Fabricius, E.F.** (2000). Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *Journal of Experimental Marine Biology and Ecology*, 252: 221–253.

Balqadi, A.A., Salama, A.J. and **Satheesh, S.** (2018). Microfouling development on artificial substrates deployed in the central Red Sea. *Oceanologia,* **60**: 219-231.

Ben-Haim, Y., Thompson, F.L., Thompson, C.C., Cnockaert, M.C., Hoste, B., Swings, J. and **Rosenberg, E.** (2003). Vibrio coralliilyticus sp. nov., a temperature-dependent pathogen of the coral Pocillopora damicornis.

International Journal of Systematic and Evolutionary Microbiology, **53**: 309–315.

Bernan, V.S., Greenstein, M. and **Maiese, W.M.** (1997). Marine micro-organisms as a source of new natural products. *Advances in Applied Microbiology*, **43**: 57–89.

Boyd, K. G., Adams, D.R. and **Burgess, J.G.** (1999). Antimicrobial and repellent activities of marine bacteria associated with algal surfaces. *Biofouling*, **14** : 227-236.

Burgess J.G., Jordan, E.M., Bregu, M., Mearns-spragg, A. and **Boyd, K.G.** (1999). Microbial antagonism: a neglected avenue of natural products research, *Journal of Biotechnology*, **1**: 27-32.

Chandramohan, D. (1997). Recent advances in Marine Microbiology: The Indian scenario. *Marine Biotechnology*, **5** :73-81.

Chelossi, E., Milanese, M., Milano, A., Pronzato, R. and **Riccardi, G.,** 2004. Characterization and antimicrobial activity of epibiotic bacteria from Petrosia ficiformis. Journal of Experimental Marine Biology and Ecology 309:21–33.

Devi, P., Wahidullah, S., Rodrigues, C. and **Souza, L.D.,** 2010. The Sponge-associated Bacterium Bacillus licheniformis SAB1: A Source of Antimicrobial Compounds. Marine Drugs 8: 1203-1212.

Fabricius, K.E. and **Alderslade, P.,** 2001. Soft Corals and Sea Fans. Australian Institute of Marine Science, Queensland, Australia.

Fenical, W., 1997. New pharmaceuticals from marine organisms. Trends in Biotechnology 15: 339–341.

Fenical, W. (1993). Chemical studies of marine bacteria; developing a new resource. *Chemical Reviews*, **93**: 1673-168.

Gil-Turnes, M.S. and **Fenical, W.** (1992). Embryos of Homarus americanus are protected by epibiotic bacteria. *The Biological Bulletin*, **182**: 105-108.

Gong, S., Jin, X., Ren, L., Tan, Y. and **Xia, X. (**2020). Unraveling Heterogeneity of Coral Microbiome Assemblages in Tropical and Subtropical Corals in the South China Sea. *Microorganisms*, **8**(4): 604.

Harvell, C.D., Kim, K., Burkholder, J.M. *et al.* (1999). Review: marine ecology-emerging marine diseases–climate links and anthropogenic factors. *Science*, **285**: 1505–1510.

Kennedy, J., Baker, P., Piper, C., Cotter, P.D., Walsh, M., Mooij, M.J., Bourke, M.B., Rea, M.C., O'Connor, P.M., Ross, R.P., Hill, C., O'Gara, F., Marchesi, J.R., and **Dobson, A.D. (**2009). Isolation and analysis of bacteria with antimicrobial activities from the marine sponge Haliclona simulans collected from Irish waters. *Marine Biotechnology*, **11**: 384–396.

Kon-Ya, K., Shimidzu, N., Otaki, N., Yokoyama, A., Adachi, K. and **Miki, W. (**1995). Inhibitory effect of bacterial ubiquinones on the settling of barnacle, Balanus amphitrite. *Experientia*, **51**: 153-155.

Koren, O. and **Rosenberg, E. (**2006). Bacteria associated with mucus and tissues of the coral Oculina patagonica in summer and winter. Applied and Environmental Microbiology 72: 5254–5259.

Kwon, K.K., Lee, H.S., Jung, S.Y., Yim, J.H., Lee, J.H. and **Lee, H.K. (**2002). Isolation and identification of biofilm-forming marine bacteria on glass surfaces in Dae-Ho Dike, Korea. Journal of Microbiology 40: 260-266.

Milinski, M. (1993). Predation risk and feeding behavior. In: T.J. Pitcher (Ed.), Behavior of teleost fishes (pp: 285-305). London, UK : Chapman & Hall.

Nehring, S. (2001). After the TBT era: alternative anti-fouling paints and their ecological risks. *Senckenbergiana maritima*, **31**: 341-351.

O'Toole, G. A. (2011). Microtiter dish biofilm formation assay. *JoVE* (*Journal of Visualized Experiments*) **47**: e2437.

Qi, S.H., Zhang, S., Yang, L.H. and **Qian, P.Y. (**2008). Antifouling and antibacterial compounds from the gorgonians Subergorgia suberosa and Scripearia gracillis. *Natural Product Research*, **22**: 154-166.

Qian, P.Y., Xu, Y. and **Fusetani, N. (**2009). Natural products as antifouling compounds: recent progress and future perspectives. *Biofouling*, **26**: 223-234.

Radjasa, O.K., Salasia, S.I.O., Sabdono, A., Weise, J., Imhoff, J.F., Lammler, C. and **Risk, M.J. (**2007). Antibacterial activity of marine bacterium Pseudomonas sp. associated with soft coral Sinularia polydactyla against Streptococcus equi subsp. zooepidemicus. *International Journal of Pharmacology*, **3**: 170-174.

Reinicke, G.B. (1997). Xeniidae (Coelenterata: Octocorallia) of the Red Sea, with descriptions of six new species of Xenia. *Fauna of Saudi Arabia*, **16**: 5–62.

Ritchie, K.B. and **Smith, W.G. (**1997). Physiological comparison of bacterial communities from various species of scleractinian corals. In: *Proc 8th Int Coral Reef Symp Publ*, **1**: 521–526.

Ritchie, K.B. (2006). Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Marine Ecology Progress Series*, **322**: 1–14.

Rohwer, F., Breitbart, M., Jara, J., Azam, F. and **Knowlton, N. (**2001). Diversity of bacteria associated with the Caribbian coral Montastraea franksi. *Coral Reefs*, **20**: 85–91.

Rosenberg, E., Koren, O., Reshef, L., Efrony, R. and **Zilber-Rosenberg, I.** (2007). The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology*, **5**: 355–362.

Rosenfeld, M., Bresler, V. and **Abelson, A. (**1999). Sediment as a possible source of food for corals. *Ecology Letters*, **2**: 345–348.

Sabdono, A. and **Radjasa, O.K. (**2006). Antifouling activity of bacteria associated with soft coral Sarcophyton sp. Against marine biofilmforming bacteria. J*ournal of Coastal Development*, **10**: 56-62.

Salama, A.J., Satheesh, S. and **Balqadi, A.A. (**2018). Antifouling activities of methanolic extracts of three

macroalgal species from the Red Sea. *Journal of Applied Phycology*, **30**: 1943-1953.

Sang, V.T., Dat, T.T.H., Vinh, L.B., Cuong, L.C.V., Oanh, P.T.T., Ha, H., Kim, Y.H., Anh, H.L.T. and **Yang, S.Y.** (2019). Coral and coral-associated microorganisms: A prolific source of potential bioactive natural products. *Marine Drugs*, **17**: 468.

Santavy, D.L., Willenz, P. and **Colwell, R.R. (**1990). Phenotypic study of bacteria associated with the caribbean scleraponge, Ceratoporella nicholsoni. *Applied and Environmental Microbiology*, **56**: 1750-1762.

Sarma, N.S., Krishna, M.S., Pasha, S.G., Rao, T.S.P., Venkateswarlu, Y. and **Parameswaran, P.S. (**2009). Marine metabolites: The sterols of soft coral. *Chemical Reviews*, **109**: 2803-2828.

Satheesh, S. and **Wesley, S. (**2010). Biofilm development on acrylic coupons during the initial 24 hour period of submersion in a tropical coastal environment. *Oceanological and Hydrobiological Studies*, **39**: 27-38.

Satheesh, S., Ba-akdah, M.A. and **Al-Sofyani, A.A. (**2016). Natural antifouling compound production by microbes associated with marine macroorganisms: A review. *Electronic Journal of Biotechnology*, **19**: 26-35.

Thiel, V. and **Imhoff, J.F. (**2003). Phylogenetic Identification of Bacteria with Antimicrobial activities Isolated from Mediterranean sponges. *Biomolecular Engineering*, **20**: 421-423.

Vacelet, J. and **Danadey, C. (**1977). Electron microscope study of the association between some sponges and bacteria. *Journal of Experimental Marine Biology and Ecology*, **30**: 301-314.

Vandecandelaere, I., Nercessian, O., Segaert, E., Achouak, W., Faimali, M. and **Vandamme, P. (**2008). *Ruegeria scottomollicae* sp. nov., isolated from a marine electroactive biofilm. *International Journal of Systematic and Evolutionary Microbiology*, **58**: 2726-2733.

Viju, N., Satheesh, S. and **Punitha, S.M.J. (**2017). Antifouling activities of antagonistic marine bacterium Pseudomonas putida associated with an octopus. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, **87**: 1113-1124.

Viju, N., Punitha, S.M.J. and **Satheesh, S. (**2020). Antibiofilm activity of symbiotic Bacillus species associated with marine gastropods. *Annals of Microbiology*, **70**: 1-12.

Ward-Rainey, N., Rainey, F. and **Stackebrandt, E. (**1996). A study of the bacterial flora associated with Holoturia atra. *Journal of Experimental Marine Biology and Ecology*, **203**: 11 –26.

Wesley, S.G. and **Satheesh, S. (**2009). Temporal variability of nutrient concentration in marine biofilm developed on acrylic panels. *Journal of Experimental Marine Biology and Ecology*, **379**(1-2): 1-7.

Zhang, X.Y., Hao, H.L., Lau, S.C.K., Wang, H.Y., Han, Y., Dong, L.M. and **Huang, R.M. (**2019). Biodiversity and antifouling activity of fungi associated with two soft corals from the South China Sea. *Archives of Microbiology*, **201**: 757-767.

النشاط المضاد للحشف للبكتيريا Epibiotic المرتبطة بالشعاب المرجانية الرخوة *Sarcophyton* sp. جمعت من وسط البحر الأحمر

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المستخلص. تعتبر المجتمعات الميكروبية المرتبطة باللافقاريات البحرية أنها مصدر غزير من المستقبلات النشطة بيولوجيا. هذه الدراسة تطرقت إلى البكتيريا المصاحبة للشعاب المرجانية الناعمة، والتي تم جمعها من البحر الأحمر بحثًا عن نشاط مضاد للحشف لتحديدها المركبات الطبيعية المضادة للحشف. أظهر مستخلص سلالة بكتيريا (*Ruegeria lacuscaerulensis*) KAU-MB3 المرتبط بالشعاب المرجانية الناعمة نشاطًا قويًا مضادًا للحشف في الاختبارات المعملية، حيث يقلل المستخلص البكتيري الخام من نمو البكتيريا المكونة للغشاء الحيوي ويثبط تشكيل biofilm بشكل ملحوظ. وأشارت مقايسة تسوية يرقات البرنكل إلى انخفاض كبير في تسوية اليرقات المعالجة بالمستخلص الخام. كما تم تحليل المستخلص الخام بواسطة GC- MS لفهم التركيب الكيميائي. وأظهرت النتائج وجود مركبات مثل: hexadecane و octatriacontyl pentafluoropropionate وcis-Z-à-bisabolene epoxide وgeranyl isovalerate. ختامًا أشارت هذه الدراسة إلى أن البكتيريا المصاحبة لشعاب الناعمة يمكن استخدامها كأحد المصادر المحتملة لمضادات التلوث الطبيعية.

الكلمات المفتاحية: الشعاب المرجانية الناعمة، التعايشين البكتيري، الحشف الحيوي، مركبات نشطة بيولوجيا، علم البيئة الميكروبي، البحر الأحمر.