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Original article

Assessing the diversity of bacterial communities from marine sponges and their bioactive compounds

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ABSTRACT

Symbiotic bacteria play vital roles in the survival and health of marine sponges. Sponges harbor rich, diverse and species-specific microbial communities. Symbiotic marine bacteria have increasingly been reported as promising source of bioactive compounds. A culturomics-based study was undertaken to study the diversity of bacteria from marine sponges and their antimicrobial potential. We have collected three sponge samples i.e. *Acanthaster carteri*, *Rhytisma fulvum* (soft coral) and *Haliclona caerulea* from north region (Obhur) of Red Sea, Jeddah Saudi Arabia. Total of 144 bacterial strains were isolated from three marine sponges using culture dependent method. Screening of isolated strains showed only 37 (26%) isolates as antagonists against oomycetes pathogens (*P. ultimum* and *P. capsici*). Among 37 antagonistic bacteria, only 19 bacterial strains exhibited antibacterial activity against human pathogens (Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 8739, *Enterococcus faecalis* ATCC 29212). Four major classes of bacteria i.e γ-*Proteobacteria*, α-*Proteobacteria, Firmicutes* and *Actinobacteria* were recorded from three marine sponges where γ-*Proteobacteria* was dominant class. One potential bacterial strain *Halomonas* sp. EA423 was selected for identification of bioactive metabolites using GC and LC-MS analyses. Bioactive compounds Sulfamerazine, Metronidazole-OH and Ibuprofen are detected from culture extract of strain *Halomonas* sp. EA423. Overall, this study gives insight into composition and diversity of antagonistic bacterial community of marine sponges and coral from Red Sea and presence of active metabolites from potential strain. Our results showed that these diverse and potential bacterial communities further need to be studied to exploit their biotechnological significance.

1. Introduction

Infectious diseases become a serious health risk to human population in many developing countries. Therefore, discovery of new compounds and antibiotics is needed to combat with these public health threats. Marine environment is diverse and represent a potent and promising source of new bioactive compounds from marine invertebrates, plants and their associated microbial communities (Adnan et al., 2018). Marine invertebrates encompass symbiotic microorganisms that produce diverse range of bioactive compounds. Symbiotic microorganisms play important role of protecting host against different predators by producing bioactive compounds. These bioactive molecules are diverse in function exhibiting immunosuppressive, antiviral, antifungal, antitumor, anti-inflammatory, antiprotozoal and many more functions of biotechnological and medical significance (Imhoff et al., 2011). Sponges provide a microhabitat for mutualistic microbes where microbes get shelter and protection and in turn byproducts of these microbes defend these host sponges against different microbial diseases (Taylor et al., 2007).

Microbial communities associated with marine invertebrates are diverse in their function and produced chemically diverse and biologically active metabolites (Livett et al., 2004). Marine sponges are promising source of biologically active metabolites

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but due to lack of their supply problem till today no compound has been approved as a drug (Thoms and Shrupp, 2005). Most of these compounds in sponges were also synthesized by symbiotic bacteria associated with them. Therefore, many researchers are focusing and paying more attention to sponge associated microbes that can be served as a renewable source for screening and isolation of bioactive metabolites (Simmons et al., 2008). Secondary metabolites help in chemical defense and this ecological role of microbes is believed to be essential for the survival of the host organisms. Sponges are sessile and soft bodied animals, mostly lacking morphological defense features such as spike, shells and spines (Faulkner, 2000). Production of these novel and unique biologically active metabolites largely rely on chemical defense of microbes.

In order to understand diversity of symbiotic bacterial communities' interactions and their function it is necessary to isolate, identify and study them phylogenetically. Until now, 25 different bacterial phyla and 2 archaeal lineages have been identified from marine sponges (Najafi et al., 2018). We therefore design a study to isolate, screen (antibacterial and antifungal activities) and identify symbiotic bacterial communities from sponges. Marine sponges *Acanthaster carteri*, *Rhytisma fulvum* (soft coral) and *Haliclona caerulea* were collected from Red Sea near Obhur region of Saudi Arabia.

2. Materials and methods

2.1. Sample collection

Sponge samples were collected at the depth of 40 m by SCUBA divers from Obhur region in Jeddah, Red Sea. Sponge samples were transferred immediately to the laboratory after collection and further processed for bacterial isolation. Identification of sponge samples was done by Dr. Mohsin from marine science department King Abdul-Aziz University by using morphological and microscopic parameters.

2.2. Isolation of bacteria from sponge samples

Sponge samples were cut finely into small pieces and grounded with sterile mortar and pestle. Sterile filtered seawater (FS) was used to made serial dilutions of sponge homogenate. Aliquots of 100 μl of 10-fold dilutions (10^{-3}, 10^{-4} and 10^{-5}) in were spread on five different isolation media i.e. half Tryptic soy agar (½ TSA), half nutrient agar (½ NA), half strength R2A (½ R2A), and marine agar (MA). To inhibit fungal contamination, cyclohexamide (50 μg/ml) was added to bacterial culture media. The culture plates were incubated for 5–7 days at 25 °C. Bacterial colonies were further purified and sub-cultured on 1/10 R2A medium.

2.3. Analysis of antagonistic activity against oomycetes

Antagonistic activity of bacterial isolates against fungal pathogens was tested using a confrontation bioassay (Bibi et al., 2012). Oomycete test pathogens, *Phytophthora capsici* and *Pythium ultimum* used in this bioassay were obtained in this laboratory. Using cross streak method on modified PDA media bacteria were screened for their antagonistic activity against oomycetes pathogens. Bacterial isolates were streaked on media perpendicular to edges of plate at 4 cm distance and freshly grown mycelial disc (6 mm) of oomycetes was placed in the centre of plate. These plates were incubated for 3–5 days at 28 °C. Positive bacterial isolates were checked twice to confirm their activity and their activity was recorded by measuring the inhibition zone of around each bacterial streak.

2.4. Screening for antibacterial activity

Bacteria isolates were checked for their antibacterial activity against human and plant pathogenic bacterial strains (Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 8739, *Enterococcus faecalis* ATCC 29212) using deferred bacterial inhibition assay. Bacterial isolates from marine sponges were cultured for 24hrs on ½ R2A media at 28 °C and then mixture of 0.1% soft agar with test strains was poured on agar plates containing bacterial strains. Before the plates were overlaid, test strains were pregrown in LB broth at 37 °C and diluted to final concentration of A600 = 0.1. After that plates were again incubated for 48hrs at 28 °C and the zone of inhibition was recorded.

2.5. DNA extraction and 16S rDNA gene analysis

Bacterial strains isolated in this study were further subjected to genomic DNA extraction. Genomic DNA extraction kit (Qiagen) was used to extract genomic DNA. For identification of strains, 16S rDNA gene analysis was performed. The 16S rDNA gene was amplified using primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′- GGTTACCTTGTTACGACTT-3′) and PCR was performed under conditions described previously (Bibi et al., 2012). Using PCR purification kit PCR products were purified (Qiagen) and sequenced commercially (Macrogen, South Korea). Bacterial isolates were identified after blast searches using the EzTaxon server (https://www.ezbiocloud.net) (Yoon et al., 2017). Alignments of 16S rRNA gene sequences was confirmed using CLUSTAL_X version 1.83 (Thompson et al., 1997) and further gaps between sequences were edited using BioEdit software version 4.7.3 (Hall, 1999). In MEGA6 Programme (Tamura et al., 2013), by using neighbor-joining method phylogenetic tree was generated based on the 16S rRNA gene sequences.

2.6. Bacterial culture conditions optimization and identification of metabolites from crude extract

Based on antagonistic activity and low 16S rRNA sequence similarity strain *Halomonas* sp. EA423 was selected for identification of secondary metabolites from culture extract. To optimize culture conditions for *Halomonas* sp. EA423, we have used four different culturing media i.e. Marine broth, ½ R2A, ½ TSB and ½NB. Strain *Halomonas* sp. EA423 was cultured and optical density (OD) was checked at different incubation times (24hrs, 36hrs and 72hrs). Antagonistic activity of the culture was checked after every 24hrs against two plant fungal pathogens, *P. capsici* and *Py. ultimum*. Further temperature (20–40 °C) and pH conditions (5–12) were optimized for *Halomonas* sp. EA423. Under optimized culture conditions strain *Halomonas* sp. EA423 was grown in culture and after 36hrs, 5 ml of bacterial culture was placed processed first at −70 °C (5mins) and then at 37 °C (5 mins). This process was repeated 5 times and then sample was centrifuged at 13000*g* (15 mins). 3 ml of supernatant after centrifugation was mixed with acetonitrile (12 ml) and vortexed (30sec). Centrifuged this sample again (13000*g* for 15 mins) and 300 μl of supernatant was used for LC-MS analysis. Samples were analyzed as described previously (Bibi et al., 2020). Agilent Mass Hunter (version B.06.00) was used for processing of raw data and for further analysis. In-house database was used for identification of metabolites from strain *Halomonas* sp. EA423. Using Gas-chromatography mass spectrometry (GC–MS) metabolites were further analyzed using Shimadzu GCMS-QP2010 Ultra as described previously (Bibi et al., 2020).

2.7. Statistical analysis

For identification of bacterial metabolites different databases (ChemSpider, SciFinder, PubChem, ChEMBL and National Institute of Standards and Technology (NIST) databases were used.

2.8. Nucleotide sequence accession numbers

Nucleotide sequences of the bacterial isolates from sponges *Acanthaster carteri*, *Rhytisma fulvum* and *Haliclona caerulea* were deposited in the GenBank database under accession numbers MK720073-MK720078, MK720079-MK720086 and MK720087-MK720101 respectively.

3. Results

3.1. Isolation of bacteria from sponges

Samples were identified as *Acanthaster carteri, Rhytisma fulvum* and *Haliclona caerulea* (Fig. 1a–c) and were further used for isolation of bacteria. Bacterial culturing was performed on four different media i.e. ½ NA, ½ R2A, MA, ½ TSA. Colony forming units (CFU) and morphology of bacteria was different on different media. We tested different media and high numbers of bacteria were seen on ½ TSA and ½ R2A indicating that low nutrient composition is favorable for recovery and culturing of bacteria from these sponge samples. While media with high concentration of nutrients didn't favor growth of bacteria hence low number of colonies were seen on ½ NA and MA. Therefore, culturing media and conditions are key factors for growth and isolation of bacteria from any environmental sample. Using four different type of culturing media, 144 different bacteria were isolated from these three marine sponges' samples. Only 37 (26%) showed inhibition against oomycetes plant pathogens (Table 1).

3.2. Phylogenetic relationship of antagonistic bacteria

Phylogenetic relationship of the antagonistic bacteria was revealed on the basis of 16S rRNA gene sequences. From sponge *A. carteri,* three different genera i.e *Bacillus, Staphylococcus*, *Microbulbifer*. For soft coral, *R. fulvum* there were eight different antagonistic bacteria belong to four different genera, *Staphylococcus, Micrococcus, Bacillus* and *Ruegeria.* From sponge *H. caerulea*, high percentage of antagonistic bacteria was recovered. These antagonistic bacteria belong to 7 different genera i.e *Halomonas*, *Pseudovibrio, Vibrio*, *Bacillus*, *Labrenzia, Microbulbifer*, *Psychrobacter*, and further belong to 3 different classes (*γ-Proteobacteria, Firmicutes* and *α-Proteobacteria*) where *γ-Proteobacteria* was dominant class (Table 1). Antagonistic bacterial isolates showed sequence similarities from 99.1–100, 96.2–100, 96.7–100% from *A. carteri,* R. *fulvum* and *H. caerulea* respectively (Table 2). Some bacterial antagonists from these three sponges with sequence similarities < than 97% were identified as new specie from respective genera. One strain of *Bacillus* sp. (EA410) from R. *fulvum* and *Labrenzia* sp. (EA419) isolated from *H. caerulea,* were identified as novel strain with low 16S rRNA gene sequence similarities (96%)

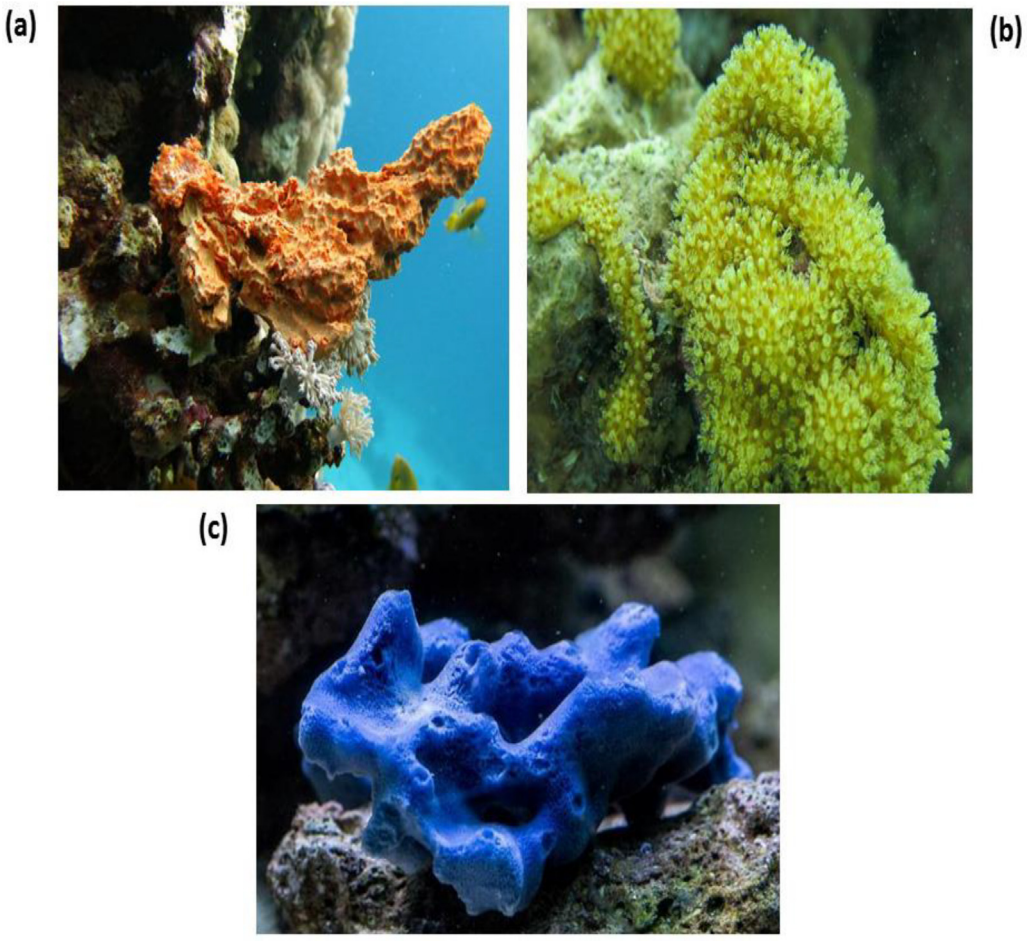

Fig. 1. Samples of three marine sponges, (a) *Acanthaster carteri* (b) *Rhytisma fulvum*, and (c) *Haliclona caerulea* collected from Red sea.

Table 1
Distribution of total number of bacteria and antagonistic one from marine sponge samples.

Sponge	Isolates	Antagonists	Antagonists (%)	Dominant phylum
Acanthaster carteri	36	6	16	NA
Rhytisma fulvum	44	8	18	*Firmicutes*
Haliclona caerulea	64	15	23	*γ-Proteobacteria*
	144	37	26	

Table 2
Taxonomic identification, antifungal and antibacterial activity of bacteria from sponges and coral, *A. carteri*, *R. fulvum* and *H. caerulea*.

Lab number	Accession Number	Similarity with closest type strain[a]	% identity[b]	Antifungal activity[c]			Antibacterial Activity[d]		
				P. capsici	*Py. ultimum*	*S. aureus*	*P. aeruginosa*	*E. coli*	*E. faecalis*
Acanthaster carteri									
EA401	MK720073	*Bacillus subtilis* subsp. *Inaquosorum* KCTC 13,429 (T)	99.7	+++	+++	+	–		+
EA402	MK720074	*Staphylococcus warneri*. ATCC 27,836 (T)	99.92	++	w	–	–	+	
EA403	MK720075	*Bacillus galliciensis*.BFLP-1 (T)	100	++	w	–	–	–	–
EA404	MK720076	*Microbulbifer variabilis*.Ni-2088 (T)	99.09	+	+	+	–	+	–
EA405	MK720077	*Microbulbifer epialgicus*.F-104 (T)	99.12	–	+	–	–	+	–
EA406	MK720078	*Microbulbifer variabilis*.Ni-2088 (T)	98.78	–	+	–	–	–	–
Rhytisma fulvum									
EA407	MK720079	*Staphylococcus haemolyticus*.ATCC 29,970 (T)	99.78	++	+++	–	+	+	–
EA408	MK720080	*Micrococcus aloeverae*.AE-6 (T)	99.81	–	–	–	+	–	–
EA409	MK720081	*Bacillus paralicheniformis*.KJ-16 (T)	98.07	–	+	+	–	+	–
EA410	MK720082	*Bacillus paralicheniformis*.KJ-16 (T)	96.25	+	+	+	–	+	–
EA411	MK720083	*Staphylococcus haemolyticus*.ATCC 29,970 (T)	100	++	++	–	–	–	–
EA412	MK720084	*Bacillus paralicheniformis*.KJ-16 (T)	99.51	–	+	–	–	–	–
EA413	MK720085	*Bacillus licheniformis*.ATCC 14,580 (T)	97.14	+	+	+	–	w	–
EA414	MK720086	*Ruegeria arenilitoris*.G-M8 (T)	99.21	++	++	–	–	–	–
Haliclona caerulea									
EA415	MK720087	*Halomonas denitrificans*.M29 (T)	97.9	+	+	–	–	–	–
EA416	MK720088	*Pseudovibrio denitrificans*.DN34 (T)	99.85	–	+	–	+	–	–
EA417	MK720089	*Vibrio owensii*.LMG 25,443 (T)	99.93	+	+	–	+	–	+
EA418	MK720090	*Bacillus gibsonii*.DSM 8722 (T)	99.93	+	–	–	+	–	–
EA419	MK720091	*Labrenzia aggregata*.IAM 12,614 (T)	96.7	+	–	–	–	–	–
EA420	MK720092	*Vibrio harveyi*.NBRC 15,634 (T)	99.92	+	+	–	–	–	+
EA421	MK720093	*Microbulbifer variabilis*.Ni-2088 (T)	99.92	–	+	–	–	–	–
EA422	MK720094	*Psychrobacter aquaticus*.CMS 56 (T)	99.77	–	+	–	–	+	–
EA423	**MK720095**	***Halomonas denitrificans*.M29 (T)**	**97.89**	**++**	**+++**	**–**	**–**	**+**	**–**
EA424	MK720096	*Microbulbifer variabilis*.Ni-2088 (T)	99.84	–	–	–	–	–	–
EA425	MK720097	*Vibrio inhibens*.BFLP-10 (T)	99.49	–	+	–	–	+	–
EA426	MK720098	*Vibrio neocaledonicus*.NC470 (T)	100	–	–	–	–	+	–
EA427	MK720099	*Halomonas nitroreducens*.11S (T)	98.22	+	+	–	–	+	–
EA428	MK720100	*Halomonas denitrificans*.M29 (T)	98.32	+	+	–	–	+	–
EA429	MK720101	*Pseudovibrio denitrificans*.DN34 (T)	99.85	w	+	–	+	+	–

[a] Identification of bacterial strain based on partial 16S rRNA gene sequence analyses.
[b] % similarity of each bacterial strain with closely related type strain.
[c] Antagonistic activity of sponge-associated bacteria. The activity was measured after 4–5 days incubation at 28 °C by measuring the clear zone of fungal growth inhibition: -, Negative, +, 3 mm; ++, between 4 and 6 mm; +++, between 7 and 9 mm.
[d] Antibacterial activity against human pathogenic bacteria: -, Negative; +, 2–3 mm.

with their respective type strains. Neighbor Joining (NJ) phylogenetic trees for bacterial isolates of three marine sponges were constructed using 16S rRNA gene sequence data (Fig. 2a–c). Bootstrap or branch values were high in all three phylogenetic trees.

3.3. Antimicrobial activity

Antagonistic activity of bacterial isolates from three sponge samples was tested against oomycetes plant pathogens (*P. capsici* and *Py. ultimum*), and pathogenic bacteria including *S. aureus, P. aeruginosa, E. coli* and *E. faecalis* (Table 2). From sponge *A. carteri,* total 6 (16%) from 36 bacterial strains were active against oomycetes. Two major classes *γ-Proteobacteria* (n = 3), *Firmicutes* (n = 3), of antagonistic bacteria from *A. carteri* were identified. *γ-Proteobacteria* was being the dominant class of bacteria from *A. carteri*. Soft coral *R. fulvum* exhibited presence of 8 (18%) antagonists from 44 bacterial isolates. Antagonistic bacterial isolates belong to three major classes, *Firmicutes* (n = 6), *α-Proteobacteria* (n = 1) and *Actinobacteria* (n = 1) where dominant class was *Firmicutes*. From sponge *H. caerulea*, 64 bacteria were screened and 15 (23%) of them showed inhibitory activity. Three classes of bacteria were identified: *γ-Proteobacteria*, *Firmicutes* and *α-Proteobacteria* where *γ-Proteobacteria* was dominant class. Using agar spot test further bacteria were checked for their antibacterial potential against human pathogenic bacteria. Isolates from sponge *A. carteri,* showed only 4 isolates positive for antibacterial activity. No isolate showed inhibition to *P. aeruginosa*. From *R. fulvum,* only five isolates were positive for antibacterial activity. No isolate showed inhibition against *E. faecalis*. Eleven isolates from sponge *H. caerulea* were active against human pathogenic bacteria tested. No one was active against *S. aureus*.

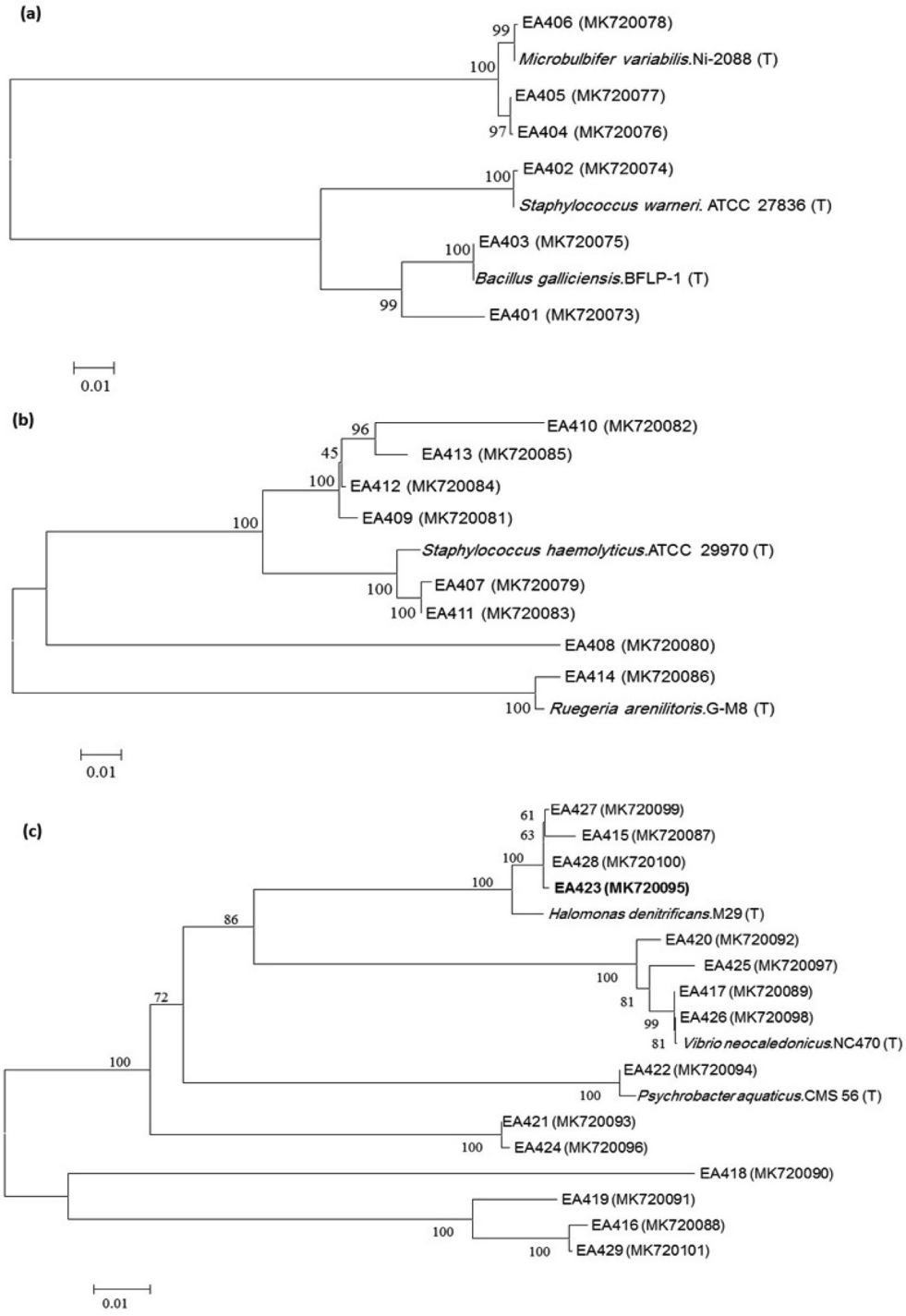

Fig. 2. Phylogenetic distribution of bacteria isolated from marine sponges and coral (a) *Acanthaster carteri* (b) *Rhytisma fulvum*, (c) *Haliclona caerulea* on the basis of 16S rRNA gene sequences of bacterial isolates and closely related sequences of the type strains of other species. The phylogenetic relationships were inferred from the 16S rRNA gene by using the neighbor-joining method from distances computed with the Jukes-Cantor algorithm. Bootstrap values (1,000 replicates) are shown next to the branches. GenBank accession numbers for each sequence are shown in parentheses. Bar, 0.01 accumulated changes per nucleotide.

3.4. Identification of bacterial metabolites using LC-MS and GC–MS analyses

Culture conditions were optimized before identification of secondary metabolites. In ½ R2A culture (pH = 7.5, temp = 28 °C), maximum inhibition against pathogens were shown by *Halomonas* sp. EA423. For identification of bioactive metabolites from *Halomonas* sp. EA423, GC and LC-MS analyses were used. Both analyses showed presence of different metabolites including some bioactive molecules from *Halomonas* sp. EA423 (Fig. 3a–b). LC-MS analysis showed peaks of 3 secondary bioactive compounds in both positive and negative mode (Fig. 3a and b). These compounds include Sulfamerazine, Metronidazole-OH and Ibuprofen. In GC–MS, hundreds of peaks are recorded but no bioactive compound have been detected from culture extract (Table S1). Using NIST library, mass spectra were compared and compounds were identified.

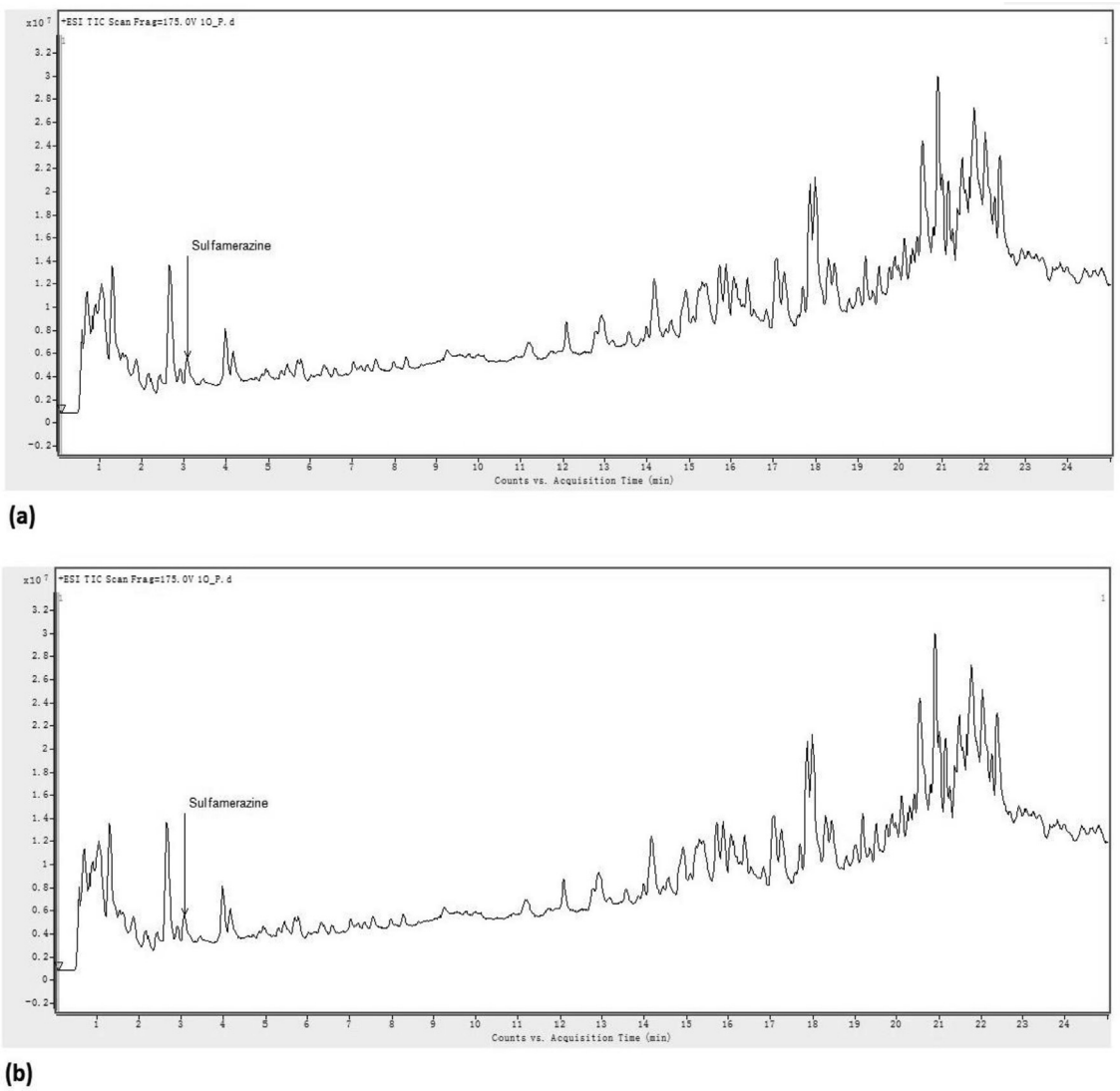

Fig. 3. Bioactive secondary metabolites detection in culture extract of strain *Halomonas* sp. EA423 detected by LC/MS analysis. (a) Positive mode and (b) negative mode LC/MS analysis.

4. Discussion

Sponges are sessile filter-feeders and harbor distinct microbial symbionts in seawater (Pita et al., 2018). Microbial symbionts are diverse, species-specific and play a pivotal role in persistence of sponges by playing functional roles such as production of secondary metabolites, nutrient cycling, photosynthesis and production of vitamins (Cárdenas et al., 2019). In the present study, three marine sponges were studied for identification of symbiotic bacterial communities. Our results showed these sponges host diverse microbial consortia. These sponges were dominated by γ-*Proteobacteria* and *Firmicutes* that is in concordance with some previous studies of sponges from marine sources (Hentschel et al. 2001; Kennedy et al. 2009; Margassery et al. 2012).

Sponge-associated microbial communities are considered a reservoir of bioactive compounds exhibiting antiviral, antiprotozoal, antimicrobial, neurosuppressive, anti-inflammatory and many other protective functions. These bioactive substances belong to different classes of chemical compounds mainly, fatty acids, peroxides, alkaloids, sterols, nucleosides, bioactive terpenes etc. (Thomas et al., 2010). Therefore, in present study we isolate, screen and identify bioactive substances from selected strain of bacteria. Cultivation of bacteria is critical step in recovery of potential bacterial isolates from different samples. Culture media mimic the natural environment of bacteria therefore, four different culturing media were used for cultivation of bacteria. High numbers of bacteria were recovered from ½TSA and ½R2A indicating that low nutrient concentration and added sea water favors the growth of bacteria from three marine sponges. Several previous studies have also confirmed that culturing media and conditions are important for recovery and growth of bacteria from different environmental samples (Medina et al., 2017; Bonnet et al., 2020).

Sponge-associated bacteria were studied phylogenetically using 16S rRNA sequences of bacteria. Antagonistic bacterial isolates belong to 10 different genera further belong to four different classes' i.e γ-*Proteobacteria, Firmicutes*, α-*Proteobacteria* and *Actinobacteria.* Members of these four classes are have been reported as potential producers of various active metabolites (Thomas et al., 2010). It highlights the potential of marine sponges as a producers of active bacterial strains in this study. They comprised of four different genera i.e *Halomonas*, *Vibrio*, *Microbulbifer, Psychrobacter*. Members of this class were consistently isolated from various marine sponges across the world (Webster and Tomas, 2016). In this study, highest number of antagonistic bacteria were

isolated from *H. caerulea* comprising of 7 genera where *Halomonas* and *Vibrio* are two dominant genera. Brominated diphenyl ethers extracted from *Vibrio* sp. associated with *Dysidea* sp. exhibited both cytotoxic and antibacterial activity (Elyakov et al., 1991). An antibacterial compound, andrimid was previously isolated from symbiotic *Vibrio* sp. from sponge *Hyatella* sp. (Oclarit et al., 1994). Previous studies of sponge genus *Haliclona* have reported 190 metabolites exhibiting different bioactivities (Yu et al., 2006). Symbiotic bacteria from *Haliclona simulans* were found to be rich source of biological activities against drug-resistant pathogenic bacteria (Li et al., 2007). Therefore, strain of *Halomonas* sp. EA423 was selected from *H. caerulea* in this study for identification of active metabolites from crude culture extract.

Marine *Halomonads* are gram-negative, halophilic and oligotrophic organisms that are pervasive to marine environments. Marine *Halomonas* have been described as a source of bioactive metabolites and surfactants (Khalifa et al., 2019; Arahal and Ventosa, 2006). Bioactive compopunds of marine *Halomonas* spp. exhibited activity against HepG2 (hepatocellular carcinoma) and HM02 (gastric adenocarcinoma) cell lines (Bitzer et al., 2006). Cytotoxic compounds exhibiting antitumor activities against T-leukemia cells were reported from *Halomonas* spp. (Ruiz-Ruiz et al., 2011). *Halomonas* spp. (EA423) was chosen on the basis of its activity and low 16S rRNA similarity to relative type strain. Many previous studies have focused on identification and adaptations of *Halomonas* to extreme environmental conditions. However, few studies have reported their bioactivities and secondary metabolites. In current study, *Halomonas* sp. EA423 was found to be active against both plant fungal pathogens and human pathogen *E.coli*. Before fermentation, different media were tested for growth and maximum yield of antimicrobial activity. Production of microbial secondary metabolite is dependent on the fermentation conditions. Growth media and conditions are two variables that are very important and effect production of secondary metabolites (Bode et al., 2002). Highest antimicrobial activity for *Halomonas* sp. EA423 was observed in ½R2A. This is because of the reason that some bacterial strains produce secondary metabolites in presence of certain nutrients they needed.

Both GC and LC-MS analyses of *Halomonas* sp. EA423 showed the presence of secondary metabolites especially Sulfamerazine, Metronidazole-OH and Ibuprofen. The sulphonamides are purely synthetic antibacterials agents and used against different bacterial diseases. Sulfamerazine is also a sulfonamide antibacterial compound identified in current studied strain. Metronidazole-OH is another bioactive compound identified. It is used widely against anaerobic micro-organisms, including bacterial and protozoal diseases in farm animals. Previous studies from *Halomonas* have reported the presence of glycolipids and glucoproteins exhibiting cytotoxic activities but such antimicrobial compounds were not reported before. Presence of these compounds highlighted important role of these symbiotic bacteria in marine environment. Current study confirmed that species of *Haliclona* are host of potential bioactive bacterial strains.

Conclusively, our data reported the important role of symbiotic bacteria from marine sponges from Red Sea. These sponges are reservoir of potential bacterial strains that are able to produce antimicrobial compounds. This study also increased our understanding about functional role of symbiotic bacteria in marine sponges. These bioactive compounds from bacteria play important role in defense of host sponge against different marine pathogens. In this regard, sponge-associated bacteria are promising source for the discovery of bioactive compounds. Further research is essential to identify the metabolites from other potential strains and to evaluate their potential for development.

CRediT authorship contribution statement

Fehmida Bibi: Methodology, Investigation, Data curation, Validation, Writing - original draft, Project administration, Supervision, Funding acquisition. **Muhammad Imran Naseer:** Methodology, Investigation, Data curation, Validation, Writing - original draft, Project administration, Supervision, Funding acquisition. **Esam Ibraheem Azhar:** Methodology, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.03.042.

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