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Antibiotic Resistance of Symbiotic Marine Bacteria Isolated from Marine Organisms in Jeju Island of South Korea

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Abstract

We investigated antibiotics resistance of bacteria isolated from marine organisms in Jeju Island of South Korea. We isolated 17 strains from a marine sponge, algaes, and sea water collected from Biyangdo on Jeju Island. Seventeen strains were analyzed by 16S rRNA gene sequencing for species identification and tested antibiotic susceptibility of strains against six antibiotics. Strain JJS3-4 isolated from *S. siliquastrum* showed 98% similarity to the 16S rRNA gene of *Formosa spongicola* A2^T and was resistant to six antibiotics. Strains JJS1-1, JJS1-5, JJS2-3, identified as *Pseudovibrio* spp., and *Stappia* sp. JJS5-1, were susceptive to chloramphenicol and these four strains belonged to the order *Rhodobacterales* in the class *Alphaproteobacteria*. *Halomonas anticariensis* JJS2-1, JJS2-2 and JJS3-2 and *Pseudomonas rhodesiae* JJS4-1 and JJS4-2 showed similar resistance pattern against six antibiotics. We could isolate bacteria from marine organisms and their antibiotic resistance investigated, and conducted this study under the premise that such bacteria could produce secondary metabolites that could bring about useful antibiotic effects, resulting in species-specific results. We have a lot of unknown marine resources that we have not been able to explore yet. Bacteria are a valuable resource that can be developed into new useful materials.

Keywords: Marine bacteria; Antibiotic resistance; Antibiotic susceptibility; Marine organisms

Introduction

Symbiotic marine bacteria chemically protect their host organisms from pathogenic organisms [1]. Additionally, symbiotic bacteria are known to have various roles, such as host health, nutrition, and antibiotic production for host organisms [2-6]. Over the past 85 years, about 50,000 natural products have been discovered from microorganisms. More than 10,000 of these compounds are biologically active and more than 8,000 are antibiotics [7]. Bacteria efficiently produce natural products that could prove to be useful drugs [8]. Over the past few decades, bacteria have evolved to resist well-known antibiotics [9]. As a result, hospitals have seen a dramatic rise in drug-resistant infections, many of which are lethal. To identify new antibiotics, scientists often make use of the natural chemical defenses of fungi and bacteria, altering these natural antibiotics to produce new ones. Bacteria have an ability to produce bioactive secondary metabolites, such as antimicrobials, antifungals, antitumorals, immunosuppressants, and antibiotics [10]. Biochemical and physiological pathways of bacteria may be responsible for antibiotic resistance [11]. Therefore, we have attempted to isolate marine bacteria that live together with marine organisms and explore their antibiotic resistance to help identify novel marine derived antibiotics such as Fijimycins and Marinopyrroles [12,13].

Materials and Methods

Collection of marine organisms

We collected a marine sponge (*Callyspongia confoederata*), brown algae (*Sargassum siliquastrum* (Merens ex Turner) C. Agardh), *S. macrocarpum* C. Agardh, and *Myagropsis myagroides* (Mertens ex Turner) Fensholt, green algae (*Cladophora wrightiana* var. *minor* C. Hoek & M. Chihara), and sea water by SCUBA diving from Biyangdo on Jeju Island in March 2016.

Isolation and culture of bacteria associated with marine organisms

These six samples are washed with sterile sea water as soon as they are collected, diluted 1:20 with sterile sea water and heated at 55°C for 10 min and 20 µl was inoculated onto A1SW isolation medium (10 g soluble starch, 4 g yeast extract, 2 g peptone, 16 g agar, 1 L filtered and sterilized sea water) and incubated at 25°C [14]. After 7 days, separated single colonies from the cultured media were transferred to new A1SW plates for pure isolation of marine bacteria, incubated at 25°C for 3-4 days, and then stored in 20% glycerol (v/v) suspensions at -80°C.

DNA extraction, PCR amplification, DNA sequencing, and phylogenetic analysis

Chromosomal DNAs of pure cultivated bacteria were isolated using a LaboPass™ tissue genomic DNA isolation kit (Cosmogenetech, Daejeon, Korea). PCR was employed to amplify 16S rRNA genes using the primers 27F and 1492R [15], and the products of which were purified with a LaboPass™ PCR purification kit (Cosmogenetech, Daejeon, Korea), according to the manufacturer's protocol, and sequenced on a capillary electrophoresis instrument (Applied Biosystems 3730XL,

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CA, USA). Similarities between the 16S rRNA gene sequence of pure isolated bacteria and those of other bacteria previously described were determined by performing BLAST searches of the GenBank/EMBL/DDBJ database [16]. Multiple sequence alignment of representative sequences was carried out using Clustal W version 2·0 [17]. Maximum-likelihood, neighbor-joining, and minimum-evolution trees were generated using MEGA 6·0·6 [18].

Disc diffusion test for Antibiotic susceptibility

Susceptibility to six antibiotics (30 µg chloramphenicol, 15 µg erythromycin, 30 µg gentamicin, 15 µg lincomycin, 30 µg tetracycline, and 30 µg vancomycin) (Liofilchem, Roseto degli Abruzzi, Italy) was determined by the disc diffusion method [19]. For all isolated strains, 100 µl (5×10^5 CFU ml^{-1}) was spread onto A1SW medium, and 6 antibiotics were inoculated onto the center of the agar plates, which were incubated at 25°C for 48 h. After incubation, clear zones of growth inhibition were measured to the nearest millimeter. The clear zone disc is the zone of inhibition that indicates the extent of the test organism's inability to survive in the presence of the test antibiotic.

Nucleotide sequence accession numbers

All bacteria 16S rRNA gene sequences isolated from marine organisms described in this study have been deposited in the GenBank nucleotide sequence database under the accession numbers MF461049-MF461065.

Results and Discussions

Culture, isolation and species identification of marine bacteria

We isolated 17 strains of marine bacteria from marine sponges (*Callyspongia confoederata*), algaes (*Sargassum siliquastrum*, *S. macrocarpum*, *Cladophora wrightiana*, *Myagropsis myagroides*), and sea water collected from Biyangdo on Jeju Island (Table 1). We found that the bacterial strains belong to six families/orders (*Halomonadaceae* / *Oceanospirillales*, *Pseudomonadaceae* / *Pseudomonadales*, *Pseudoalteromonadaceae* / *Alteromonadales*, *Rhodobacteraceae* / *Rhodobacterales*, *Flavobacteriaceae* / *Flavobacteriales*, and *Bacillaceae* / *Bacillales*) and four classes (*Gammaproteobacteria*, *Alphaproteobacteria*, *Flavobacteria*, and *Bacilli*) (Table 1). Analysis of strain JJS3-4 isolated from *S. siliquastrum* revealed 98% similarity to the 16S rRNA gene of *Formosa spongicola* $A2^T$ [20].

Phylogenetic analysis of marine bacteria isolated from marine organisms

Analysis of the 16S rRNA sequences from the 17 isolates, along with similar type strains, revealed a significant level of diversity (Figure 1). This approach identified species that have been exclusively reported from marine habitats. These bacteria consist of four classes (*Alphaproteobacteria* and *Gammaproteobacteria*, *Flavobacteria*, and *Bacilli*) based on 16S rRNA gene sequencing and phylogenetic analysis (Figure 1).

Antibiotic susceptibility of isolated marine bacteria

We tested the antibiotic resistance of the 17 strains against 6 antibiotics (30 µg chloramphenicol, 15 µg erythromycin, 30 µg gentamicin, 15 µg lincomycin, 30 µg tetracycline, and 30 µg vancomycin) by disc diffusion test (Table 2). In contrast to the other bacteria tested, strain JJS3-4, identified as *Formosa* sp. and isolated from *S. siliquastrum*, was resistant to the six antibiotics, indicating that the need to study the useful secondary metabolites production of this strain (Table 2). Strain JJS1-1, JJS1-5, and JJS2-3, which were identified as *Pseudovibrio* spp., and *Stappia* sp. JJS5-1 were susceptive to chloramphenicol (Table 2). In addition, *Halomonas anticariensis* (strains JJS2-1, 2-2, and 3-2) and *Pseudomonas rhodesiae* (strain JJS4-1 and JJS4-2) showed similar resistance pattern against six antibiotics (Table 2). We performed this experiment three times and then calculated the mean value except for the bounce value and are shown to Table 2 as mean values.

Conclusion

Many marine bacteria are associated with marine sponges and marine algae and such isolated bacteria have useful physiological activities [21,22]. The media and culture conditions for bacteria can significantly influence the isolation and cultivability of distinct bacterial isolates [23]. In this study, we obtained 17 cultivable heterotrophic bacteria isolates from marine organisms using a culture-dependent method. Strain JJS1-1, JJS1-5 and JJS 2-3, *Pseudovibrio* spp. isolated from sea water and sponge were shown

#	Strain	The closest species (16S rRNA gene similarity, %)	Origin source
1	**JJS1-1**	*Pseudovibrio* sp. (100)	Sea water
2	**JJS1-2**	*Pseudoalteromonas* sp. (100)	
3	**JJS1-3**	*Bacillus velezensis* (100)	
4	**JJS1-4**	*Bacillus firmus* (100)	
5	**JJS1-5**	*Pseudovibrio* sp. (100)	
6	**JJS2-1**	*Halomonas anticariensis* (100)	*Callyspongia confoederata*
7	**JJS2-2**	*Halomonas anticariensis* (100)	
8	**JJS2-3**	*Pseudovibrio ascidiaceicola* (100)	
9	**JJS3-1**	*Bacillus pumilus* (100)	*Sargassum siliquastrum* (Merens ex Turner) C. Agardh
10	**JJS3-2**	*Halomonas anticariensis* (100)	
11	**JJS3-3**	*Bacillus pumilus* (100)	
12	**JJS3-4**	*Formosa spongicola* (98)	
13	**JJS4-1**	*Pseudomonas rhodesiae* (100)	*Cladophora wrightiana* var. *minor* C. Hoek & M. Chihara
14	**JJS4-2**	*Pseudomonas rhodesiae* (100)	
15	**JJS5-1**	*Stappia* sp. (100)	*Myagropsis myagroides* (Mertens ex Turner) Fensholt
16	**JJS5-2**	*Cobetia* sp. (100)	
17	**JJS6-2**	*Bacillus amyloliquefaciens* (100)	*Sargassum macrocarpum* C. Agardh

Table 1: Sources for isolation of marine bacteria.

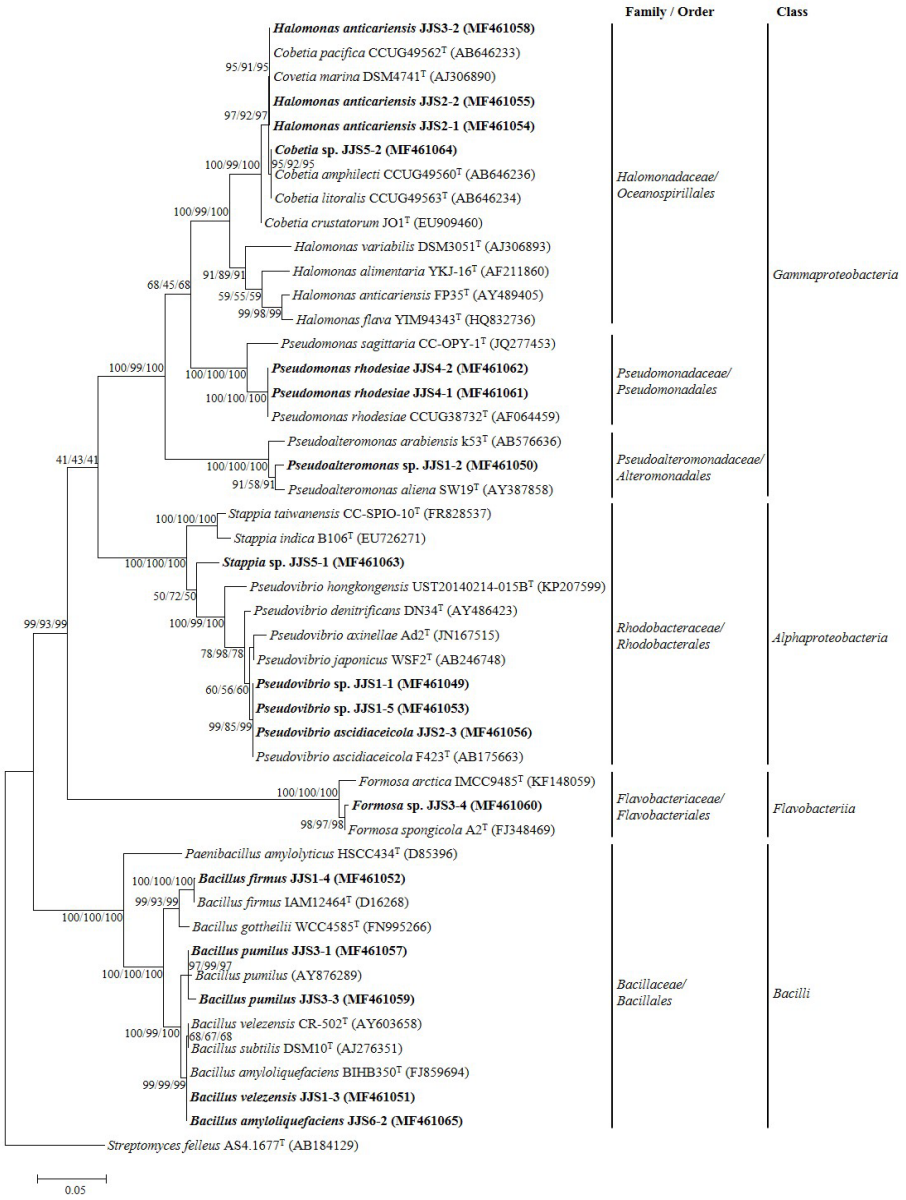

Figure 1: Phylogenetic tree conducted using 16S rRNA gene sequences of 17 marine-derived bacteria and type strains in this study. Bootstrap values 40% (from 1000 replicates) from maximum-likelihood / neighbor-joining / minimum-evolution analyses are shown at branch points. Type strain *Streptomyces felleus* AS4.1677^T was used as outgroup. Bar 0.05 substitutions per nucleotide position.

susceptibility against chloramphenicol. Among these bacteria, *Pseudovibrio* spp. Strain JJS1-1 and JJS1-5 showed high antioxidant activities and are considered to be useful marine resources as antioxidant-producing strains (Table 3). Bacterial isolates belonging to the genera *Pseudovibrio* are known for their ability to produce antibiotics. *Pseudovibrio* isolates originating from marine invertebrates such as tunicates, corals, and sponges showed antimicrobial activity [24-26]. The antioxidants generally produced by marine environment include polyphenolic metabolites such as flavonoids, cinnamic acid, furan, and phlorotannins [27,28]. So, we are doing to search bioactive compounds from these results now.

Also, strain JJS3-4 was shown 98% similarity in 16S rRNA gene sequence with *Formosa spongicola* A2^T that was represented strong resistance against all antibiotics. In addition to, this strain is considered to be a new strain based on the 16S rRNA gene results. By Challinor and Bode, unexplored bacterial strains from unusual sources could play in the search for such novel compounds [9]. So, it will be worth exploring the potentially useful materials that these bacteria produce via large-scale culture approaches.

Based on our findings, our results show that 17 marine bacteria derived from marine resources have species-specific results for antibiotics although the isolated sources are different. Our study not only suggests the possibility of novel antibiotic materials that can be used as a source for natural products (chemically identical to the pure natural product) but also indicates that this approach can be useful in delineating the taxonomic composition of associated microbial communities.

Strain	The closest Species (16S rRNA gene similarity, %)	Family/Order	Class	Zone of inhibition (mm)*					
				1†	2	3	4	5	6
JJS3-2	*Halomonas anticariensis* (100)	*Halomonadaceae /Oceanospirillales*	*Gammaproteobacteria*	17.8	8.8	21.9	GW‡	15.1	GW
JJS2-2	*Halomonas anticariensis* (100)			16.8	8.1	20.9	GW	16.5	GW
JJS2-1	*Halomonas anticariensis* (100)			18.9	8.3	21	GW	14.8	GW
JJS5-2	*Cobetia* sp. (100)			19.8	8.5	24.8	GW	GW	GW
JJS4-2	*Pseudomonas rhodesiae* (100)	*Pseudomonadaceae /Pseudomonadales*		GW	GW	34.8	GW	9.5	GW
JJS4-1	*Pseudomonas rhodesiae* (100)			GW	GW	26.8	GW	12	GW
JJS1-2	*Pseudoalteromonas* sp. (100)	*Pseudoalteromonadaceae /Alteromonadales*		21	7.6	14.1	GW	14.9	GW
JJS5-1	*Stappia* sp. (100)	*Rhodobacteraceae /Rhodobacterales*	*Alphaproteobacteria*	47.8	10.5	32.3	GW	GW	GW
JJS1-1	*Pseudovibrio* sp. (100)			44.8	25.4	18.5	11.1	25.9	GW
JJS1-5	*Pseudovibrio* sp. (100)			44.3	25.5	19.8	GW	24.1	GW
JJS2-3	*Pseudovibrio ascidiaceicola* (100)			45.8	20.8	18.9	GW	24.5	GW
JJS3-4	*Formosa spongicola* (98)	*Flavobacteriaceae /Flavobacteriales*	*Flavobacteriia*	GW	GW	GW	GW	GW	GW
JJS1-4	*Bacillus firmus* (100)	*Bacillaceae /Bacillales*	*Bacilli*	37.3	27.5	29	13.8	32	28
JJS3-1	*Bacillus pumilus* (100)			20.8	18.9	21	GW	20.6	19.3
JJS3-3	*Bacillus pumilus* (100)			18.4	17.1	20.4	8.6	22	21.3
JJS1-3	*Bacillus velezensis* (100)			28.1	24.9	20.3	GW	24	20.9
JJS6-2	*Bacillus amyloliquefaciens* (100)			25.8	42	36.3	GW	35	30.8

*Each antibiotic was loaded onto a disk (8 mm in diameter).

†Concentration: 1; 30 μg chloramphenicol, 2; 15 μg erythromycin, 3; 30 μg gentamicin, 4; 15 μg lincomycin, 5; 30 μg tetracycline, 6; 30 μg vancomycin.

‡GW: Bacterium grows well on agar plate with antibiotics.

Table 2: The result of 17 strains for disc diffusion antibiotic susceptibility test.

Strain	Species (Similarity, %)	IC_{50} (μg ml^{-1})
JJS1-1	*Pseudovibrio* sp. (100)	23
JJS1-2	*Pseudoalteromonas* sp. (100)	448
JJS1-3	*Bacillus velezensis* (100)	116
JJS1-4	*Bacillus firmus* (100)	437
JJS1-5	*Pseudovibrio* sp. (100)	37
JJS2-1	*Halomonas anticariensis* (100)	296
JJS2-2	*Halomonas anticariensis* (100)	208
JJS2-3	*Pseudovibrio ascidiaceicola* (100)	126
JJS3-1	*Bacillus pumilus* (100)	85
JJS3-2	*Halomonas anticariensis* (100)	587
JJS3-3	*Bacillus pumilus* (100)	209
JJS3-4	*Formosa spongicola* (98)	326
JJS4-1	*Pseudomonas rhodesiae* (100)	297
JJS4-2	*Pseudomonas rhodesiae* (100)	511
JJS5-1	*Stappia* sp. (100)	829
JJS5-2	*Cobetia* sp. (100)	>2500
JJS6-2	*Bacillus amyloliquefaciens* (100)	1871

Table 3: ABTS radical scavenging activities of 17 strains of marine bacteria.

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Refrences

1. Gil-Turnes MS, Hay ME, Fenical W (1989) Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus. Science 246: 116-118.
2. Beinart RA, Nyholm SV, Dubilier N, Girguis PR (2014) Intracellular Oceanospirillales inhabit the gills of the hydrothermal vent snail *Alviniconcha* with chemosynthetic, γ-proteobacterial symbionts. Environ Microbiol Rep 6: 656-664.
3. Rua CP, Trindade-Silva AE, Appolonario LR, Venas TM, Garcia GD, et al. (2014) Diversity and antimicrobial potential of culturable heterotrophic bacteria associated with the endemic marine sponge *Arenosclera brasiliensis*. Peer J 2: e419.
4. Gardéres J, Bedoux G, Koutsouveli V, Crequer S, Desriac F, et al. (2015) Lipopolysaccharides from commensal and opportunistic bacteria: characterization and response of the immune system of the host sponge *Suberites domuncula*. Mar Drugs 13: 4985-5006.
5. Katharios P, Seth-Smith HM, Fehr A, Mateos JM, Qi W, et al. (2015) Environmental marine pathogen isolation using mesocosm culture of sharpsnout seabream: striking genomic and morphological features of novel *Endozoicomonas* sp. Sci Rep 5: 17609.
6. Neave MJ, Apprill A, Ferrier-Pagés C, Voolstra CR (2016) Diversity and function of prevalent symbiotic marine bacteria in the genus *Endozoicomonas*. Appl Microbiol Biotechnol 100: 8315-8324.
7. Fenical W (1993) Chemical studies of marine bacteria: developing a new resource. Chem Rev 93: 1673-1683.
8. Gupta C, Prakash D, Gupta S (2014) Natural useful therapeutic products from microbes. J Microbiol Exp 1: 00006.

9. Challinor VL, Bode HB (2015) Bioactive natural products from novel microbial sources. Ann N Y Acad Sci 1354: 82-97.

10. Procópio RE, Silva IR, Martins MK, Azevedo JL, Araújo JM (2012) Antibiotics produced by *Streptomyces*. Braz J Infect Dis 16: 466-471.

11. Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Microbiol Spectr 4.

12. Sun P, Maloney KN, Nam SJ, Haste NM, Raju R, et al. (2011) Fijimycins A-C, three antibacterial etamycin-class depsipeptides from a marine derived *Streptomyces* sp. Bioorg Med Chem 19: 6557-6562.

13. Hughes CC, Prieto-Davo A, Jensen PR, Fenical W (2008) The marinopyrroles, antibiotics of an unprecedented structure class from a marine *Streptomyces* sp. Org Lett 10: 629-631.

14. Gontang EA, Fenical W, Jensen PR (2007) Phylogenetic diversity of Gram-positive bacteria cultured from marine sediments. Appl Environ Microbiol 73: 3272-3282.

15. Brosius J, Palmer ML, Kennedy PJ, Noller HF (1978) Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. Proc Natl Acad Sci USA 75: 4801-4805.

16. Altschul SF, Gish W, Miller W, Myers EW, Lipman, DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403-410.

17. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948.

18. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30: 2725-2729.

19. Bauer AW, Kirby WM, Serris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45: 493-496.

20. Yoon BJ, Oh DC (2011) *Formosa spongicola* sp. nov., isolated from the marine sponge *Hymeniacidon flavia*. Int J Syst Evol Micobiol 61: 330-333.

21. Taylor MW, Tsai P, Simister RL, Deines P, Botte E, et al. (2012) 'Sponge-specific' bacteria are widespread (but rare) in diverse marine environments. ISME J 7: 438-443.

22. Boyd KG, Adams DR, Burgess JG (2009) Antibacterial and repellent activities of marine bacteria associated with algal surfaces. Biofouling 14: 227-236.

23. Sipkema D, Schippers K, Maalcke WJ, Yang Y, Salim S, et al. (2011) Multiple approaches to enhance the cultivability of bacteria associated with the marine sponge *Haliclona (gellius)* sp. Appl Environ Microbiol 77: 2130-2140.

24. Sertan-de Guzman AA, Predicala RZ, Bernardo EB, Neilan BA, Elardo SP, et al. (2007) *Pseudovibrio denitrificans* strain Z143-1, a heptylprodigiosin-producing bacterium isolated from a Philippine tunicate. FEMS Microbiol Lett 277: 188-196.

25. Rypien KL, Ward JR, Azam F (2010) Antagonistic interactions among coral-associated bacteria. Environ Microbiol 12: 28-39.

26. Santos OC, Pontes PV, Santos JF, Muricy G, Giambiagi-deMarval M, et al. (2010) Isolation, characterization and phylogeny of sponge-associated bacteria with antimicrobial activities from Brazil. Res Microbiol 161: 604-612.

27. Al-Saif SS, Abdel-Raouf N, El-Wazanani HA, Aref IA (2014) Antibacterial substances from marine algae isolate from Jeddah coast of Red sea, Saudi Arabia. Saudi J Biol Sci 21: 57-64.

28. Ding LJ, Gu BB, Jiao WH, Yuan W, Li YX, et al. (2015) New furan and cyclopentenone derivatives from the sponge-associated fungus *Hypocrea koningii* PF04. Mar Drugs 13: 5579-5592.