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ANTIBACTERIAL ACTIVITY OF MARINE BACTERIA ASSOCIATED WITH SPONGES FROM PHU QUOC ISLAND IN VIETNAM

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SUMMARY

Sponge associated marine microbes recognised as potential candidate for screening and isolation of bioactive compounds. In this work, thirty-one marine bacterial strains associated with seven species of sponges collected from Phu Quoc Island, Vietnam were isolated and screened for antimicrobial activity against selected human and animal pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Vibrio harveyi, Bacillus cereus*, *Streptococcus faecalis*, *Listeria monocytogenes*, *Proteus mirabilis* and *Klebsiella pneumoniae* using agar well diffusion assay. Twenty-six percent of bacterial strains were found to be antibacterial producers and their activities ranged from broad spectral to species specific (*P. aeruginosa, E. coli, P. mirabilis, B. cereus,* and *L. monocytogenes*). Out of the 31 marine bacterial strains subjected to preliminary screening for antibacterial activity, 8 isolates exhibited antibacterial activity against at least two tested pathogens, that 55% against *P. mirabilis*, 22% against *P. aeruginosa*, 10% against *E. coli* and 6% against *K. pneumoniae, B. cereus* and *L. monocytogenes.* A strain coded 045-203-4 was found to be highly potent and was cultured for further study. The strain produced metabolites with good activity in medium contained yeast extract (0.8% w/v), glucose (0.5%) at pH 7.0 and after incubated shaking for 30 h at 150 rpm. In addition, strain 045-203-4 was analyzed for morphological and physiological characteristics. Analysis of the nucleotide sequence of 16s rRNA gene of strain 045-203-4 showed a strong similarity (99%) with that of the 16s rRNA gene of *Bacillus subtilis*. The present investigation reveals that the marine bacteria isolated from sponges at Phu Quoc Island can be a definite source for novel antibiotics.

Keywords: *Antibacterial activity, Bacillus subtilis, Marine bacteria, Metabolites, Sponges, Pathogenic bacteria*

INTRODUCTION

Marine environment has extreme conditions for the survival of organisms including microorganisms due to low temperature, salinity and pressure. Therefore, in order to adapt to the harsh living conditions requires microorganisms must change the way metabolism to adapt and survive (Jafarzade *et al.*, 2013). Besides, the marine environment with the diversity of the marine species and complex habitat conditions, the marine microbial biosynthesis metabolites have more interesting biological activity than terrestrial organisms (Carte *et al.*, 1996, Rinehart *et al.*, 2000, Schwartsmann *et al.*, 2001).

Currently, infectious diseases caused by bacteria, fungi and viruses are still a major threat to public health, although human medicine has reached a certain level of progress. This situation is common in developing countries due to lack of drug and the emergence of multi-drug resistance. The researchers discovered new alternative antibiotics, especially antibiotics derived from marine microorganisms are attracting the interest of many scientists (Joseph *et al.*, 2009).

During the past 40 years, many scientists over the world have focus on discovery novel natural products from marine organisms. The results showed that more than 12,000 novel chemicals with hundreds of new compound are still being discovered every year (Pettit *et al.*, 2004). So far most of these chemicals have been found from marine organisms including sponges dominate (Lie *et al.*, 2002).

Many collected compounds from marine sponges expressed strong biological activity such as anticancer, antibacterial and anti-inflammatory activities, and usually used in medicine (Pettit *et al.*, 2004; Faulkner *et al.*, 2002; Sonnenschein *et al.*, 2004). Some research showed that compounds previously extracted from sponges are biosynthesized through associated microorganisms or actually be produced by microorganisms (Bewley *et al.*, 1998; Unson *et al.*, 1993; Unson *et al.*, 1994). In recent years, many new compounds with diverse biological activities have been discovered from microorganisms isolated from marine sponges (Jayatilake *et al.*, 1996; Mitova *et al.*, 2003; Suzumura *et al.*, 2003). With the goal of finding compounds have antibiotic activity from marine microorganisms, we isolated several strains of bacteria associated with marine sponges collected from Phu Quoc Island, Vietnam.

MATERIALS AND METHODS

Isolation of marine bacteria

Bacterial strains were isolated from seven species of marine sponges from Phu Quoc Island, Vietnam. Collected samples were rinsed three times with sterile seawater in order to remove the non-attached bacteria. One g sample was triturated with sterile sea water and spread on the entire surface of Marine Agar (MA) (peptone 5 g, yeast extract 1 g, $MgSO_4$ 0.1 g, KH_2PO_4 0.1 g and agar 18 g dissolved in 500mL sea water and 500mL distilled water, pH 7.0-7.2). After incubation at 28^{o}C for 24 h, all colonies with different pigmentation and morphology were picked out. The isolated colonies were repeatedly streaked to obtain pure cultures and stored at -80^{o}C in Marine Broth supplemented with 30% (v/v) glycerol for further studies.

Test bacteria

Ten clinical bacterial pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Vibrio harveyi, Bacillus cereus*, *Streptococcus faecalis*, *Listeria monocytogenes*, *Proteus mirabilis* and *Klebsiella pneumoniae* were used in this study. The strains were obtained from Pacific Institute of Bio-Organic Chemistry, Far-Eastern Branch, Russian Academy of Science (PIBOC FEBRAS), Russia.

Preliminary screening of antibacterial substance from marine bacteria

Primary screening of antibacterial activity of 31 marine bacterial strains were determined by agar well diffusion method described by Bauer *et al.,* (1966) with modification. One hundred µL contained cell masses of each isolated bacteria in 0.9% NaCl solution for 24 h was inoculated into wells (8 mm in diameter) in MA plates containing the pathogenic bacteria. All the plates were incubated at 37^{o}C for 24 h. The zone of inhibition was measured and expressed in diameter in millimeter. Based on the results of preliminary screening, marine bacterial isolates with high activity were selected as potential strains for further investigations.

Preparation of crude extract

Selected marine bacteria were cultured in 300mL Marine Broth (peptone 5 g, yeast extract 1 g, $MgSO_4$ 0.1 g and KH_2PO_4 0.1 g, dissolved in 500mL distilled water and 500mL seawater, pH 7.0-7.2) to produce secondary metabolites in 500mL Erlenmeyer flasks. Flasks were incubated on a rotatory shaker at 150 rpm. After 24 h of cultivation, the broth was centrifuged at 8000 rpm for 20 min to remove the cell and extract equal volume of ethyl acetate (EtOAc) for two times. The solvent layers were collected and then evaporated to obtained crude required for second antibacterial screening.

Secondary screening of antibacterial activity

Antibacterial activity assay of the extracts was done using the disk diffusion method performed on MA according to Bauer *et al.,* (1966). The crude extract is weighed and dissolved in EtOAc (0.2 mg/mL) for antibacterial studies. The sensitivity of the test bacteria strains to the EtOAc extracts of the isolates was determined of measuring the sizes of inhibitory zones on the agar surface around the disks and expressed in diameter in millimeter.

Identification of isolate 045-203-4

Morphological and physiological characterizations

The strain 045-203-4 was morphologically and physiologically characterized by the methods described by Sneath *et al.,* (1986).

Molecular identification

Genomic DNA was extracted from strain 045-203-4 in logarithm phrase as a template for polymerase chain reaction (PCR), and then 16S

rRNA gene was amplified by PCR. PCR amplification of the 16S rRNA gene was performed using universal primers 16SF (5'-AGAGTTTGATCCTGGCTCAG-3') and 16SR (5-TACGGTTACCTTGTTACGACTT-3'). Sequences were compared with other 16S rRNAs obtained from GenBank using the BLAST program. Alignments and similarity comparison were done using the CLUSTALW programmed at European Bioinformatics site (http://www.ebi.eic.uk/clustalw). The phylogenetic tree was constructed using MEGA with neighbor-joining method.

Table 1. Antibacterial activity of marine bacteria (zone of inhibition in mm).

Strain no	Pathogens									
	SA	**PA**	**EC**	**VP**	**PM**	**VH**	**KP**	**BC**	**SF**	**LM**
045-203-2	-	-	-	-	-	-	-	-	-	-
045-203-3	-	-	-	-	-	-	-	-	-	-
045-203-4	**-**	**22**	**12**	**-**	**-**	**11**	**11**	**09**	**09**	**-**
045-203-5	-	10	-	-	15	-	-	-	-	-
045-206-1	**-**	**12**	**20**	**-**	**10**	**-**	**-**	**-**	**-**	**-**
045-206-2	-	-	-	-	-	-	-	-	-	-
045-206-3	-	-	-	-	-	-	-	-	-	-
045-230-1	-	-	-	-	-	-	-	-	-	-
045-230-2	-	-	-	-	15	-	-	-	-	-
045-230-3	-	-	-	-	18	-	-	-	-	-
045-236-1	-	-	-	-	-	-	-	11	-	-
045-236-2	-	12	-	-	-	-	-	11	-	-
045-236-3	-	11	-	-	15	-	-	-	-	-
045-236-4	-	11	-	-	12	-	-	-	-	-
045-236-5	-	-	-	-	12	-	-	-	-	-
045-236-6	-	-	-	-	-	-	-	-	-	23
045-255-1	-	-	22	-	12	-	-	-	-	-
045-255-2	-	-	-	-	12	-	-	-	-	-
045-255-3	-	-	-	-	12	-	-	-	-	-
045-255-4	-	-	-	-	20	-	-	-	-	-
045-255-5	-	-	-	-	15	-	-	-	-	-
045-255-6	-	-	-	-	15	-	-	-	-	-
045-273-1	**-**	**20**	**-**	**12**	**18**	**-**	**-**	**-**	**-**	**12**
045-273-2	-	-	-	-	25	-	-	-	-	-
045-273-3	-	-	-	-	18	-	-	-	-	-
045-273-4	-	-	-	-	15	-	-	-	-	-
045-273-5	-	-	-	-	-	-	-	-	-	-
045-274-2	-	-	-	-	-	-	-	-	-	-
045-274-3	-	-	-	-	-	-	12	-	-	-
045-274-4	-	+	-	-	-	-	-	-	-	-
045-274-5	-	+	-	-	-	-	-	-	-	-

Note: - : no inhibition zone

Medium optimization

All experiments in this study were carried out in shaking cultures to determine the physiological and physical conditions that would affect the antibacterial agent production of the selected strain six various nitrogen sources at a concentration of 1% (w/v) to study the effects of different nitrogen

sources on antibacterial agent production by isolate 045-203-4. The effect of addition of equimolar amounts of different inorganic and organic nitrogen sources to enhance the antibacterial agent production by isolate 045-203-4 was also studied. Since, yeast extract might be considered as a growth factor and nitrogen source. So, different concentrations of yeast extract were added to test their effect on the antibacterial agent production.

In addition, the effect of equimolar amounts of different carbon sources and different concentrations of selected carbon source on antimicrobial activity was also studied.

RESULTS AND DISCUSSION

Antibacterial activity of marine bacteria

Microorganisms play a central role in sponge biology, as they are associated with many sponges either extracellularly, intracellularly or both. Isolation is a mandatory approach to obtain novel microbes and also for evaluating their biochemical characteristics to understand the ecophysiological and environmental functions with their potential applications (Sfanos *et al.*, 2005).

Out of the 31 marine bacterial strains associated with sponges subjected to preliminary screening for antibacterial activity, 8 isolates exhibited antibacterial activity against at least two tested pathogens, that 55% against *P. mirabilis*, 22% against *P. aeruginosa*, 10% against *E. coli* and 6% against *K. pneumoniae, B. cereus* and *L. monocytogenes* (Table 1). Among them, 3 isolates (045-203-4, 045-206-1, and 045-273-1) showed good antibacterial activity to at least three tested pathogens. Therefore, they were selected as potential strains for secondary screening.

A total of 8 tested pathogens were chosen for secondary screening of antibacterial activity using EtOAc crude extracts of three selected isolates. In the present study, the highest activity was exhibited from the strain 045-203-4, with an inhibition zone of 29 mm against *S. aureus* and 38 mm against *L. monocytogenes* (Table 2).

The discovery of enormous microbial diversity in marine sponges provides unprecedented research opportunities. Increased metabolic capabilities of sponge-associated bacteria were directly correlated with increased levels of potentially available nutrients in the sponge. Sponges filter seawater and accumulate copious amount of organic matter within the choanocytic chambers along with bacteria (Santavy *et al.*, 1990).

Jafarzade *et al.,* (2011), Guo *et al.,* (2011) and Kennedy *et al.,* (2009) also reported the microorganism isolated from the marine sponges, showed potent antimicrobial activity against bacterial pathogens.

Table 2. Antibacterial activity of EtOAc extracts from strain 045-203-4, 045-206-1 and 045-273-1.

Test bacteria	Extracts (zone of inhibition in mm)		
	045-203-4	045-206-1	045-273-1
S. aureus	**29**	20	15
P. aeruginosa	**25**	13	22
E. coli	**30**	22	-
K. pneumoniae	**25**	-	-
P. mirabilis	**21**	12	19
B. cereus	**16**	12	-
S. faecalis	**20**	10	10
L. monocytogenes	**38**	25	14

It has been reported that the production and potential of bioactive compounds by different microorganisms can be strongly influenced by the source of isolation. The antibiotic activity of marine bacteria is well-known and has been demonstrated in a number of studies the vast diversity of microorganisms in the marine niches, continue to yield many novel bioactive compounds. Hence,

exploration of biotechnological potentials of microbes associated with invertebrates still remains a very important and untapped resource. From all these observations the bacterial isolates from the sponges are found to be most prolific marine producers of novel compounds (Devi *et al.*, 2010).

Identification of strain 045-203-4

The results of morphological, physiological and biochemical characteristics of strain 045-203-4 were shown in Table 3. The 045-203-4 strain was able to grow at the 20-45^{o}C, pH 5-11 and it could tolerate concentration of 90 g L^{-1} NaCl solution. It has positive results for catalase, oxidase, ONPG, gelatinase, Voges-Proskauer and utilization of citrate as a source of carbon. It could not produce hydrogen sulfide as well as indole. According to investigated results on the morphological, physiological and biochemical characteristics of the strain, 045-302-4 preliminary classified to be *Bacillus* genus.

Table 3. Morphological and physiological characteristics of strain 045-203-4.

Properties	Results	Properties	Results
Shape	Rods	H_2S production	-
Flagellum	+	Urease	-
Spores	+	Indole production	-
Motility	+	VP test	+
Growth temperature	20-45^{o}C	Gelatinase	+
Growth pH	5-11	Growth on sole carbon sources	
NaCl concentration for growth	0-90 g L^{-1}	D-Glucose	+
Catalase	+	D-Mannitol	+
Oxidase	+	Inositol	-
ONPG	+	Sorbitol	+
Decarboxylation of Amino acids		Rhamnose	+
Arginine	-	Saccharose	+
Lysine	-	Melibiose	+
Ornithine	-	Amylase	+
Citrate utilization	+	L-Arabinose	+

Note: Cultivating 045-203-4 at 28^{o}C for 24h; + positive results; - negative results

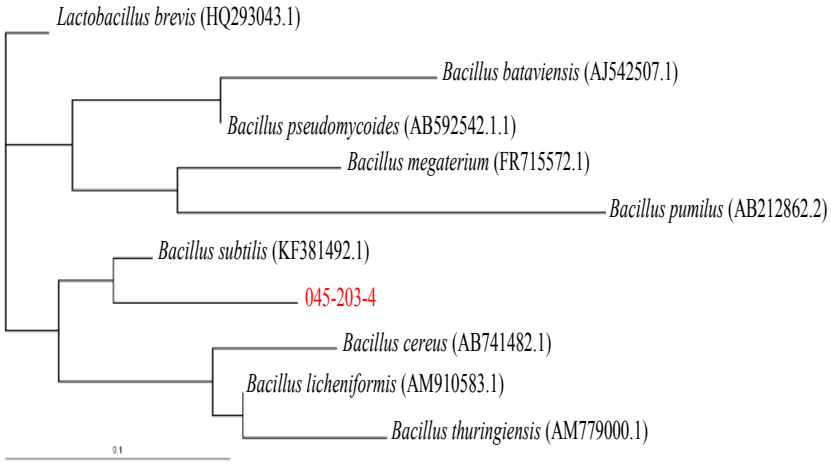

Figure 1. Phylogenetic tree based on 16S rRNA gene sequences and closely related members of the genus *Bacillus*. Numbers at nodes are levels of bootstrap support based on neighbor-joining analyses of 1000 replications.

The analysis of the 16S rRNA gene is an important tool for correct identification of microbial species. The length of the partial 16S rRNA gene sequence of the isolate was 1500 bp. The phylogenetic tree, which was constructed for comparison of the 16S rRNA gene sequences, indicated that strain 045-203-4 belonged to the genus of *Bacillus*. The levels of similarity between the 16S rRNA gene of *Bacillus* 045-203-4 and the 16S rRNA gene of other *Bacillus* species are summarized in Figure 1. It was found that *Bacillus* 045-203-4 has the highest similarity with *B. subtilis,* in fact over 99% (NCBI accession no. 381492.1).

It has been estimated that over 99% of the marine sponge-associated microbes have yet to be cultured in the laboratory with bacteria isolated from the sponges containing diverse *Bacillus* species being one of the most divergent forms. Among all isolates *Bacillus* species was found to be predominant in symbiotic association with sponges. Many members of the *Bacillus* group continue to be dominant bacterial workhorses in microbial fermentation for the production of novel compounds (Kennedy *et al.*, 2009).

The terrestrial *Bacillus* sp. is widely recognized as a rich source of antimicrobial agents (Gebhardt *et al.*, 2002). The occurrence of *Bacillus* sp. in the marine environment has been well documented (Ivanova *et al.*, 1999). Many antibiotics including cyclic peptides, cyclic lipopeptides and novel thiopeptides have been reported from marine *Bacillus* sp. (Nagai *et al.*, 2003). Until 2002, 12 bioactive compounds were reported from marine *Bacillus* sp. (Dobler *et al.*, 2002). *Bacillus* sp. isolates produce structurally diverse classes of secondary metabolites that exhibit a wide range of biological activities (Mandol *et al.*, 2013). To 2014, Tareq *et al.* discovered four new non-cytoxic lipopeptides, gageopeptides A-D with good antimicrobial activity from a marine-derived bacterium *Bacillus subtilis*. In 2015, two new glycolipids, ieodoglucomide C ieodoglycolipid were isolated from *Bacillus licheniformis* (Tareq *et al*., 2015).

Medium optimization

The condition of incubation influenced quantitatively the biosynthesis of antibiotics as well as biomass. It has been reported that nutritional requirement play an important role during metabolite synthesis (Tabbene *et al.*, 2009). The part of work aims at the optimization of some culture conditions to attain maximum antibacterial agent production. Of all the tested nitrogen sources (1% w/v) in the MB medium inoculated with *B. subtilis* 045-203-4 and incubated shaken for 24 h, yeast extract supported the highest level of antibacterial agent production (Table 4).

Table 4. Effect of different inorganic and organic nitrogen sources for antibacterial agent production by *B. subtilis* 045-203-4.

Test bacteria	Inorganic and organic nitrogen sources (zone of inhibition in mm)					
	Peptone	Casein	Peptone meat	Yeast extract	$(NH_4)_2SO_4$	Na NO_3
S. aureus	30	15	25	**33**	12	20
P. aeruginosa	20	-	13	**22**	-	-
E. coli	19	19	22	**32**	19	21
V. parahaemolyticus	18	11	19	15	-	-
P. mirabilis	13	17	14	**25**	22	14
K. pneumoniae	**24**	13	13	14	-	17
V. harveyi	11	10	13	12	12	18
B. cereus	16	19	15	**22**	13	18
S. faecalis	15	**19**	14	14	13	14
L. monocytogenes	27	25	24	**45**	30	27

The effects of different nitrogen sources on antibacterial agent production by *B. subtilis* 045-203-4 were also studied. Zero point eight percent (w/v) was the best concentration of yeast extract for optimum antibacterial agent production by *B. subtilis* 045-203-4 (Table 5). The requirements for specific nitrogen supplement differ from one microorganism to another. In most microorganisms both inorganic

and organic forms of nitrogen are metabolized to produce amino acids, nucleic acids, proteins and cell wall components. However, it was found that some nitrogen sources had an inhibitory effect on the antibacterial agent production and this may be due to organic acid accumulation, oxygen depletion or sugar catabolic repression.

Sreerag *et al.,* (2014) reported that yeast extract was the best nitrogen sources for antibiotics production by *Bacillus* sp. isolated from a rhabditid entomopathogenic nematode, *Rhabditis (Oscheius)* sp.. The nature of the nitrogen source used has a notable effect on the production of the antimicrobial metabolite in the bacterium. Depending on the biosynthetic pathways involved, nitrogen sources may significantly affect antibiotic formation. It was noted by Sanchez and Demain (2002) that ammonium salts did not favor biosynthesis of novobiocin, actinomycin, neomycin, kanamycin and others, but for rapamycin ammonium sulfate was the best nitrogen source.

In addition, different carbon sources including starch, maltose, manitol, glucose, sucrose were used at the concentration 0.4% (w/v) in the MB medium to study their effect on antimicrobial activity of the strain. After shaking for 24 h, glucose supported the highest level of antibacterial agent production. The effect of different concentrations of glucose on antimicrobial activity was also studied. Maximum antimicrobial activity of *B. subtilis* 045-203-4 was recorded at glucose concentration of 0.5 % (w/v). Antimicrobial metabolite production by *Bacillus* sp. for other studies was also optimally produced with glucose or sucrose in the medium (Abdel-Aziz *et al.*, 2013).

Table 5. Effect of different yeast extract concentrations for antibacterial agent production by *B. subtilis* 045-203-4.

Test bacteria	Different yeast extract concentrations (%w/v) (zone of inhibition in mm)						
	0.4	**0.6**	**0.8**	**1.0**	**1.2**	**1.4**	**1.6**
S. aureus	-	-	20	22	24	28	22
P. aeruginosa	-	10	**14**	12	-	-	-
E. coli	23	25	**28**	22	-	-	-
P. mirabilis	12	20	**34**	24	13	13	12
K. pneumoniae	12	13	**16**	14	13	10	10
B. cereus	13	15	**20**	18	13	13	13
S. faecalis	14	14	14	15	14	13	13
L. monocytogenes	36	38	**42**	40	39	38	32

Time course of the antibacterial agent production on the optimized medium

Time course from 12 to 48 h was followed in shaking incubated flask containing the optimized culture conditions inoculated with *B. subtilis* 045-203-4. The relationship between antibacterial activity and cell density is shown in Figure 2. It is evident that strain *B. subtilis* 045-203-4 could produce antibacterial substances only when the OD_{660} value was above the threshold value of 1.0, at the beginning of the stationary phase. Antibacterial activity was highest when strain *B. subtilis* 045-203-4 was cultured in MB after 30 h incubated shaken. It is reported that antibiotic production usually occurs in stationary phase (Tabbene *et al.*, 2009).

As show in Figure 2, OD and pH values had a close correlation with the growth conditions of *B. subtilis* 045-203-4. At the beginning of fermentation, the biomass content and pH were low. Then, along with the growth of the bacterium, after 12 h fermentation the pH and OD values increased quickly. When the strain was in stationary phase, the pH value began to stabilize (pH = 7.8) and unchanged the value during the stationary phase.

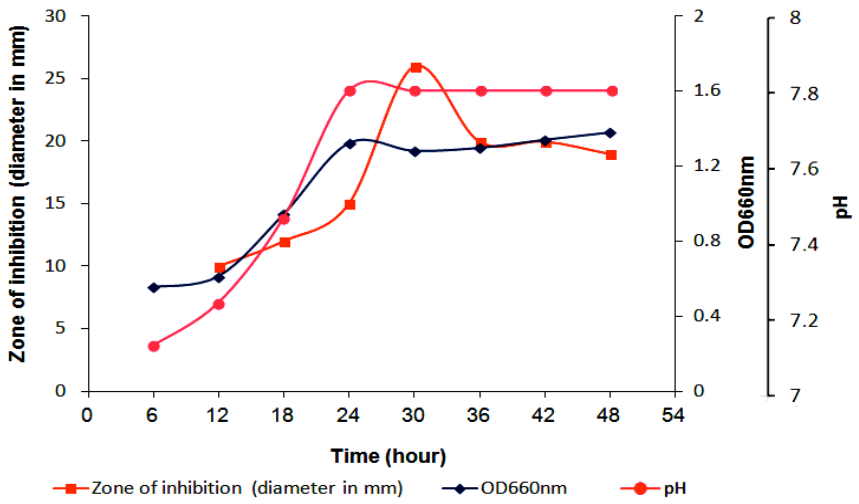

Figure 2. Time course of the antibacterial agent production against *S. aureus* and the growth by *Bacillus subtilis*. 045-203-4.

CONCLUSION

The present study indicated that isolated bacteria from marine sponges remain an interesting source for new antibacterial metabolites and also suggested that *B. subtilis* 045-203-4 producing secondary metabolites with high antibacterial activity. Marine environment in Phu Quoc Island can be a potential source for natural products with biological activities in order to discover new compounds for application of marine microbial sources in Vietnam.

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HOẠT TÍNH KHÁNG KHUẨN CỦA VI KHUẨN BIỂN CỘNG SINH VỚI BỌT BIỂN THU TỪ ĐẢO PHÚ QUỐC, VIỆT NAM

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TÓM TẮT

Vi sinh vật cộng sinh với bọt biển được xem là nguồn tiềm năng cho việc sàng lọc và phân lập các hợp chất có hoạt tính sinh học. Trong nghiên cứu này, ba mươi mốt chủng vi khuẩn biển cộng sinh với bảy loài bọt biển được thu từ đảo Phú Quốc, Việt Nam đã được phân lập và sàng lọc hoạt tính kháng khuẩn đối với các chủng vi khuẩn gây bệnh cho người và động vật bao gồm *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Vibrio harveyi, Bacillus cereus*, *Streptococcus faecalis*, *Listeria monocytogenes*, *Proteus mirabilis* và *Klebsiella pneumoniae* theo phương pháp khuếch tán trên đĩa thạch. Kết quả nghiên cứu cho thấy 26% chủng vi khuẩn biển có khả năng sinh chất kháng khuẩn và thể hiện hoạt tính kháng khuẩn đối với một số loài vi khuẩn gây bệnh (*P. aeruginosa, E. coli, P. mirabilis, B. cereus,* and *L. monocytogenes*). Trong số 31 chủng vi khuẩn được sàng lọc hoạt tính kháng khuẩn, 8 chủng thể hiện hoạt tính kháng khuẩn đối với ít nhất 2 chủng vi khuẩn kiểm định, cụ thể 55% kháng *P. mirabilis*, 22% kháng *P. aeruginosa*, 10% kháng *E. coli* và 6% kháng *K. pneumoniae, B. cereus* và *L. monocytogenes.* Chủng 045-203-4 thể hiện hoạt tính kháng khuẩn mạnh nên được tuyển chọn cho nghiên cứu sâu hơn. Chủng vi khuẩn này sinh chất chuyển hóa có hoạt tính kháng khuẩn cao trong môi trường chứa dịch chiết nấm men (0,8%), glucose (0,5%), ở pH 7.0 và sau nuôi lắc với tốc độ 150 vòng/phút trong 30 giờ. Bên cạnh đó, chủng 045-203-4 cũng được xác định một số đặc điểm hình thái và sinh lý. Định danh loài dựa trên so sánh trình tự 16s rRNA với một số chủng trên ngân hàng gen kết luận chủng này tương đồng 99% với trình tự 16s rRNA của chủng *Bacillus subtilis.* Nghiên cứu cho thấy các chủng vi khuẩn phân lập từ bọt biển ở đảo Phú Quốc có thể xem là nguồn tiềm năng các chất kháng sinh mới.

Từ khóa: *Hoạt tính kháng khuẩn, Bacillus subtilis, Vi khuẩn biển, Chất chuyển hóa, Bọt biển, Vi khuẩn gây bệnh*

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