Microbiol. Biotechnol. Lett. (2017), 45(2), 124–132
http://dx.doi.org/10.4014/mbl.1702.02002
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Identification of a Bioactive Compound, Violacein, from *Microbulbifer* sp. Isolated from a Marine Sponge *Hymeniacidon sinapium* on the West Coast of Korea

Nam-Il Won[1], Ga-Eun Lee[2], Keebeom Ko[3], Dong-Chan Oh[3], Yang Ho Na[4]*, and Jin-Sook Park[2]*

[1]*Water Resources Research Center, K-water Institute, Daejeon 34045, Republic of Korea*
[2]*Department of Bioscience and Biotechnology, Hannam University, Daejeon 34430, Republic of Korea*
[3]*Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*
[4]*Department of Advanced Materials, Hannam University, Daejeon 34430, Republic of Korea*

Received: February 10, 2017 / Revised: March 28, 2017 / Accepted: March 28, 2017

Microbial secondary metabolites of marine organisms are regarded as major sources of structurally and biologically novel compounds with numerous potential uses. Sponge-microbe associations are among the most interesting sources for exploring bioactive compounds. In this study, the bacterial strain *Microbulbifer* sp. (127CP7-12) was isolated from the Asian marine sponge *Hymeniacidon sinapium* collected at an intertidal zone on the west coast of Korea. Cultured bacteria produced a violet pigment, and optimal culture conditions for violet pigment production were investigated. Maximum production of the violet pigment from the strain culture was observed under the conditions of 25℃, pH 6.0, and 3% NaCl. Acetone provided better extraction of the pigment from fermented broth compared with ethanol and methanol. The proposed structure of the major component in the extracted crude pigment was determined via high-performance liquid chromatography, nuclear magnetic resonance, mass spectrometry, and UV spectra analyses, which showed that the metabolite was the promising bioactive compound violacein. This study describes the examination of marine bioactive materials from microbe-engaged metabolites and the ecological implications of the sponge-microbe association in a changing ocean.

Keywords: **Marine sponge, bacterial production, violacein, violet pigment, Korean waters**

Introduction

Food and bioactive materials are part of most important ecosystem services. These services can be produced by photosynthetic organisms and originate from carbon dioxide, water, and sunlight, which are being affected by climate change. There have been increasing reports on critical environmental stressors on marine ecosystems from both anthropogenic and climate forcing [1, 2]. Remarks on the environmental impact of the marine ecosystem have been made often in terms of ecosystem structure (e.g., species range shift) [3–5]. Services provided by marine ecosystems, such as fisheries and biomaterials, are important areas that will be affected by climate change, which could lead to possible threats against access to and the sustainable use of bioactive compounds from marine organisms.

Natural products have become important resources for human well-being amidst recent global concerns, such as increasing outbreaks of epidemics including avian influenza and the emergence of antibiotic resistance, as well

***Corresponding authors**
Y. H. N.
Tel: +82-42-629-8905, Fax: +82-42-629-8853
E-mail: yhna@hnu.kr
J.-S. P.
Tel: +82-42-629-8771, Fax: +82-42-629-8751
E-mail: jspark@hnu.kr

as for clinical trials for anticancer and antimicrobial agents [6, 7]. Many candidate bioactive products have been reported from various taxa, including from bacteria and plants. Marine organisms are one of the groups attracting the most focus for the examination of natural products [8, 9]. Especially marine benthic organisms have been a major target group because they harbor useful bacterial communities for the production of bioactive materials owing to complex prey-predator interactions among large marine organisms as well as the microorganisms present in biofilms. Recent attention has focused on the bioactive materials produced by microorganisms living on marine benthic organisms and in biofilm. Among them, violacein is one promising natural product that has been reported from diverse microbe-organism interactions as well as from a microbe-sponge interaction, as recently reviewed by Choi *et al*. [10]. Despite the well-documented studies on violacein, there is still limited understanding of the ecological function of this material for both producer microbes and host organisms, and many studies are needed to investigate its clinical applications. This study examined microbial production originating from a marine sponge, and violacein was one of the natural products expected to be found during the research.

Marine sponges (phylum Porifera) are among the oldest and simplest animals, and they grow in every ocean and have a great capacity for withstanding extreme temperatures and pressures. They have no true tissues or organs and are just constructed with layers of cells without even a nervous system. As filter feeders, their bodies are full of pores and channels that allow water to circulate through them, allowing interactions with various organisms. These structural features for sequestering food by filtering make sponge a suitable habitat for symbiotic microorganisms. From their interactions with these various species as well as their long biological history, sponges are well-known for their production of secondary metabolites that constitute an effective defense mechanism against foreign predators [11]. Since the beginning of the exploration of marine natural products in the 1970s, investigations of secondary metabolites from marine sponge have been reported throughout the world. The barrel sponge, belonging to *Xestospongia* sponges (family *Petrosiidae*), is one of the most studied species [12] with many studies having been carried out successively in several regions around the world. This sponge has been recognized as a rich source of different chemical classes and its crude extracts and isolated compounds display remarkable bioactivities [13].

Among sponge-related biological interactions, sponge-microbe associations have been important sources for exploring natural products. Various microorganisms have been found in sponges. These microorganisms may occupy more than half of the sponge body volume, exceeding microorganisms in seawater by 2–4 orders of magnitude, and include a diverse range of green algae, heterotrophic bacteria, cyanobacteria, archaea, cryptophytes, red algae, dinoflagellates, and diatoms. The symbiotic microbial community is a highly diverse society. One host sponge can possess diverse symbionts. Some of the symbionts inhabit specific sponges while others do not. Moreover, sponge-associated microbes are important in terms of marine biodiversity because some microbes are found only in a sponge and not in seawater, as also reported for biofilm-associated microbes [14, 15]. For example, a species of α-proteobacteria dominates in sponge *Rhopaloeides odorabile* over various habitats, but it is not detected in seawater, which is an indication that the symbiont is sponge-specific. Analogously, the violacein-producers living on biofilm are prevalent in the ocean, but the violacein gene cluster has not been detected in the currently largest metagenomics sequence database of pelagic ocean waters [16, 17].

As part of continued interest in identifying bioactive compounds from microorganisms isolated from marine benthic organisms, we thoroughly investigated the pigments produced from a sponge-microbe association. The marine sponge *Hymeniacidon sinapium* used in this study is native to Korea and Japan [18, 19]. It is reported to inhabit other countries as an invasive species [20]. In this paper, we report the production and characterization of pigment extract produced by microbial activity isolated from the marine sponge *H. sinapium* collected in Korean coastal water. Also, the growth characterization of the isolated strain and the production of pigment are evaluated against various culturing parameters. Finally, we discuss briefly the marine sponge-microbe association in terms of the changing ocean around Korean coastal waters.

Materials and Methods

Marine sponge collection and microorganism

The sponge, *H. sinapium*, was collected by hand from an intertidal rocky shore on the western coast of Korea. The organism was transported to the laboratory after washing with sterilized artificial seawater and used for isolation of bacteria within 12 h [21]. The isolated marine bacterium was examined and maintained by subculturing at a regular interval of 3 days on Marine Broth 2216 (MB; Difco, USA) at 25℃ and stored at 4℃.

Fermentation of pigment

Influence of process parameters on pigment production by the bacterium was studied in a medium [22]. The MB was used for the production of violet pigment by the bacterium. The pre-culture was carried out by inoculating the cells into 0.1 L of medium in a 0.5 L conical flask. After incubation on a shaker (100 rpm) at 25℃ for 3 days, the culture was poured into a 2 L conical flask containing 1 L of the fresh medium. For the study on the optimum concentration of NaCl, the microbial solution was cultured on modified agar ($MgSO_4 \cdot 7H_2O$ 4.8 g, $MgCl_2 \cdot 6H_2O$ 3.5 g, KCl 1.0 g, $CaCl_2 \cdot 2H_2O$ 0.18 g, $NaHCO_3$ 0.03 g, NaBr 0.013 g, Bacto Peptone (Difco, USA) 2.5 g, yeast extract (Difco, USA) 5.0 g, glucose (Sigma, USA) 1.0 g, D.W. 1 L, pH 7.2), which was based on a previous report [23].

Extraction of pigment from the fermented broth

For extraction of pigment, the bacterium was incubated on a shaker in MB for 3 days. Supernatant was removed from culture broth by centrifugation for 20 min at 11,200 ×*g* and extracted using ethanol unless stated. This process was repeated three times. Extracted crude pigment was filtered through a filter paper (BioFACT Membrane Filter, Biofact, Korea). Filtered pigment extracts were dried at 35℃ under reduced pressure in a rotary evaporator (EYELA, Japan) and stored at −20℃ until analysis.

Determination of pigment concentration

Pigment concentration in the supernatant was determined by measuring the absorption at k_{max} (573 nm) using a UV-visible spectrophotometer (UV-3600, Shimadzu, Japan).

Characterization of the pigment by HPLC, NMR, MS, and UV

UV and MS spectra were obtained on an Agilent Technologies 6130 quadrupole mass spectrometer coupled with an Agilent Technologies 1200-series HPLC using a reversed-phase C_{18} (2) column (Phenomenex Luna, 100 × 4.6 mm). LC/MS analysis was performed under the gradient solvent conditions from 10% acetonitrile in water to 100% acetonitrile with 0.1% formic acid over 20 min. The major pigment was purified using reversed-phase HPLC through a Kromasil column (5 μm, C_{18}, 250 × 10 mm) under isocratic conditions (39:61 acetonitrile/water, UV 360-nm detection, flow rate: 2 ml/min). The entire purification procedure was repeated three times and pigment was isolated as pure compound at the retention time of 29 min under the HPLC conditions. ^{1}H NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer at the National Center for Inter-University Research Facilities (NCIRF) at Seoul National University.

Results

Characteristics of bacterial strain 127CP7-12

The characteristics of the bacterium isolated from the marine sponge *H. sinapium* are shown in Table 1. Several taxonomic characteristics of the pigment-producing strain were consistent with the bacterium *Microbulbifer* sp. reported in previous studies [21, 24]. Colonies form-

Table 1. Taxonomic characteristics of the pigment-producing *Microbulbifer* sp.

Strain	127CP7-12
Gram stain	-
Colony color	purple
Motility	+
Shape	rod
Oxidase	+
Catalase	+
Utilization of mannitol	+
Ranges for growth	
Temp (℃)	20–30
NaCl (%)	2–4
pH	6–10
Absorbance (nm)	573
Isolation site	marine sponge

http://dx.doi.org/10.4014/mbl.1702.02002

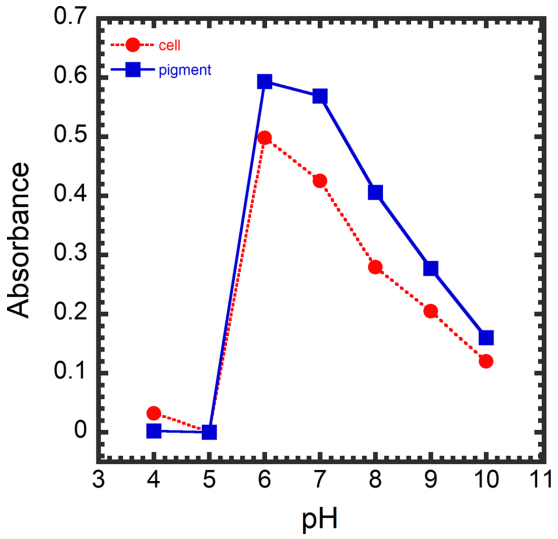

Fig. 1. Effect of pH on cell growth and pigment production (Temperature: 25℃, NaCl concentration: 3%, Culturing time: 104 h).

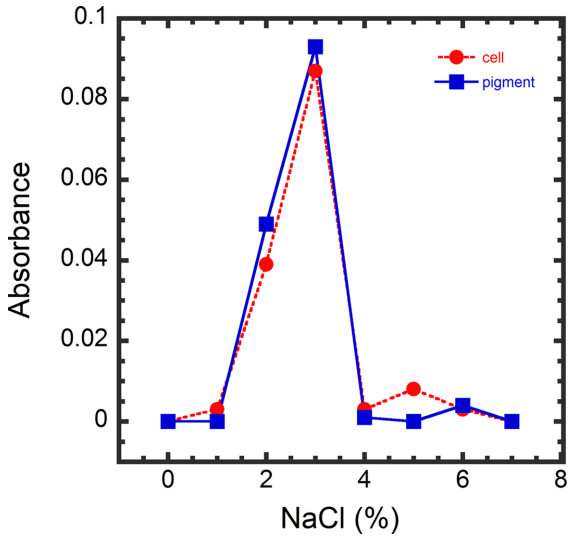

Fig. 2. Effect of NaCl concentration on cell growth and pigment production (Temperature: 25℃, pH: 6, Culturing time: 60 h).

ing on Marine Broth 2216 (MB; Difco, USA) are rodlike, smooth, raised, and dark purple in color. The isolated strain (127CP7-12) is kept in the bacterial culture collection of the Laboratory of Microbial Taxonomy and Ecology, Hannam University.

Effect of pH on pigment production

Pigment production was found to be strongly dependent on the cell growth of the bacterium *Microbulbifer* sp., implying that the pigment production originates from the bacterial production. The bacterium showed the best growth performance around pH 6 and 7 with almost no growth at pH 4 and 5, indicating a relatively narrow optimal range of pH (Fig. 1). Above the optimum pH, growth gradually decreased until pH 10, the tested upper limit. Over all the tested pH range, pigment production was closely synchronized with bacterial growth.

Effect of NaCl concentration on pigment production

The peak harvest was shown at 3% NaCl and the intermediate harvest at 2% NaCl among the tested concentrations from 0 to 7% NaCl (Fig. 2).

Effect of temperature on pigment production

For bacterial pigment production, a good harvest was shown in high temperature regimes (28℃ and 30℃) compared with a low temperature regime (20℃) with an

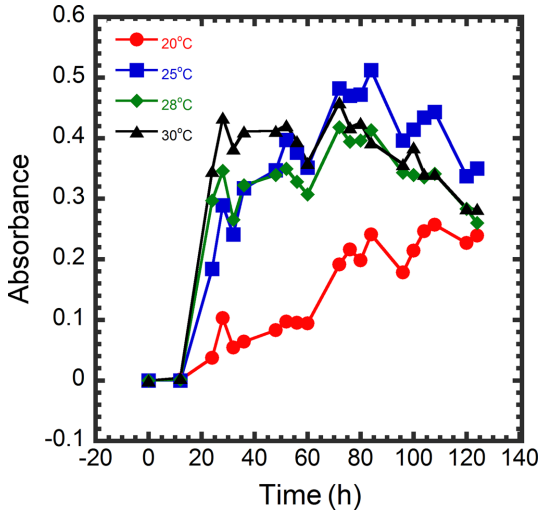

Fig. 3. Effect of temperature on pigment production in the dark at 20, 25, 28, and 30℃ (pH 6).

intermediate feature at 25℃ (Fig. 3). After 24 h of bacterial inoculation, pigment production was detected from all the temperature treatments and showed the first peak production at 30 h. An abrupt increase was only noted at the three high temperature treatments, while the lowest 20℃ treatment showed a gradual increase.

Effect of solvents on pigment extraction

Different solvents, namely distilled water (D.W.), ethanol, methanol, acetone, and methanol:acetone (7:2, v/v),

were used to extract the pigment from the broth. The pigment was extracted with every type of organic solvent used in this study, indicating that the produced pigment is lipid-soluble. Although the extraction efficiencies were similar among the tested solvents, acetone showed the highest efficiency.

Analysis of the pigment by HPLC, NMR, MS, and UV

The chemical profile of the extracted pigment mixture by LC/MS indicated the existence of a major compound (Fig. 4). The major pigment was further purified and analyzed by LC/MS to obtain its UV and MS spectra. The UV spectrum of the purified compound showed λ_{max} values at 210, 250, 360, and 570 nm (Fig. 5), which is consistent with those of violacein based on our in-house UV spectral library and the literature [25]. The molecular mass of the major pigment was deduced to 343 on the

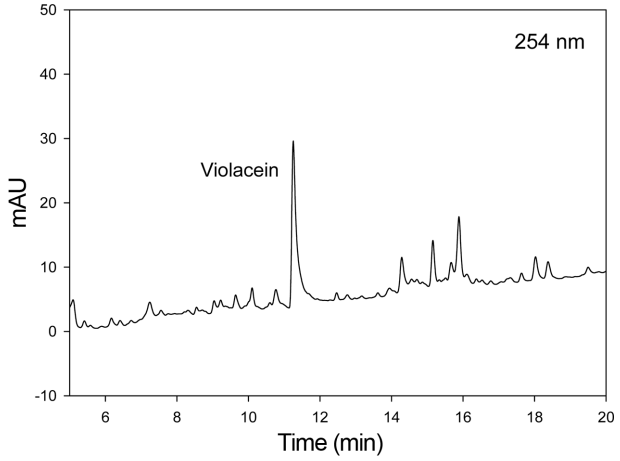

Fig. 4. HPLC chromatogram of extracted pigment acquired in LC/MS analysis (UV detection: 254 nm). Violacein was detected as a major compound at the retention time at 11.5 min

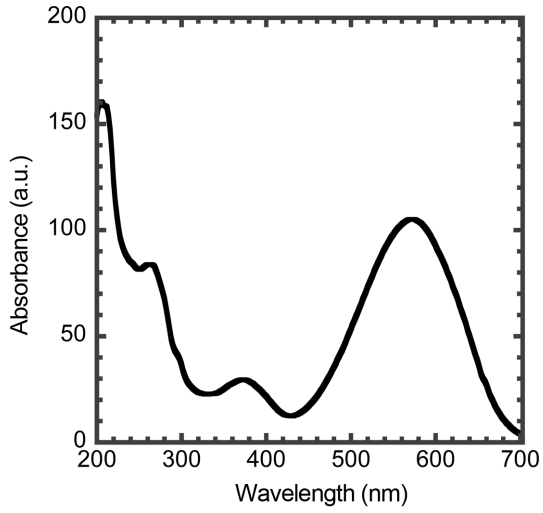

Fig. 5. Observed UV spectrum of the major pigment (127CP7-12). UV spectrum was consistent with that of violacein

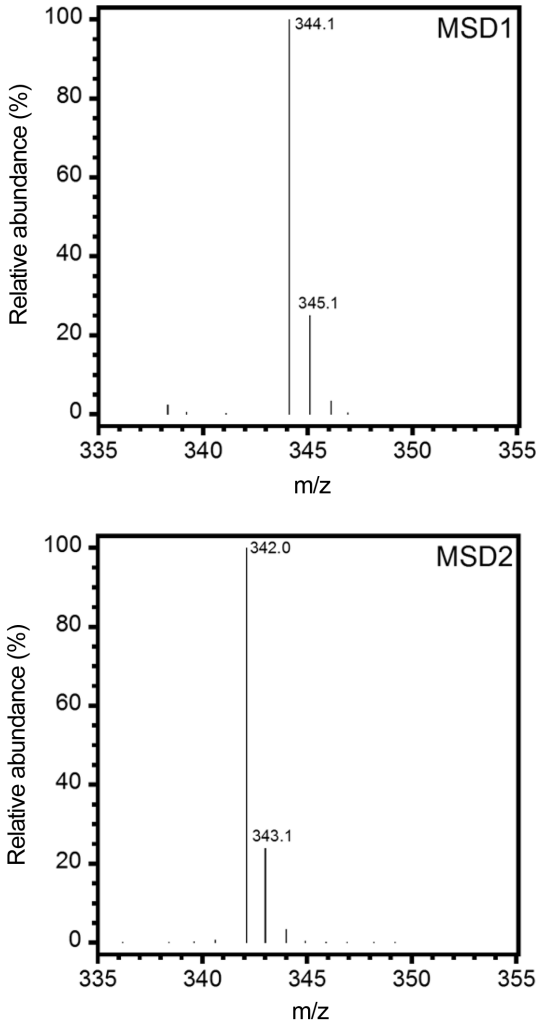

Fig. 6. ESI mass spectra (MSD1 in the positive ionization mode and MSD2 in the negative ionization mode) of the major pigment (127CP7-12). The mass spectral data also showed good agreement with the mass of violacein

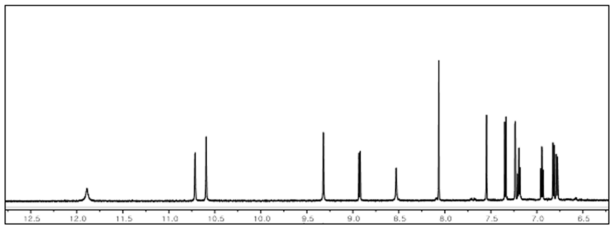

Fig. 7. ^{1}H NMR spectrum of the major pigment (127CP7-12) in DMSO-d_6. The ^{1}H NMR spectrum was consistent with that of violacein

http://dx.doi.org/10.4014/mbl.1702.02002

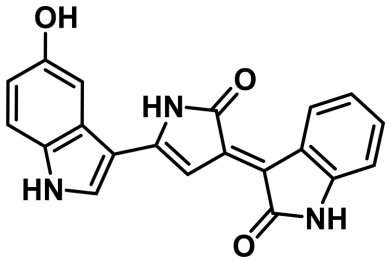

Fig. 8. Molecular structure of violacein (molecular mass = 343.34).

basis of electrospray ionization (ESI) low-resolution mass spectrometric data ($[M+H]^+$ at m/z 344 and $[M-H]^-$ at m/z 342) (Fig. 6). The ^{1}H NMR spectrum showed that the pigment structure was coincident with that of violacein. The molecular mass also supported that the major pigment is violacein [25, 26]. For further unequivocal identification, the ^{1}H NMR spectrum of the purified pigment was analyzed (Fig. 7). Based on the ^{1}H NMR spectrum, the pigment was clearly identified as violacein [25, 26]. The molecular structure of violacein is shown in Fig. 8.

Discussion

Violet pigment as bacterial production

The overall production level indicates that bacterial pigment production is optimal at high temperature; however, the highest production peak was found at 25℃ with a gradual increase shown after the abrupt increase and first peak. Furthermore, the two highest and the lowest temperatures showed a similar level of pigment production at the end of the experiment, while the intermediate temperature showed a slightly higher final production. The relatively similar production levels imply that the pigment production is limited to the broth quantity available for bacterial growth. The overall patterns of pigment production such as peak appearance were closely synchronized and the final production level was also similar among all the temperature treatments. This result indicates that the pigment production depends on bacterial activities limited by the availability of organic resources in the natural environment. The optimal production in relatively high temperatures implies that the presented bacterial pigment production could work effectively in the warming ocean.

The major component in the extracted crude pigment was violacein, as supported by the experimental results of NMR, MS, and UV spectra analyses. To our best knowledge, this is the first report of violacein from a marine bacterium, *Microbulbifer* sp., associated with a marine sponge *H. sinapium*, which is native to Korea and other East Asian countries.

This optimum range of bacterial growth falls into the range from previous studies [24, 27] on the same bacterium genus *Microbulbifer*. It is also consistent with the narrow optimum range reported for the bacterium *M. elongates* [27]. With a similar result for the pH effect, the close relationship between bacterial growth and pigment production indicates that this bacteria-pigment association is present in relatively stable environmental conditions such as those of biofilm and cellular fluid rather than in the changing conditions of the open ocean.

The specific production of violacein as a primary component of violet pigment, mg product per gram of cells, was not presented in this study and is beyond the scope to be discussed from the presented results; however, the production level can be inferred from the production of violet pigment as a proxy of violacein. In the study, pigment production was closely related with bacterial growth. The optimal incubation conditions were consistent with previous results using *Microbulbifer* species [24]. In a study on violacein production by a psychrotrophic bacterium, the optimal conditions for violet pigment production were reported as 20℃ and pH 6 and the maximum concentration and the productivity of violet pigment were 3.7 g/l and 0.12 $g \cdot l^{-1} \cdot h^{-1}$, respectively [26]. As reviewed by Choi *et al*. [10], there are many factors affecting the violacein production level, and these should be considered carefully in comparing various published values. During the present study, the preliminary yield of violet pigment was 0.25 g/l (unpublished) and further studies need to be performed to suggest both the maximum concentration and the productivity of violacein from the suggested *Microbulbifer* strain.

Ecological implications of sponge-microbe association in a changing ocean

Many bioactive products are derived from microorganism-engaged activities. In the marine environment, chemical defense mechanisms of attached organisms are prevalent and various microorganism-mediated pro-

cesses occur in biofilm. This study reports the successful production of a bioactive pigment produced by a marine bacterium *Microbulbifer* sp., which was isolated from a marine sponge *H. sinapium* native to Asian coastal waters. The yield of the violet pigment was found to be dependent on bacterial growth after testing under various culturing conditions, thereby indicating that violacein is produced by the bacterium *Microbulbifer* sp. The main component of the pigment was identified as violacein, which has been reported as one of the potent antibiotic materials from marine bacteria [10].

The sponge community has been considered as one of the important benthic fauna for indicating environmental impacts such as climate changes. Sponge-microbe associations have been one of the most popular marine resources for obtaining natural products, and changing environmental conditions also could have a considerable effect on optimal bacterial activities and production as well as the ecological status of the sponge community [28]. Therefore, climate effect studies on well-known sponge-microbe interactions and latitudinal sponge distribution can provide valuable insights for understanding how climate changes will affect ecosystem services that provide bioactive resources as well as primary marine production.

In the context of the issue of general ocean acidification (OA), the sponge community as one of the marine benthic communities has been reported as being affected by OA [29], and the impact of OA on marine microorganisms was also discussed in consideration of benthic organisms as host organisms [30]; however, the results of this study for the acidic optimum pH range of the bacterium and its pigment harvest indicate that it is unlikely to be affected directly by recent OA concerns about global ocean conditions. Additionally, considering the usually acidic condition of the sponge cellular fluid, the experimental results on pH dependency indicate that this bacteria-pigment relationship could be relatively stable in the host sponge in the coming acidic ocean.

There are still increasing numbers of reports and progress on natural products obtained from marine ecosystems. The identification of a bioactive material is not a novel approach in scientific communities; however, marine symbiotic microbial diversity is still not fully explored. Pharmaceutical metabolites from the marine ecosystem are some of the most promising and challenging subjects. As reviewed by Li [13], there are many useful cases of marine sponge-microbe related pharmaceutical metabolites from the South China Sea. Korean marine waters are one of the most affected and changing oceans in the world, providing increasing evidence of the northward range shift of tropical marine organisms. In this context, the present evidence of violacein production from a sponge-microbe association is not only one case report of many typical relations to be found in the Korean coastal ecosystem, but it is also a result that may be valuable for exploring marine-origin bioactive and pharmaceutical compounds in the changing ocean.

Acknowledgments

This work was partially supported by the 2016 Hannam University Research Fund, Korea and by the Ministry of Education, Korea through the fostering project of "Hannam University Industry-Academy Cooperation Campus". The author (N.-I.W) was also supported by the research project "Study on coastal marine ecosystem structures and suspended sediment in the EEZ sand mining zone" funded by K-water.

References

1. Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, *et al.* 2008. A global map of human impact on marine ecosystems. *Science* **319**: 948-952.
2. Belkin IM. 2009. Rapid warming of large marine ecosystems. *Progress in Oceanography* **81**: 207-213.
3. Poloczanska ES, Brown CJ, Sydeman WJ, Kiessling W, Schoeman DS, Moore PJ, *et al.* 2013. Global imprint of climate change on marine life. *Nature Climate Change* **3**: 919-925.
4. Morán XAG, Alonso-Sáez L, Nogueira E, Ducklow HW, González N, López-Urrutia Á, *et al.* 2015. Presented at the Proc. R. Soc. B.
5. Barton AD, Irwin AJ, Finkel ZV, Stock CA. 2016. Anthropogenic climate change drives shift and shuffle in North Atlantic phytoplankton communities. *Proceedings of the National Academy of Sciences* **113**: 2964-2969.
6. Dias DA, Urban S, Roessner U. 2012. A historical overview of natural products in drug discovery. *Metabolites* **2**: 303-336.
7. Harvey AL, Edrada-Ebel R, Quinn RJ. 2015. The re-emergence of natural products for drug discovery in the genomics era. *Nature Reviews. Drug Discovery* **14**: 111-129.
8. D'Orazio N, Gammone MA, Gemello E, De Girolamo M, Cusenza S, Riccioni G. 2012. Marine bioactives: pharmacological properties and potential applications against inflammatory diseases. *Marine Drugs* **10**: 812-833.
9. Skropeta D, Wei L. 2014. Recent advances in deep-sea natural products. *Natural Product Reports* **31**: 999-1025.

10. Choi SY, Kim S, Lyuck S, Kim SB, Mitchell RJ. 2015. High-level production of violacein by the newly isolated Duganella violaceinigra str. NI28 and its impact on *Staphylococcus aureus*. *Scientific Reports* **5**: 15598.
11. Laport MS, Santos OC, Muricy G. 2009. Marine sponges: potential sources of new antimicrobial drugs. *Curr. Pharm. Biotechnol.* **10**: 86-105.
12. El-Shitany NA, Shaala LA, Abbas AT, Abdel-Dayem UA, Azhar EI, Ali SS*, et al.* 2015. Evaluation of the anti-inflammatory, antioxidant and immunomodulatory effects of the organic extract of the red sea marine sponge xestospongia testudinaria against carrageenan induced rat paw inflammation. *PLoS One* **10**: e0138917.
13. Li Z. 2009. Advances in marine microbial symbionts in the china sea and related pharmaceutical metabolites. *Marine Drugs* **7**: 113-129.
14. Matz C, Kjelleberg S. 2005. Off the hook--how bacteria survive protozoan grazing. *Trends Microbiol.* **13**: 302-307.
15. Matz C, Webb JS, Schupp PJ, Phang SY, Penesyan A, Egan S*, et al.* 2008. Marine biofilm bacteria evade eukaryotic predation by targeted chemical defense. *PLoS One* **3**: e2744.
16. Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, Yooseph S*, et al.* 2007. The Sorcerer II Global Ocean Sampling expedition: northwest Atlantic through eastern tropical Pacific. *PLoS Biol.* **5**: e77.
17. Seshadri R, Kravitz SA, Smarr L, Gilna P, Frazier M. 2007. CAMERA: a community resource for metagenomics. *PLoS Biology* **5**: e75.
18. Park MH, Sim CJ, Baek J, Min GS. 2007. Identification of genes suitable for DNA barcoding of morphologically indistinguishable Korean Halichondriidae sponges. *Mol. Cells* **23**: 220-227.
19. Hoshino S, Saito DS, Fujita T. 2008. Contrasting genetic structure of two Pacific Hymeniacidon species. *Hydrobiologia* **603**: 313-326.
20. Fuller T, Hughey J. 2013. Molecular investigation of the invasive sponge *Hymeniacidon sinapium* (de Laubenfels, 1930) in Elkhorn Slough, California. *Aquatic Invasions* **8**: 59-66.
21. Jeong J-B, Park J-S. 2012. Seasonal differences of bacterial communities associated with the marine sponge, *hymeniacidon sinapium*. *The Korean J. Microbiol.* **48**: 262-269.
22. Yang LH, Xiong H, Lee OO, Qi SH, Qian PY. 2007. Effect of agitation on violacein production in *Pseudoalteromonas luteoviolacea* isolated from a marine sponge. *Lett. Appl. Microbiol.* **44**: 625-630.
23. Ventosa A, Márquez MC, Ruiz-Berraquero F, Kocur M. 1990. *Salinicoccus roseus* gen. nov., sp. nov., a New Moderately Halophilic Gram-Positive Coccus. *Syst. Appl. Microbiol.* **13**: 29-33.
24. Wakabayashi M, Sakatoku A, Noda F, Noda M, Tanaka D, Nakamura S. 2012. Isolation and characterization of Microbulbifer species 6532A degrading seaweed thalli to single cell detritus particles. *Biodegradation* **23**: 93-105.
25. Rettori D, Durán N. 1998. Production, extraction and purificationof violacein: an antibiotic pigment producedby *Chromobacterium violaceum*. *World J. Microbiol. Biotechnol.* **14**: 685-688.
26. Nakamura Y, Sawada T, Morita Y, Tamiya E. 2002. Isolation of a psychrotrophic bacterium from the organic residue of a water tank keeping rainbow trout and antibacterial effect of violet pigment produced from the strain. *Biochem. Eng. J.* **12**: 79-86.
27. Yoon JH, Kim H, Kang KH, Oh TK, Park YH. 2003. Transfer of *Pseudomonas elongata* Humm 1946 to the genus Microbulbifer as Microbulbifer elongatus comb. nov. *Int. J. Syst. Evol. Microbiol.* **53**: 1357-1361.
28. Webster NS, Cobb RE, Negri AP. 2008. Temperature thresholds for bacterial symbiosis with a sponge. *The ISME J.* **2**: 830-842.
29. Goodwin C, Rodolfo-Metalpa R, Picton B, Hall-Spencer JM. 2014. Effects of ocean acidification on sponge communities. *Mar. Ecol.* **35**: 41-49.
30. Das S, Mangwani N. 2015. Ocean acidification and marine microorganisms: responses and consequences. *Oceanologia* **57**: 349-361.

국문초록

한국 서해안에 서식하는 주황해변해면에서 분리된 해양세균 *Microbulbifer* sp.으로부터 생리활성물질 비올라세인의 규명

원남일[1], 이가은[2], 고기범[3], 오동찬[3], 나양호[4]*, 박진숙[2]*

[1]*한국수자원공사 K-water 연구원*

[2]*한남대학교 생명시스템과학과*

[3]*서울대학교 약학대학 천연물과학연구소*

[4]*한남대학교 신소재공학과*

오늘날 해양생물로부터 얻어진 미생물유래의 이차대사물질은 구조적, 생물학적으로 새로운 화합물의 주요한 자원이다. 그 중에서 해면동물과 미생물 관계는 생리활성 물질을 탐색하는데 가장 흥미있는 자원 중 하나로서 주목받아 왔다. 본 연구에서는 서해안 조간대에서 채집된 주황해변해면(*Hymeniacidon sinapium*)으로부터 분리된 세균 균주(*Microbulbifer* sp., 127CP-12)를 검토하였다. 배양된 세균은 자주색 색소를 생산하였으며, 색소생산의 최적 배양조건을 조사하였다. 최대 색소생산을 위한 미생물 배양조건은 25℃, pH 6.0, 3% NaCl임을 알 수 있었다. 추출용매는 에탄올과 메탄올에 비해 아세톤이 더 적절한 것으로 나타났다. 추출된 색소의 주요성분은 HLPC, NMR, MS, 그리고 UV 스펙트럼의 구조 분석을 통해 유용한 생리활성물질인 비올라세인으로 밝혀졌다. 본 연구는 해양미생물이 관여한 대사물질로부터 생리활성물질을 조사하는 연구기법을 서술함과 동시에 오늘날 변화하는 해양환경에서 해면동물과 미생물 관계의 생태학적 의의를 제시하고 있다.

http://dx.doi.org/10.4014/mbl.1702.02002