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Original Article

Isolation and production of prodigiosin and cycloprodigiosin from marine sponges-associated bacteria of the Andaman coast of Thailand

Tharanan Kongkapan[1], Naraid Suanyuk[1, 2*], and Suphada Kiriratnikom[3]

[1] *Kidchakan Supamattaya Aquatic Animal Health Research Center, Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand*

[2] *Discipline of Excellence in Sustainable Aquaculture, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand*

[3] *Biological Science and Environment, Faculty of Science, Thaksin University, Phatthalung Campus, Pa Phayom, Phatthalung, 93110 Thailand*

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Abstract

During October 2015 to December 2016, eight marine sponge-associated bacteria (PSU-KSAAHRC MS1-8) with red, reddish orange, yellow, reddish pink, and dark red pigments were isolated from five species of marine sponge, namely *Callyspongia* sp., *Callyspongia diffusa*, *Haliclona* sp., *Dysidea* sp. and *Stylissa carteri*, sampled from Satun and Phang-Nga provinces on the Andaman coast of Thailand. These bacteria produced pigments in the range 55.65-5,619.67 µg/g. The highest pigment content was found in the dark red-pigmented bacterial isolate PSU-KSAAHRC MS2 isolated from *Haliclona* sp. sampled in Satun province. Thin layer chromatography of a red amorphous pigment extract from the bacterial isolate PSU-KSAAHRC MS2 revealed two fractions with respective Rf value of 0.65 and maximum absorbance at 535, and Rf value of 0.57 and maximum absorbance at 539 nm. Analyses of each TLC fraction by liquid chromatograph quadrupole time of flight mass spectrometer revealed the molecular weights of 323 (*m/z* 324, $[M+H]^+$) and 321 (*m/z* 322, $[M+H]^+$), in the same order. Comparing to published data, the compound with maximum absorbance at 535 and molecular weight of 323 (*m/z* 324, $[M+H]^+$) was identified as prodigiosin while the compound with maximum absorbance at 539 nm and molecular weight of 321 (*m/z* 322, $[M+H]^+$) was identified as cycloprodigiosin. Based on morphological and biochemical characteristics as well as phylogenetic analysis obtained from the present study, the bacterial isolate PSU-KSAAHRC MS2 was identified as *Zooshikella* sp. To our knowledge, this is a first report on identification of prodigiosin and cycloprodigiosin from *Zooshikella* sp. isolated from marine sponge, *Haliclona* sp., in Thailand.

Keywords: marine sponge, *Zooshikella* sp., prodigiosin, cycloprodigiosin

1. Introduction

Marine sponges, the members of the phylum Porifera, are benthic organisms living in a wide range of habitats from polar regions to the tropics (Bergquist, 2001). These multicellular organisms are sessile filter feeders and play important roles in marine nutrient cycles as vital source of dissolved inorganic nitrogen (Jackson *et al.*, 2015). It is well-known that the tissues of sponges carry diverse communities of microorganisms, such as fungi, archaea, and bacteria. Of these microorganisms, bacteria were the dominant group of microbial associates in marine sponges, with densities up to 10^8-10^{10} bacteria/g wet weight (Hentschel, Usher, & Taylor, 2006). To date, numerous studies have revealed several bioactive compounds produced by the marine sponge-associated bacteria. Some of these compounds may

*Corresponding author
Email address: naraid.s@psu.ac.th

serve beneficial purposes as antimicrobial compounds and natural pigments (Anand *et al.*, 2006; Araújo, Fukushima, & Takaki, 2010; Ibrahim, Nazari, Kassim, & Lim, 2014; Saravanakumar, Ronald, Ramesh, & Maheswari, 2011). Natural pigments are involved in physiological or metabolic processes of organisms (Britton, 1983). They can be obtained from ores, plants, insects, animals, and microorganisms (Venil, Zakaria, & Ahmad, 2013). Among the microbes, bacteria are known as a potential source of pigment for applying in food, textiles, cosmetics, and pharmaceuticals (Venil *et al.*, 2013). Example of natural pigments produced by bacteria, such as astaxanthin produced by *Paracoccus haeundaensis* isolated from seawater collected from the Haeundae coast, Korea (Lee, Kim, Choi, Lee, & Kim, 2004), carotenoids produced by *Psychrobacter celer* isolated from sponge collected from Cemara Besar Island, Indonesia (Abfa, Radjasa, Susanto, Nuryadi, & Karwur, 2017) and *Methylobacterium mesophilicum* isolated from marine sponge in the Gulf of Thailand (Thawornwiriyanun, Dechasakul watana, Suntornsuk, & Suntornsuk, 2009), violacein by *Pseudoalteromonas amylolytica* isolated from seawater of the Arabian Sea (Wu *et al.*, 2017), and prodigiosin by *Serratia marcescens* isolated from semi-arid soil (Araújo *et al.*, 2010). To gain further knowledge on pigmented bacteria, the present study aims to isolate and characterize marine sponge-associated bacteria that effectively produce natural pigments. Obtained data as well as pigmented bacteria derived from the present study will be a potential source of pigment for applications in aquaculture, food, textiles, cosmetics, and pharmaceuticals in Thailand.

2. Materials and Methods

2.1 Sample collection

During October 2015 to December 2016, twenty-nine samples of marine sponges were collected from Satun and Phang-Nga provinces on the Andaman coast of southern Thailand (Table 1). Sponge samples were cut and put into sterile plastic bags underwater, stored on ice in a cooler box, and transferred to the laboratory for further study. They were identified to genus and species level based on morphological characteristics, using the method described by Putchakarn (2012) and by Dr. S. Putchakarn, Institute of Marine Science, Burapha University, Thailand.

2.2 Isolation of marine sponge-associated bacteria

Pigmented bacteria were isolated from marine sponges by using a method modified from Lee *et al.* (2011). Briefly, the marine sponges were cut into small pieces of approximately 1 g fresh weight, cleaned with sterile sea water and grounded with mortar and pestle. Samples were then serially diluted to 10 and 100 folds with sterile sea water, spread onto Marine agar 2216 (MA; Himedia) and incubated at 30 ºC for 24-48 hrs under fluorescent light. After incubation, red, orange and yellow single presumptive colonies were selected and reisolated by streaking onto MA and incubating at 30 ºC for 24-48 hrs under fluorescent light. All the purified bacterial isolates were inoculated into Marine broth (MB; Himedia) supplemented with 15% glycerol, and stored at -80 ºC.

2.3 Analysis of pigment production

Pigmented bacteria were selected and analyzed for pigment production by using a method modified from Britton, Liaaen-Jensen, and Pfender (1995) and Williams, Green, and Rappoport (1956). The bacteria were streaked onto MA and incubated at 30 ºC for 24-48 hrs under fluorescent light. After incubation, bacterial cells were transferred into flasks containing 100 ml of MB and incubated at 30 ºC for 24-48 hrs under fluorescent light. Then cells were harvested by centrifugation at 10,000 rpm for 10 min at 4 ºC, and washed twice with sterile 0.85% NaCl. Prior to the analysis of pigment, pellet cells were dried in a vacuum freeze dryer (Labcongo Corporation, USA) until constant weight. The dried cells were rehydrated with distilled water and then pigments were extracted by adding acetone, followed by several re-extractions with fresh acetone until no visible pigment remained in the bacterial debris. The acetone extracts were pooled and subjected to phase separation with an equal

Table 1. Source of isolate, number, color, and pigment content of bacteria isolated from marine sponges sampled in the Andaman coast of Thailand.

No.	Date	Sample location	Marine sponge	Bacterial isolate	Color	Pigment content (µg/g)
1	Oct 2015	Takua Pa, Phang-nga (8°58'52.4" N, 98°15'25.8" E)	*Callyspongia* sp.	PSU-KSAAHRC MS1	Reddish pink	267.56 ± 90.58^{bc}
2	Dec 2015	La-ngu, Satun (6°46'21.4" N, 99°49'32.8" E)	*Haliclona* sp.	PSU-KSAAHRC MS2	Dark red	$5{,}619.67 \pm 831.68^{a}$
3	Jan 2016	Takua Thung, Phang-nga (8°10'53.9" N, 98°20'36.4 "E)	*Dysidea* sp.	PSU-KSAAHRC MS3	Red	943.42 ± 225.40^{b}
4	Dec 2016	Takua Thung, Phang-nga (8°10'53.9" N, 98°20'36.4" E)	*S. carteri*	PSU-KSAAHRC MS4	Red	667.05 ± 168.92^{bc}
			S. carteri	PSU-KSAAHRC MS5	Reddish orange	762.29 ± 165.32^{bc}
			S. carteri	PSU-KSAAHRC MS6	Reddish orange	79.73 ± 8.58^{c}
			S. carteri	PSU-KSAAHRC MS7	Yellow	nd
			C. diffusa	PSU-KSAAHRC MS8	Red	55.65 ± 5.73^{c}

Different superscripts indicate significant differences ($p<0.05$). nd = not detected.

volume of diethyl ether. The diethyl ether extracts were repeatedly washed with an equal volume of distilled water to remove trace contaminants in the solution. To determine pigments content in the extract, the absorbance of extract in diethyl ether was spectrophotometrically determined over the range 400-600 nm for maximum absorbance (Multiskan Go, Thermo Fisher Scientific, Finland). The pigment content was measured using the extinction coefficient of prodigiosin at 0.1397 $mM^{-1}cm^{-1}$ (Elahian *et al.*, 2013) and calculated according to the following formula:

$$\text{Pigment content } (\mu g/g) = (A \times V)/(E \times G)$$

where A is the absorbance at maximum wavelength, V is the amount of extract (ml), E is the extinction coefficient of prodigiosin (0.1397) and G is the sample weight (g)

2.4 Pigment separation and identification

The bacterial isolate that produced the highest amount of pigment was chosen for pigment separation by thin layer chromatography (TLC) (Britton *et al.*, 1995) and identification by liquid chromatograph quadrupole time of flight mass spectrometer (LC-QTOF-MS). Briefly, the pigment extract was evaporated with nitrogen flushing and then blended with petroleum ether. This solution was spread in band onto TLC aluminum plate with silica gel 60 F_{254} and then placed in the chromatographic chamber containing petroleum ether: diethyl ether at 1:3 ratio as the mobile phase (Britton *et al*., 1995). The solvent was allowed to move up to about 80% coverage of the plate. The retention factor (Rf) value of the chromatogram was calculated according to Hussan and Authaman (2015). Each band on the TLC plate was scraped and eluted in a vial containing diethyl ether. This solution was filtered before measuring the maximum absorbance with UV-visible spectrophotometry (Evolution 300LC, Thermo Electron Corporation, England) over the range 400-600 nm (Araújo *et al.*, 2010) and subsequent characterized by using a LC-QTOF-MS (Agilent Q-TOF G6545A, Agilent technologies, USA).

2.5 Bacterial identification

The bacterial isolate that produced the highest amount of pigment was tentatively identified from its morphological and biochemical characteristics, including gram stain, oxidase test, and catalase test. This bacterial isolate was further classified to genus level based on molecular analysis as follows.

2.5.1 DNA extraction

Nucleic acid was extracted from the selected bacterial strain using the method of Berridge *et al.* (1998). Briefly, the bacteria were cultured on MA for 48 hrs at 30 ºC under fluorescent light. After incubation, bacterial cells were harvested by centrifugation at 10,000 rpm for 10 min at 4 ºC. The pellet cells were resuspended into 100 µl of lysis solution (100 mM NaCl, 10 mM Tris-HCl (pH 8.3), 1 mM EDTA (pH 8.0), 1% Triton X-100), boiled for 10 min in hot water, and then cooled to room temperature. The lysates were diluted to 500 µl with sterile distilled water.

2.5.5 16S rDNA sequencing and phylogenetic analysis

The forward primer 20F (5´-AGAGTTTGATCA TGGCTCAG-3´) and reverse primer 1500R (5´-GGTT ACCTTGTTACGACTT-3´) described by Weisburg, Barns, Pelletier, and Lane (1991) were used to amplify the 16s rDNA gene of the bacteria. PCR was amplified in the MJ Mini™ thermal cycler (Bio-Rad) using 7.5 µl of PCRBIO HS Taq Mix Red (PCR BIOSYTEM), 0.5 µl of each 10 µM primer, 2 µl of DNA template, and 4.5 µl of distilled water. The thermal program had initial denaturation at 94 ºC for 4 min; 29 cycles of 94 ºC for 40 seconds, 55 ºC for 1 min, and 72 ºC for 1 min; and a final extension at 72 ºC for 5 min. The PCR-amplified products were separated by electrophoresis (30 min, 110V) in a 1.5% agarose gel with TAE buffer (40 mM Tris-acetate, 1 mM ethylenediaminetetraacetic acid, pH 8.0) and visualized by ethidium bromide staining, alongside a 100 bp DNA ladder (Genedirex).

The PCR amplified product was purified with a gel extraction kit (Qiagen) and sequenced using PCR primers 20F and 1500R. The generated sequences were used to search a database for homologous sequences using the Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.gov) and the found homologous sequences were included in a phylogenetic analysis using the clustalW application of the Molecular Evolutionary Genetics Analysis (MEGA) package (version 7.0). The phylogenetic tree was constructed using neighbor-joining method, and with bootstrap analysis with 1,000 replications to assess the strength of nodes in the tree.

2.6 Statistical analysis

All testing was done in triplicates and the averages and standard deviation of total pigment content are reported. Analysis of variance (ANOVA) and Duncan's multiple range test (Steel & Torrie, 1980) were used to determine significant differences in the total pigment contents between bacterial strains.

3. Results

3.1 Isolation of marine sponge-associated bacteria

A total of eight pigmented bacteria (PSU-KSAAHRC MS1-8) (Table 1) were isolated from the five marine sponge species *Callyspongia* sp., *Callyspongia diffusa*, *Haliclona* sp., *Dysidea* sp. and *Stylissa carteri* sampled in Satun and Phang-Nga provinces (Table 1). All bacterial isolates from marine sponges exhibited circular, convex, glistening and smooth edge colonies with reddish pink, dark red, red, reddish orange or yellow in color (Table 1).

3.2 Analysis of pigment production

Eight pigmented bacteria were analyzed for pigment production by spectrophotometry. The pigment contents ranged within 55.65-5,619.67 µg/g. Pigment was not detectable from the bacterial isolate PSU-KSAAHRC MS7 which was isolated from *S. carteri* collected from Phang-nga province, while significantly highest 5,619.67 µg/g pigment content ($p<0.05$) was found in the dark red-pigmented

bacterial isolate PSU-KSAAHRC MS2 which was isolated from *Haliclona* sp. sampled in Satun province (Table 1).

3.3 Pigment separation and identification

A red amorphous pigment extracted from bacterial isolate PSU-KSAAHRC MS2 revealed two fractions separated on the TLC plate. Fraction 1 was reddish orange with Rf value of 0.65 and maximum absorbance at 535 nm, and fraction 2 was light pink with Rf value of 0.57 and maximum absorbance at 539 nm (Figure 1). Analysis of these two TLC fractions by LC-QTOF-MS revealed the molecular weights of 323 (*m/z* 324, $[M+H]^+$) and 321 (*m/z* 322, $[M+H]^+$). Comparing to published data, the compound with maximum absorbance observed at 535 nm with molecular weight of 323 (*m/z* 324, $[M+H]^+$) corresponding to prodigiosin while the compound with maximum absorbance observed at 539 nm with molecular weight of 321 (*m/z* 322, $[M+H]^+$) corresponding to cycloprodigiosin (Figure 1) (Faraag, El-Batal, & El-Hendawy, 2017; Lee *et al.*, 2011; Oh *et al.*, 2016; Ramaprazad, Bharti, Sasikala, & Ramana, 2015).

3.4 Bacterial Identification

On assessing the morphological and biochemical characteristics, the bacterial isolate PSU-KSAAHRC MS2 was gram-negative, rod-shaped, and oxidase and catalase positive. The sequence of its 16S rDNA gene was 99.52% similar to three *Zooshikella ganghwensis* sequences stored in GenBank with accession numbers AY130995.2, AY130994.2 and NR_025668.1 and was 99.28% identity to *Zooshikella marina* NR_145895.1. The phylogenetic tree constructed using the neighbor-joining method, identified bacterial isolate PSU-KSAAHRC MS2 as *Zooshikella* sp. (Figure 2).

4. Discussion

Marine sponges are the oldest extant metazoan animals (Jackson *et al.*, 2015; Maloof *et al.*, 2010). They are sessile animals, derive nutrition by filter-feeding from seawater and can be hosts for several microbes, including eukarya, archaea, viruses and bacteria (Jackson *et al.*, 2015). Marine sponge-associated bacteria are potentially rich sources of pigment (Abfa, *et al.*, 2017; Ibrahim *et al.*, 2014; Jafarzade, Yahya, Mohamad, Usup, & Ahmad, 2013; Sivaganesh & Packiasamy, 2016; Thawornwiriyanun *et al.*, 2009). In this study, eight bacterial isolates that produced red, orange and yellow pigments were isolated from marine sponges sampled in Satun and Phang-Nga provinces, the Andaman coast of Thailand. The results are consistent with a report by Thawornwiriyanun *et al.* (2009), in which 24 pigmented bacteria that produce orange-pink, brown, white, yellow, red, and orange pigments were isolated from marine sponges. Additionally, it should be noted that, some other pigmented bacteria isolated from the present study was unable to grow in MA medium after subculture indicated that specific nutrients may be required for their growth. Therefore, further study on screening for suitable media for culture of marine sponge-associated bacteria needed to be investigated.

The present study revealed a bacterial isolate, PSU-KSAAHRC MS2 from *Haliclona* sp., produced the highest amount of pigment at 5,619.67 µg/g. Although sponges have been reported as good sources for bioactive compound discovery, they are not available in large quantities in nature,

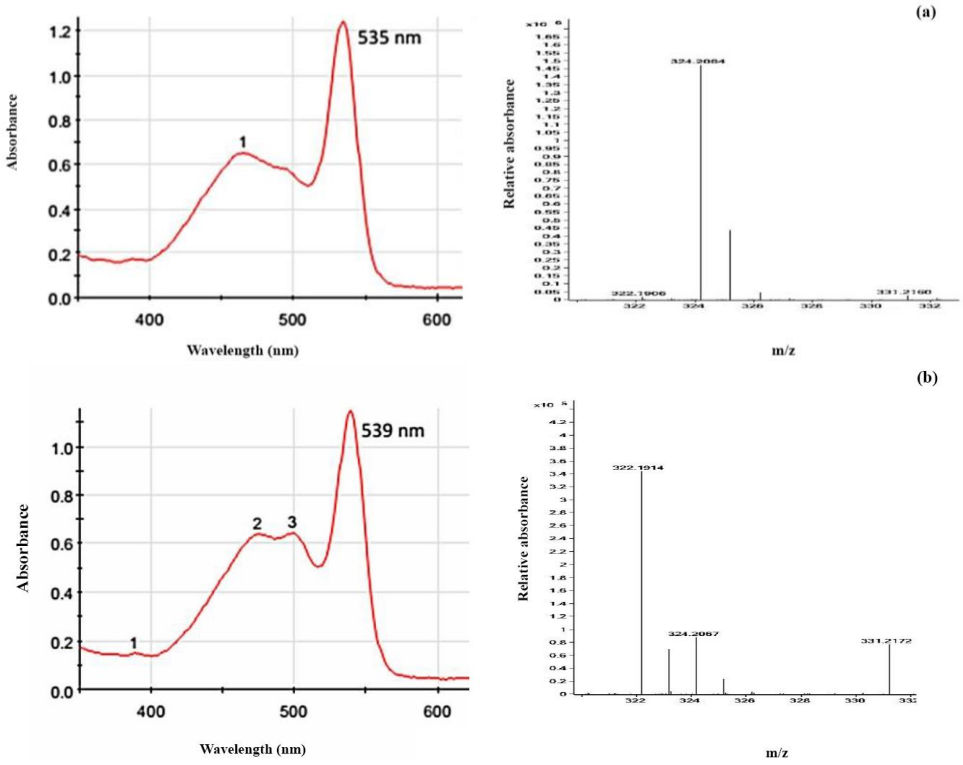

Figure 1. Maximum absorbance and mass spectra of pigment fraction 1 (a) and fraction 2 (b), from TLC

Figure 2. Phylogenetic tree calculated from 16s rDNA sequences for the *Zooshikella* sp. PSU- KSAAHRC MS2 in the present study and similar sequences in the Genbank database.

and they are difficult to culture in laboratory conditions. Therefore, marine sponge-associated bacteria are a new alternative target in the search for natural bioactive compounds (Leal *et al.*, 2014; Muscholl-Silberhorn, Thiel, & Imhoff, 2008).

Characterization of marine sponge-associated bacteria capable of producing bioactive compounds is of high current interest. Manikandan, Ganesapandian, Sangeetha, and Kumaraguru (2014) reported antibacterial activity of *Stenotrophomonas* sp. and *Bacillus subtilis* isolated from marine sponges in the Gulf of Mannar against gram positive and negative bacteria. Furthermore, Asagabaldan *et al.* (2016) reported antibacterial activity of *Chromohalobacter salixigens* isolated from *Haliclona* (*Reniera*) sp. against multidrug resistant bacteria. Based on biochemical characteristics and phylogenetic analysis obtained from the present study, the bacterial isolate PSU-KSAAHRC MS2 was identified as *Zooshikella* sp. BLAST analysis based on 16S rDNA gene sequence indicated that the bacterial isolate PSU-KSAAHRC MS2 was closely related to *Z. ganghwensis* (99.52% identity). However, it should be noted that BLAST analysis of the bacterial isolate PSU-KSAAHRC MS2 also showed high sequence similarity to *Z. marina* (99.28%) and phylogenetic analysis clustered the bacterial isolate PSU-KSAAHRC MS2, *Z. ganghwensis* and *Z. marina* nearly to be the same group. Similarly, Ramaprazad *et al.* (2015) reported that phylogenetic analyses based on the 16S rRNA gene sequence of *Z. marina* strain JC333^{T} showed highest sequence similarity to *Z. ganghwensis* JC2044^{T} (99.24 %). Therefore, more molecular techniques such as DNA-DNA hybridizations between PSU-KSAAHRC MS2, *Z. ganghwensis* and *Z. marina* may be needed to identify the bacterial isolate PSU-KSAAHRC MS2 to the species level. In the present study, the bacterial isolate PSU-KSAAHRC MS2 was gram-negative, rod-shaped, and oxidase and catalase positive. This was in agreement with Yi, Chang, Oh, Bae, and Chun (2003) who reported that *Z. ganghwensis* was a gram-negative, halophilic bacterium, oxidase and catalase positive, and rod-shaped. It grows on marine agar, medium B, or yeast extract agar as circular colonies that are yellowish-red or red. This bacteria have been isolated from sediment samples in Korea (Yi *et al.*, 2003), Malaysia (Jafarzade *et al.*, 2013), and Saudi Arabia (Rehman, Alam, Kamau, Bajic, & Leiknes, 2018). To our knowledge, this is a first report on isolation of *Zooshikella* sp. from marine sponge, *Haliclona* sp., in Thailand.

Analysis of the pigments produced by *Zooshikella* sp. PSU-KSAAHRC MS2 identified its red pigments as cycloprodigiosin and prodigiosin with molecular weights of 321 (*m/z* 322, $[M+H]^{+}$) and 323 (*m/z* 324, $[M+H]^{+}$), respectively. The result from the present study were identical to the previous studies of Lee *et al.* (2011) and Ramaprasad *et al.* (2015) which identified both cycloprodigiosin and prodigiosin from *Z. rubidus* isolated from tidal flat sediment of the Yellow Sea, Korea and *Z. marina* isolated from a sand sample collected from Shivrajpur–Kachigad beach, Gujarat, India, respectively. In this study, the red amorphous pigment from *Zooshikella* sp. PSU-KSAAHRC MS2 with the Rf value of 0.65, maximum absorbance at 535 nm and the molecular weight of 323 (*m/z* 324, $[M+H]^{+}$) was identified as prodigiosin. The Rf value of prodigiosin at 0.60 and 0.62 was reported previously (Aruldass, Venil, Zakaria, & Ahmad, 2014; Metwally, El-Sersy, El Sikaily, Ghozlan, & Sabry, 2017). In addition, Gulani, Bhattacharya, and Das (2012) reported the Rf value of 0.27, 0.64 and 0.82 observed in three different fractions of prodigiosin. Other studies reported the Rf value ranged from 0.85-0.94 (Gondil, Asif, & Bhalla, 2017; Hussan, & Authaman, 2015; Phatake & Dharmadhikari, 2016; Sulaiman, Ibrahim, & Noordin, 2019). Prodigiosin is a natural red pigment with a maximum absorbance of 535–540 nm (Allen, 1967). It is a secondary metabolite alkaloid containing three pyrrole rings in its chemical structure, with molecular weight of 323 (*m/z* 324, $[M+H]^{+}$) (Araújo *et al.*, 2010; Lee *et al.*, 2011). The maximum absorbance and molecular weight of the prodigiosin in this study was identity to prodigiosin produced by *Z. ganghwensis* isolated from mud flats in Kanwondo in Korea (Oh *et al.*, 2016), *Z. rubidus* isolated from tidal flat sediment of the Yellow Sea, Korea (Lee *et al.*, 2011), *Z. marina* isolated from a sand sample collected from Shivrajpur–Kachigad beach, Gujarat, India (Ramaprasad *et al.*, 2015) and *S. marcescens* isolated from irrigation water in Egypt (Faraag, *et al.*, 2017). Prodigiosin can be also produced by *Vibrio psychoerythrus*, *Rugamonas rubra* and *Streptoverticillium rubrireticuli* (Shaikh, 2016). This pigment exhibited antifungal, antimicrobial, antitumor

and antimalarial properties (Lapenda, Silva, Vicalvi, Sena, & Nascimento, 2015; Shaikh, 2016). Recently, Lapenda *et al.* (2015) reported that prodigiosin extracted from *S. marcescens* exhibited significant inhibition zones against *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus pyogenes.* In addition, prodigiosin possesses antifungal properties, inhibiting the growth of *Pythium myriotylum, Rhizoctonia solani, Sclerotium rolfsii, Phytophthora infestans,* and *Fusarium oxysporum,* according to Jimtha, Jishma, Sreelekha, Chithra, and Radhakrishnan (2017).

In this study, the pigment from *Zooshikella* sp. PSU-KSAAHRC MS2 with the Rf value of 0.57, maximum absorbance at 539 nm and the molecular weight of 321 (*m/z* 322, $[M+H]^+$) were identified as cycloprodigiosin. Using different solvents in the purification and separation of pigment resulted in different Rf value (Hussan & Authaman, 2015). The Rf value of cycloprodigiosin at 0.20 was reported by Kim *et al.* (1999) using benzene:diethylether (1:1) as solvent. In this study, similar Rf value (0.22) was observed when using the same solvent indicating that our pigment was cycloprodigiosin (data not shown). Cycloprodigiosin, a compound with a molecular weight of 321 (*m/z* 322, $[M+H]^+$) (Lee *et al.*, 2011), was produced by a few marine bacteria such as *Z. rubidus* (Lee *et al.*, 2011), *Z. marina* (Ramaprasad *et al.*, 2015), *Pseudoalteromonas rubra* (Xie *et al.*, 2012) and *Pseudoalteromonas denitrificans* (Kawauchi *et al.*, 1997). This compound has shown an immunosuppressive (Kawauchi *et al.*, 1997) and anticancer activities (Yamamoto *et al.*, 1999). Interestingly, Lee *et al.* (2011) reported that cycloprodigiosin extracted from *Z. rubidus* exhibited higher antimicrobial activity than prodigiosin. Further study on the effect of prodigiosin and cycloprodigiosin extracted from *Zooshikella* sp. from the present study on inhibitory activities against pathogenic microorganisms may provide deep insight to understand the antimicrobial activities of these pigments.

5. Conclusions

The present study isolated *Zooshikella* sp. PSU-KSAAHRC MS2 from *Haliclona* sp. sampled in the Satun province of Thailand. This bacterium produced pigment content of 5,619.67 µg/g. Analysis of the red amorphous pigment by TLC and LC-QTOF-MS revealed that this bacterium harbored prodigiosin with the Rf value of 0.65, maximum absorbance observed at 535 nm and molecular weight of 323 (*m/z* 324, $[M+H]^+$), and cyloprodigiosin with the Rf value of 0.57, maximum absorbance observed at 539 nm and molecular weight of 321 (*m/z* 322, $[M+H]^+$). The antimicrobial activities of prodigiosin and cycloprodigiosin produced by *Zooshikella* sp. need further studies to investigate more in details.

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