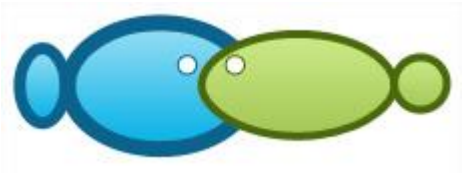

Genetic screening of a marine pigmented NRPS-producing bacterium associated with brown algae exhibiting anti-*Vibrio* activity

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Abstract. The *Vibrio* spp. bacterium has caused many problems in the aquaculture industry and human health, which led to the discovery of new compounds from marine microorganisms. Thus, the study was conducted to isolate and identify a symbiotic bacterium from brown algae, evaluate its anti-*Vibrio* properties against three *Vibrio* bacteria (*V. harveyi*, *V. vulnificus* and *V. parahaemolyticus*) and characterize its non-ribosomal peptide synthetases (NRPS) gene, which is amplified using specific primers. Out of 45 pigmented bacterial symbionts isolated from three brown algae, only 11 isolates exhibited anti-*Vibrio* activity against at least one *Vibrio* bacteria, and there was no pathogenic pattern when those selected bacteria were inoculated on blood agar. Moreover, all those 11 bacteria were subjected for fermentation and extraction using ethyl acetate to assess their crude extract against three *Vibrio* isolates. The results also showed that all crude extracts from the selected bacteria exhibited zones of inhibition ranging from 1 to 3 mm for at least one *Vibrio* bacteria, except the karimun B (KRB) isolate that presents no inhibition. Furthermore, the karimun G (KRG) isolate, a bright yellow bacterium, which demonstrated a broad halo inhibiting zone, was identified as *Aurantimonas coralicida* according to 16SrRNA and detected to have NRPS fragments when it is visualized in electrophoresis. However, the sequences of its NRPS are close to *Pseudomonas psychrotolerans*.
Key Words: *Aurantimonas coralicida*, brown algae associated bacteria, marine pigmented bacteria, NRPS gene, vibriosis.

Introduction. Diseases in shrimp industries result in a significant loss of productivity. *Vibrio* bacteria are predicted to be responsible for many outbreaks in the aquaculture industry. Recently, *Vibrio parahaemolyticus* was found to be able to colonize the stomach of shrimp and releases toxins identic with Pir toxins (Joshi et al 2014; Xiao et al 2017), causing acute hepatopancreatic necrosis disease (AHPND) (Soto-Rodriguez et al 2010). *Vibrio harveyi* also releases toxins (Nakayama et al 2006), leading to luminous shrimp (Soonthornchai et al 2015; Teo et al 2000). AHPND and luminous shrimp lead to early mortality syndrome (EMS), a condition where the mortality of post-larvae shrimp could reach 100% after stocking (Tran et al 2013). Therefore, a strategy should be developed to tackle vibriosis by applying antagonistic bacterial candidates.

Brown algae secrete many metabolism products to protect themselves from the extreme marine environment. These are very important in biotechnological applications, from antibacterial activities to anticancer agents (Kizhakkekalam & Chakraborty 2019; Manivannan et al 2011; Palanisamy et al 2018). Furthermore, bacteria that are symbiotic with the algae are also interesting to explore, because they can produce the same or even unique compounds with the ability to suppress the growth of bacterial pathogens. For example, Karthick & Mohanraju (2018) observed that brown algae associated bacteria, *Exiguobacterium profundum*, exhibited antibacterial activity against 13 pathogenic bacteria and produced many cellular fatty acids. Also, several studies revealed that *Bacillus pumilus*

(Susilowati et al 2015), *Vibrio owensii* (Karthick & Mohanraju 2018) and *Pseudomonas* sp. (Ismail et al 2016) isolated from brown algae have an antibacterial activity against diverse bacterial pathogens. Moreover, in case of symbiotic bacteria producing pigment, many compounds have been isolated and reported to have an antibacterial effect (Kalinovskaya et al 2017). Thus, exploration of antibacterial candidates from pigmented bacterial symbionts has increased over time.

Non-ribosomal peptide synthetases (NRPS) are enzymes involving the biosynthesis of biological essential compounds by microorganisms, including bacteria (Felnagle et al 2008). The molecular detection of NRPS fragments is an entirely new and rapid way to confirm the biological ability of bacteria against other pathogenic bacteria, because the gene could be involved in the biosynthesis of antibacterial agents. For instance, Mohamad et al (2018) observed that 16 bacterial isolates from the Chinese medicinal plant, *Glycyrrhiza uralensis*, showed NRPS genes as well as a broad antimicrobial activity. Sibero et al (2019) also reported the presence of the NRPS gene and antibacterial activity in *Vibrio owensii*, isolated from coral reefs. Therefore, the study aims to isolate and identify a potential algal associated bacterium, evaluate its anti-*Vibrio* properties and characterize its NRPS fragment.

Material and Method

Sample collection and bacterial isolation. Three types of brown algae (*Sargassum* sp., *Padina* sp. and *Turbinaria* sp.) were collected from the coastal area of Karimunjawa Island, Indonesia (Figure 1). The samples were stored in a sterilized zip-lock plastic bag within a cool box for further study. Furthermore, the isolation of bacteria was carried out using serial dilution methods. Fresh samples were initially cut to approximately 1 cm^2 and grounded with a mortar before they were homogenized in sterilized seawater. The dilutions from 10^{-1} to 10^{-6} were transferred into marine agar (MA) and incubated at room temperature (27°C) for 3–14 days with daily checking to acquire the pigmented bacteria.

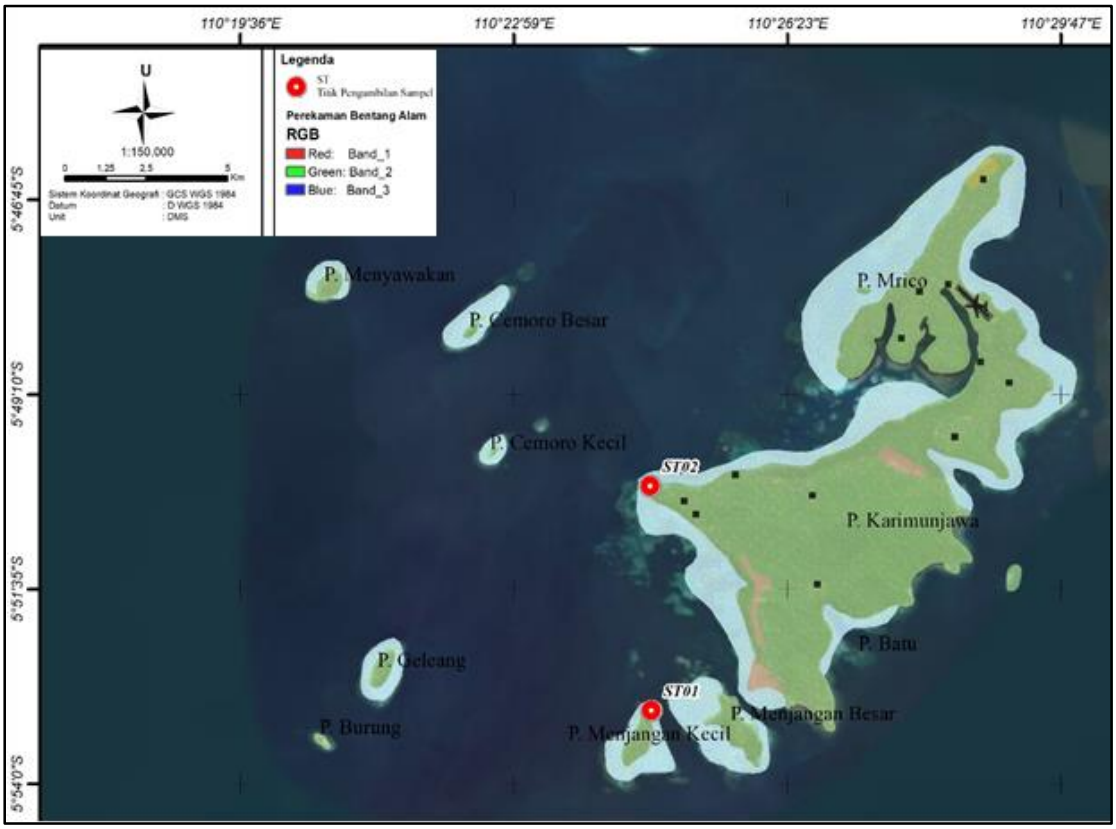

Figure 1. Sampling area in Karimunjawa island, Central Java, Indonesia.

Screening of pigmented bacteria against three Vibrio spp. and pathogenicity test. The antibacterial potency of the isolated symbiotic bacteria was assessed against three types of *Vibrio* bacteria, namely *V. harveyi*, *V. vulnificus,* and *V. parahaemolyticus*. These three bacteria were obtained from the Center for Brackish Water Aquaculture (BBPBAP), Jepara, Indonesia. 200 µL of *Vibrio* culture suspension and 10 mL of nutrient agar (NA) were poured onto a sterile plate. After the plate solidified, the potential bacterial symbionts (aged 18 h) were inoculated by a point method and incubated in the plate for 24 hours at 27°C. The presence of a clear zone around the symbiotic bacteria inoculated colony indicated the potential antibacterial properties of the isolates and they would be examined for the further study.

The potential of pigmented marine bacteria associated with brown algae capable of inhibiting three *Vibrio* spp. was tested for pathogenicity test. This test is done by inoculating pigmented bacteria on a blood agar medium and incubating at room temperature for 24 hours. Clear zones and greenish colored zones formed indicate that bacterial isolates can lyse blood cells, which suggests that the bacteria are pathogenic.

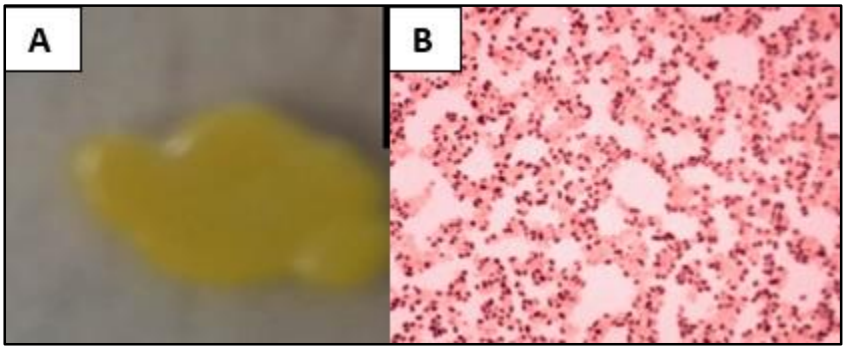

Figure 2. (A) A Colony morphology and (B) gram-staining of karimun G (KRG) isolate after 24 hours of incubation at room temperature (1000x).

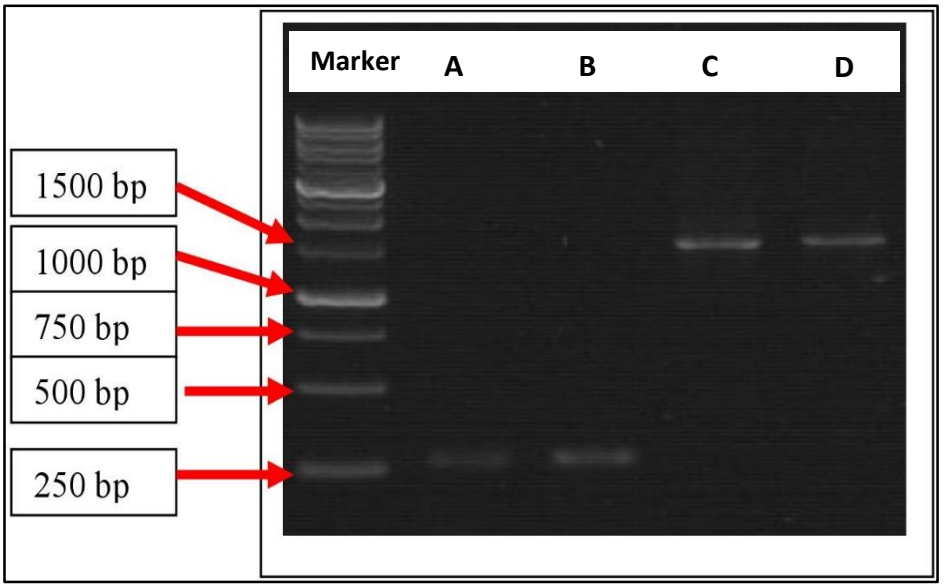

Figure 3. non-ribosomal peptide synthetases (NRPS) gene of (A) karimun H (KRH) and (B) karimun G (KRG) isolates. Partial genes of 16S rRNA of (C) KRH and (D) KRG isolate on 1% agarose gel.

Extraction of potential bacteria and assessing their antibacterial activity. Bacterial symbionts that produced the broadest clear zone against three types of *Vibrio* spp. from the screening were used for mass production. 10 mL of a 15-hour-old bacterial suspension was inoculated into a 500 mL nutrient broth (NB) medium and incubated for 48 hours at room temperature with 100 rpm agitation. Then, bacterial culture was extracted by adding ethyl acetate 1:1 and stirring continuously for 20 minutes. The solvent layer was separated

and evaporated using a rotary evaporator at 42°C to gain the crude bacterial extract. It was then stored in -4°C.

Three similar *Vibrio* bacteria were used as bacterial tests and refreshed onto NA for 24 hours at 27°C. The crude bacterial extract was dissolved in dimethyl sulfoxide (DMSO). 20 µL of crude extract was inoculated onto a sterile paper disk that had been previously inoculated with the target bacteria. 5% DMSO and 100 ppm ampicillin were used as a negative and positive control, respectively. The plate was incubated for 24 hours at 27°C and the formation of the halo zone attested the antibacterial potency of symbiotic bacteria.

Amplification of 16S rRNA gene of a potential bacterium. Bacterial DNA was extracted using the PrestoTM Mini gDNA (Geneaid, New Taipei City, Taiwan) bacterial kit by the established protocol. The amplification of the 16S ribosomal RNA gene was performed using two common primers: 27F as a forward primer (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R as a reverse primer (5'-GGT TAC CTT GTT ACG ACT T-3'). The final volume of the PCR mix (50 µL) consisted of 5 µL of DNA template, 25 µL GoTaq™ Master Mix (Promega, Wisconsin, USA), 1.5 µL 27F primer, 1.5 µL 1492R primer and 17 µL of nuclease-free water. The PCR program implemented was pre-denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 56°C for 1 minute and extension at 72°C for 1 minute, then post cycling in 72°C extension for 7 minutes and the process was stopped at 4°C.

Amplification of NRPS genes of a potential bacterium. NRPS genes from potential isolates were amplified using PCR with two primers: A2gamF (5´-AAG GCN GGC GSB GCS TAY STG CC-3´) and A3gamR (5´-TTG GGB IKB CCG GTS GIN CCS GAG GTG-3´) (Radjasa et al 2007). The PCR mix for NRPS detection contained a total of 50 µL, comprised of 5 uL 10x Buffer, 1 uL of 10 mM dNTP Mix, 3 uL of 25 mM $MgCl_2$, 1µL of both 10 uM forward and reverse primers, 4 µL of DNA template and 35 µL of ddH2O. PCR cycles were carried out with pre-denaturation (94°C for 3 minutes) and 35 cycles of denaturation (94°C for 1 minute), annealing (60°C for 1 minute) and extension (72°C for 2 minutes). The process was terminated at 4°C.

Table 1. Anti-*Vibrio* activity of 11 symbiotic bacteria against three types of *Vibrio* and pathogenicity test

Isolates	*Vibrio isolates*			*Hemolysis*
	V. harveyi	*V. parahaemolyticus*	*V. vulnficus*	
KRA	-	+	-	-
KRB	-	-	-	-
KRC	++	+	++	-
KRD	-	+	+	-
KRE	++	+	-	-
KRF	+	+	+	-
KRG	+++	++	+++	-
KRH	++	+	++	-
KRI	-	-	+	-
KRJ	-	++	-	-
KRK	+	-	-	-

Note: (-) no inhibition observed; (+) 0.1-2.0 mm; (++) 2.1-3 mm; (+++)>3 mm; KRA to KRK – karimun A to karimun K.

UV visualization and bioinformatic study. The PCR amplicon of both 16S rRNA and NRPS genes was analyzed with electrophoresis in 1% agarose gel and visualized under ultraviolet illumination (UV) with Geldoc. The amplification results were sequenced by 1st Base Laboratories Sdn Bhd, Malaysia. The raw sequence of 16S rRNA was trimmed, edited and aligned using MEGA 7.0 (Kumar et al 2016) to obtain complete sequences before it was deposited to GenBank for data availability of the sequence. Species homology was

carried out using BLASTn from the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). For the NRPS gene, sequences were translated using the Sequence Manipulation Suite (SMS) (https://www.bioinformatics.org/sms2/) and compared to the NCBI protein sequence. Finally, the phylogenetic tree of closely related sequences was built using MEGA 7.0, with the neighbor-joining package and 1000 bootstraps resampling.

Results and Discussion

Isolation symbiotic bacteria from brown algae. Through random purposive sampling, three types of fresh brown algae (*Sargassum* sp., *Padina* sp. and *Turbinaria* sp.) were successfully collected from Karimunjawa Island, Semarang, Indonesia. Moreover, a total of 45 symbiotic bacteria were successfully isolated from the three algae and all the bacteria grew well in the MA medium after 24 hours of incubation, being different in both morphology and pigment.

Antibacterial and hemolysis assay. Of the 45 pigmented bacterial isolates obtained, 11 strains can inhibit the growth of at least one of the *Vibrio* spp., as indicated by the formation of halo zones around bacterial colonies. When the crude extract is applied to the bacterial tests, all extracts from the symbiotic bacteria exhibit a zone of inhibition for at least one isolate after 24 hours of incubation (Table 1). The best isolate was KRG (Figure 2), a bright yellow bacterial isolate that is isolated from *Padina* sp., with a halo zone for more than 3 mm against three *Vibrio* bacteria. However, the crude extract from the KRB strain shows no inhibition against the three *Vibrio* isolates, even though it demonstrated a zone of inhibition during the screening. Furthermore, there are no bacterial symbionts showing any clear zone or greenish-colored zones around the inoculated area, which indicates hemolysis on the blood agar.

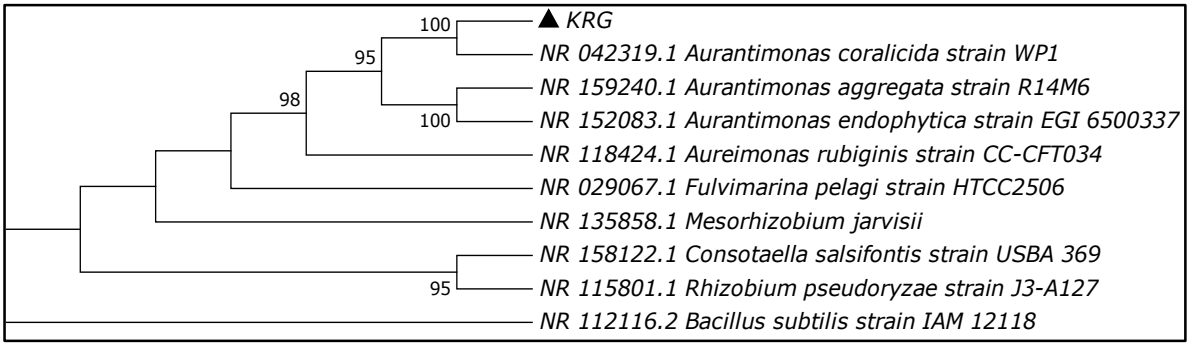

Figure 4. Phylogenetic tree of karimun G (KRG) isolate on its 16S rRNA gene.

Amplification of 16S rRNA. The amplification of the 16S rRNA sequence with a pair of primers named 27F and 1492R, produced a single band measuring around 1.5 kb by the marker and was sequenced from two directions. The quality of the DNA of the KRG isolate is shown in Figure 3D. Furthermore, the isolate was identified as *Aurantimonas coralicida* NR_042319.1, with 99.50% similarity and the phylogenetic tree is presented in Figure 4. The current strain was deposited in GenBank with access number MN165449.

Characterization of NRPS genes. The detection of NRPS-producing symbiotic bacteria was carried out using a specific pair of primers, called A2gamF/A3gamR. The molecular weight of the fragments was 300 bp (Figure 3B). Moreover, the fragments were also sequenced from two directions and successfully identified as a type of NRPS produced by *Pseudomonas psychrotolerans*, WP_058789425.1 (Figure 5).

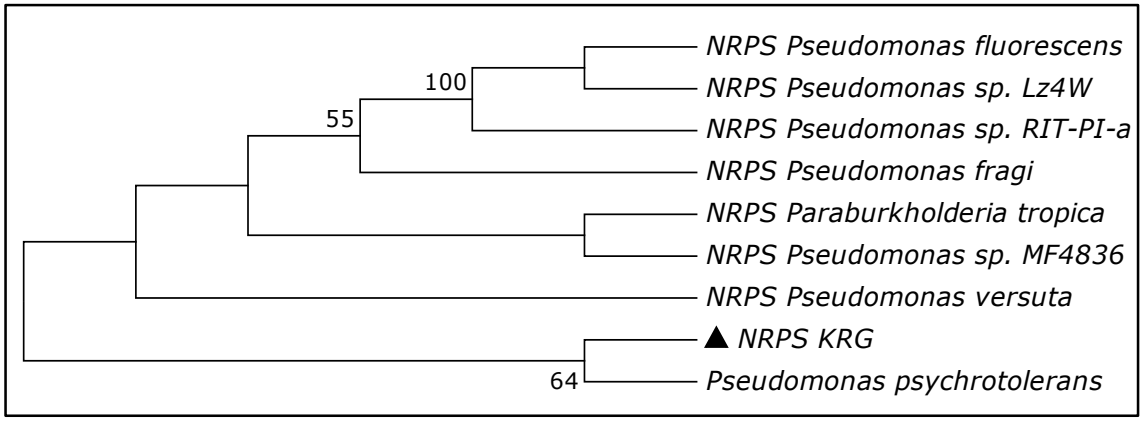

Figure 5. Phylogenetic tree constructed based on NRPS gene coding fragment of karimun G (KRG) isolate.

Based on the analysis of 16S rRNA sequences, the KRG isolate was identified as *Aurantimonas coralicida* and the isolate is, for the first time to our knowledge, reported as a symbiotic bacterium of *Padina* sp. and first isolated in the Indonesian Archipelago. *A. coralicida* is known as a marine bacterium isolated from a coral named *Dichocoenia stokesi* in the northern Florida Key, experiencing white plague type II (Denner et al 2003). We believe that the bacterium does not cause any diseases to the macroalgae because of its host specificity to coral, which experiences white plague disease. The characteristics of the bacteria are rods and gram-negative bacterium, have a golden-orange pigment and have a slightly circular and smooth colony (Denner et al 2003; Richardson & Voss 2005), similar to the isolate in this study. According to Figure 4, the KRG strain is included in the same clade as *A. aggregata* and *A. endophytica*. This result was somehow in agreement with several studies (Li et al 2017; Liu et al 2016). According to 16S rRNA, the sequences of *A. aggregata* and *A. endophytica* are in the same clade as *A. coralicida*, *A. manganoxydans*, and *A. litoralis*.

The zone of inhibition exhibited by the crude extract of *Aurantimonas coralicida* KRG isolate showed moderate antibacterial activity against three *Vibrio* bacteria and did not show any pathogenic pattern. Interestingly, the isolate also produced NRPS fragments when it was visualized on the agarose gel. Thus, NRPS could be responsible for producing antimicrobial peptides (AMP) that could give *A. coralicida* KRG antibacterial properties against bacterial tests. In addition, other studies also proposed that the presence of NRPS fragments in microorganisms is related with their antibacterial activity (Chaskraborty et al 2017; Falanga et al 2016), because the fragment is involved in the biosynthesis of secondary metabolites (Felnagle et al 2008). Many marine-associated bacteria, such as isolates from coral (Kvennefors et al 2012; Sibero et al 2018) and algae (Chakraborty et al 2017), demonstrate antibacterial activities to especially pathogenic bacteria. Therefore, the exploration of AMP from marine bacteria would be fascinating for discovering new antimicrobial constituents.

As mentioned previously, based on the 16S rRNA analysis, KRG isolate had a 99.50% similarity with *A. coralicida*. However, when the NRPS fragments of the KRG isolate were sequenced and analyzed with the NCBI database, it had close similarity to NRPS produced by *Pseudomonas psychrotolerans*. *P. psychrotolerans* was, for the first time, identified as an endophytic bacteria isolated from *Clerodendrum colebrookianum* (Passari et al 2016) and from a dog cage water (Hauser et al 2004). Moreover, Tziros et al (2007) revealed the biological activity of *Pseudomonas* sp. as a biocontrol agent against fusarium wilt that infects watermelons. Another study also investigated the potency of *Pseudomonas* spp. to protect tomatoes from *F. oxysporum* (Yiğit & Dikilitaş 2009). Thus, the genus has many biotechnological prospects, particularly antibacterial and biocontrol. Nevertheless, the in-depth study regarding its NRPS fragment remains unclear and this study could be the first to identify *Aurantimonas coralicida* that has slightly similar NRPS fragments with *Pseudomonas psychrotolerans*.

Conclusions. A yellow pigmented bacterium from brown algae was successfully obtained and identified, according to 16S rRNA, as *Aurantimonas coralicida* KRG, with the access number MN165449. Moreover, this bacterium also has NRPS fragments for 300 bp and is characterized as a NRPS type of *Pseudomonas psychrotolerans*. The crude extract of the isolate can inhibit the growth of three *Vibrio* isolates, *V. harveyi*, *V. vulnificus* and *V. parahaemolyticus*. It did not show any pathogenic pattern in the blood agar. Therefore, this KRG isolate could be a quite promising discovery in the effort to overcome vibriosis in both the aquaculture industry and human health.

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References

Chakraborty K., Thilakan B., Chakraborty R. D., Raola V. K., Joy M., 2017 O-heterocyclic derivatives with antibacterial properties from marine bacterium *Bacillus subtilis* associated with seaweed, *Sargassum myriocystum*. Applied Microbiology and Biotechnology 101:569–583.

Denner E. B. M., Smith G. W., Busse H. J., Schumann P., Narzt T., Polson S. W., Lubitz W., Richardson L. L., 2003 *Aurantimonas coralicida* gen. nov., sp. nov., the causative agent of white plague type II on Caribbean scleractinian corals. International Journal of Systematic and Evolutionary Microbiology 53:1115–1122.

Falanga A., Lombardi L., Franci G., Vitiello M., Iovene M. R., Morelli G., Galdiero M., Galdiero S., 2016 Marine antimicrobial peptides: Nature provides templates for the design of novel compounds against pathogenic bacteria. International Journal of Molecular Sciences 17:785, 18 p.

Felnagle E. A., Jackson E. E., Chan Y. A., Podevels A. M., Berti A. D., McMahon M. D., Thomas M. G., 2008 Nonribosomal peptide synthetases involved in the production of medically relevant natural products. Molecular Pharmaceutics 5:191–211.

Hauser E., Kämpfer P., Busse H. J., 2004 *Pseudomonas psychrotolerans* sp. nov. International Journal of Systematic and Evolutionary Microbiology 54:1633–1637.

Ismail A., Ktari L., Ahmed M., Bolhuis H., Boudabbous A., Stal L. J., Cretoiu M. S., El Bour M., 2016 Antimicrobial activities of bacteria associated with the brown alga *Padina pavonica*. Frontiers in Microbiology 7:1072, DOI: 10.3389/fmicb.2016.01072.

Joshi J., Srisala J., Truong V. H., Chen I. -T., Nuangsaeng B., Suthienkul O., Lo C. F., Flegel T. W., Sritunyalucksana K., Thitamadee S., 2014 Variation in *Vibrio parahaemolyticus* isolates from a single Thai shrimp farm experiencing an outbreak of acute hepatopancreatic necrosis disease (AHPND). Aquaculture 428–429:297–302.

Kalinovskaya N. I., Romanenko L. A., Kalinovsky A. I., 2017 Antibacterial low-molecular-weight compounds produced by the marine bacterium *Rheinheimera japonica* KMM 9513T. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology 110:719–726.

Karthick P., Mohanraju R., 2018 Antimicrobial potential of epiphytic bacteria associated with seaweeds of little Andaman, India. Frontiers in Microbiology 9:611, DOI: 10.3389/fmicb.2018.00611.

Kizhakkekalam V. K., Chakraborty K., 2019 Pharmacological properties of marine macroalgae-associated heterotrophic bacteria. Archives of Microbiology 201:505–518.

Kumar S., Stecher G., Tamura K., 2016 MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33:1870–1874.

Kvennefors E. C. E., Sampayo E., Kerr C., Vieira G., Roff G., Barnes A. C., 2012 Regulation of Bacterial Communities Through Antimicrobial Activity by the Coral Holobiont. Microbial Ecology 63:605–618.

Li A. Z., Lin L. Z., Zhang M. X., Zhu H. H., 2017 *Aurantimonas aggregata* sp. nov., isolated

from deep-sea sediment. International Journal of Systematic and Evolutionary Microbiology 67:5056–5061.
Liu B. B., Wang H. F., Li Q. L., Zhou X. K., Zhang Y. G., Xiao M., Li Q. Q., Zhang W., Li W. J., 2016 *Aurantimonas endophytica* sp. nov., A novel endophytic bacterium isolated from roots of *Anabasis elatior* (C. A. Mey.) Schischk. International Journal of Systematic and Evolutionary Microbiology 66:4112–4117.
Manivannan K., Karthikai devi G., Anantharaman P., Balasubramanian T., 2011 Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. Asian Pacific Journal of Tropical Biomedicine 1:114–120.
Mohamad O. A. A., Li L., Ma J. B., Hatab S., Xu L., Guo J. W., Rasulov B. A., Liu Y. H., Hedlund B. P., Li W. J., 2018 Evaluation of the Antimicrobial Activity of Endophytic Bacterial Populations from Chinese Traditional Medicinal Plant Licorice and Characterization of the Bioactive Secondary Metabolites Produced by *Bacillus atrophaeus* Against *Verticillium dahliae*. Frontiers in Microbiology 9:924, DOI: 10.3389/fmicb.2018.00924.
Nakayama T., Nomura N., Matsumura M., 2006 Study on the relationship of protease production and luminescence in Vibrio harveyi. Journal of Applied Microbiology 101:200–205.
Palanisamy S., Vinosha M., Manikandakrishnan M., Anjali R., Rajasekar P., Marudhupandi T., Manikandan R., Vaseeharan B., Prabhu N. M., 2018 Investigation of antioxidant and anticancer potential of fucoidan from *Sargassum polycystum*. International Journal of Biological Macromolecules 116:151–161.
Passari A. K., Mishra V. K., Leo V. V., Gupta V. K., Singh B. P., 2016 Phytohormone production endowed with antagonistic potential and plant growth promoting abilities of culturable endophytic bacteria isolated from *Clerodendrum colebrookianum* Walp. Microbiological Research 193:57–73.
Radjasa O. K., Marterns T., Grossart H. P., Brinkhoff T., Sabdono A., Simon M., 2007 Antagonistic activity of a marine bacterium *Pseudoalteromonas luteoviolacea* TAB4.2 associated with coral *Acropora* sp. International Journal of Biological Sciences 7:239–246.
Richardson L. L., Voss J. D., 2005 Changes in a coral population on reefs of the northern Florida Keys following a coral disease epizootic. Marine Ecology Progress Series 297:147–156.
Sibero M. T., Bachtiarini T. U., Trianto A., Lupita A. H., Sari D. P., Igarashi Y., Harunari E., Sharma A. R., Radjasa O. K., Sabdono A., 2019 Characterization of a yellow pigmented coral-associated bacterium exhibiting anti-Bacterial Activity Against Multidrug Resistant (MDR) Organism. Egyptian Journal of Aquatic Research 45:81–87.
Sibero M. T., Herdikiawan D., Radjasa O. K., Sabdono A., Trianto A., Triningsih D. W., 2018 Antibacterial activity of sponge associated fungi against vibriosis agents in shrimp and its toxicity to *Litopenaeus vannamei*. AACL Bioflux 11(1):10–18.
Soonthornchai W., Chaiyapechara S., Jarayabhand P., Söderhäll K., Jiravanichpaisal P., 2015 Interaction of *Vibrio* spp. with the Inner Surface of the Digestive Tract of *Penaeus monodon*. PLoS One 10(8):e0135783 DOI: 10.1371/journal.pone.0135783.
Soto-Rodriguez S., Gomez-Gil B., Lozano R., 2010 'Bright-red' syndrome in Pacific white shrimp *Litopenaeus vannamei* is caused by *Vibrio harveyi*. Diseases of Aquatic Organisms 92:11–19.
Susilowati R., Sabdono A., Widowati I., 2015 Isolation and Characterization of Bacteria Associated with Brown Algae *Sargassum* spp. from Panjang Island and their Antibacterial Activities. Procedia Environmental Sciences 23:240–246.
Teo J. W. P., Suwanto A., Poh C. L., 2000 Novel β-lactamase genes from two environmental isolates of *Vibrio harveyi*. Antimicrobial Agents and Chemotherapy 44:1309–1314.
Tran L., Nunan L., Redman R., Mohney L., Pantoja C., Fitzsimmons K., Lightner D., 2013 Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Diseases of Aquatic Organisms 105:45–55.
Tziros G. T., Lagopodi A. L., Tzavella-Klonari K., 2007 Reduction of fusarium wilt in

watermelon by *Pseudomonas chlororaphis* PCL1391 and *P. fluorescens* WCS365. Phytopathologia Mediterranea 46:320–323.
Xiao J., Liu L., Ke Y., Li X., Liu Y., Pan Y., Yan S., Wang Y., 2017 Shrimp AHPND-causing plasmids encoding the PirAB toxins as mediated by pirAB-Tn903 are prevalent in various *Vibrio* species. Scientific Reports 7:1–11.
Yiğit F., Dikilitaş M., 2009 Control of *Fusarium Wilt* of Tomato by Combination of Fluorescent *Pseudomonas*, Non-pathogen *Fusarium* and *Trichoderma harzianum* T-22 in Greenhouse Conditions. Plant Pathology Journal 6:159–163.
***https://blast.ncbi.nlm.nih.gov/Blast.cgi
***https://www.bioinformatics.org/sms2/

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