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Molecular Networking Analysis and Antibacterial Potential of Ethyl Acetate Extracts of *Sinomicrobium* sp. PAP.21 using OSMAC Method

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ABSTRACT

Challenges in drug discovery include biosynthetic gene clusters which remain silent under standard laboratory culture conditions. On the other hand, the rediscovery of the known compounds is inevitable. Accordingly, One Strain-MAny Compounds (OSMAC) approach and molecular networking analysis are currently applicable to discovering new bioactive compounds. *Sinomicrobium* sp. PAP.21 isolated from marine sediment collected in Cenderawasih Bay, West Papua, was added to the culture. Then, the bacterium was cultured in five different liquid media (RL1, A1BFe+C, NB, LB, and seawater) and incubated for 4, 5, and 7 days. The bacterial cultures were extracted using ethyl acetate (EtOAc) separately for each medium and incubation period, followed by LC-HRMS measurement. A total of 45 ethyl acetate extracts were assayed for *in vitro* antibacterial activity against *Micrococcus luteus* and *Escherichia coli*. Molecular networking analysis through GNPS indicated that three putative compounds possess antibacterial properties. EtOAc extracts from the A1BFe+C medium demonstrated antibacterial activity against *M. luteus*. However, none of them were active against *E. coli*. Collectively, *Sinomicrobium* sp. PAP.21 produced bioactive compounds exhibiting antibacterial potential, particularly against Gram-positive bacteria.
Keywords: antibacterial; LC-HRMS; molecular networking; OSMAC; *Sinomicrobium* sp. PAP.21

INTRODUCTION

The level of resistance of pathogenic bacteria to antibiotics is increasing due to overuse and misuse of antibiotics (Thawabteh et al., 2023). Antimicrobial Resistance Collaborators, (2022) reported that 1.72 million deaths in 2019 were caused by antibiotic resistance. Marine microorganisms, including bacteria, are an important source for discovering and developing new antibiotics (Thawabteh et al., 2023; Tortorella et al., 2018). However, drug discovery is challenging as exceed 99% of marine microorganisms cannot be grown under standard laboratory culture conditions (Wang et al., 2021). It means that biosynthetic gene clusters (BGCs) encode the production of secondary metabolites are often silent or cryptic. Culture-based approaches such as an OSMAC (One Strain-MAny Compounds) method can activate the silent BGCs, leading to increased discovery of novel lead structures. The critical factors in the biosynthesis of secondary metabolites using the OSMAC method include culture media and incubation period (Tangerina et al., 2020).

On the other hand, the rediscovery of known compounds also hinders the discovery of novel bioactive substances (Pinedo-Rivilla et al., 2022). However, a comprehensive strategy is urgently needed to overcome the historical and complex bottlenecks, including the timescale and complexity of traditional procedures and small amounts of pure metabolites. For instance, untargeted metabolomics using mass spectrometry with liquid chromatography enables the analysis of minor compounds in samples (Tangerina et al., 2020). The development of molecular networking allows rapid comparison of many metabolites expressed under different conditions. Molecular networking is an effective strategy to assess the effects of different culture conditions and to shorten dereplication time (Esposito et al., 2021).

The most abundant marine bacteria originated from the phylum Bacteroidetes (Li et al., 2021). However, studies on secondary metabolites from this bacterial phylum are still limited (Brinkmann et al., 2021). In this study, a bacterium *Sinomicrobium* sp. PAP.21 was isolated from marine sediments collected in the Cenderawasih Bay, West Papua, Indonesia. *Sinomicrobium* is a new genus of bacteria in the phylum Bacteroidetes belonging to the Flavobacteriaceae family (Riyanti et al., 2023). The Flavobacteriaceae family is known to afford antibacterial compounds such as β-lactam and quinolones (Gavriilidou et al., 2020).

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Based on GC-MS analysis by Pringgenies et al., (2023), methanol extracts of *S. oceani* and *S. pectinilyticum* contained bioactive metabolites with antibacterial potential.

However, related studies on the *Sinomicrobium* genus are still focusing on the isolation of new species and the classical analysis of their compounds by using GC-MS (Pringgenies et al., 2023). Therefore, in this study, we implement the OSMAC method on bacterial cultures of *Sinomicrobium* sp. PAP.21 uses a variety of media and incubation periods, analyzes secondary metabolites through molecular networking, and assesses *in vitro* antibacterial activity.

MATERIALS AND METHODS

Materials

Sinomicrobium sp. PAP.21 was isolated from marine sediment collected in the Cenderawasih Bay, West Papua, from 10 m depth and taken from the 5–10 cm top layer, Marine Broth medium (Zobell Marine Broth Himedia), RL1 medium, A1BFe+C medium, Nutrient Broth medium (Himedia), Luria Bertani medium (LB), seawater, aquadest (Prima Chemical), Nutrient Agar medium (Himedia), trace metals, vitamin B12, ethyl acetate (EtOAc) (Prima Chemical), dimethyl sulfoxide (DMSO) (Merck), methanol, *Micrococcus luteus* (ATCC 4698) and *Escherichia coli* from the Microbiology Laboratory, Faculty of Biology Jenderal Soedirman University, and Phenoxymethylpenicillin (Erela, Semarang).

The tools used in this study were micropipettes 1-10 uL (Eppendorf Research) and 100-1000 uL (Dragon Lab), Bunsen lamp, 50 mL centrifuge tube (Biologix), ose needle, vernier caliper, Biological Safety Cabinet (BSC) (Myco7-Mycolab), petri dish (Anumbra), centrifuge tube racks (Biologix), hair dryer, autoclave (All American Model 1925X), shaker (Cleaver Scientific), LC-HRMS instrument (HPLC Dionex Ultimate 3000 Thermo Scientific combination Micro-TOF-Q II MS Bruker) and glassware.

Methods

Bacterial preculture

The preculture was prepared by dissolving Zobell Marine Broth (MB) 2216 in aquadest. A total of 1.6 g of MB was transferred into a 100 mL Erlenmeyer and dissolved in a 40 mL aquadest. Preculture was made based on a modified method from Riyanti *et al.* (2020a). Single colonies were transferred to MB medium, and the preculture was incubated at 30°C and shaken at 160 rpm for 48 hours.

Bacterial culture

Cultures were performed using the following five different sterile liquid media per 1 L seawater: (1) RL1:2 g yeast extract (Himedia), 3 g peptone (Himedia), and 0.5 g potassium nitrate (Carl Roth GmbH, Germany) (Spyere et al., 2003); (2) A1BFe+C:10 g starch (Merck), 4 g yeast extract (Himedia), 2 g peptone (Himedia), 1 g CaCO3 (Merck), 40 mg Fe2(SO4)3.4H2O, and 100 mg KBr (Merck) (Choi et al., 2015); (3) NB:13 g nutrient broth; (4) LB: 10 g yeast extract (Himedia) and 5 g peptone (Himedia) (Hamill et al., 2020); and (5) seawater medium. For each medium, secondary metabolite extractions were carried out based on variations of the incubation period, and the culture was grown in triplicate; then, 9 of 250-mL Erlenmeyer flasks were used for one type of media. A total of 1 mL of preculture was added to each Erlenmeyer flask containing 100 mL of media (Riyanti et al., 2020a). 0,1% each of trace metals and vitamin B12 were added to the culture. The strains were grown for the specified incubation period and shaken at 160 rpm.

Extraction of secondary metabolites

Extraction was performed using the liquid-liquid extraction (LLE) method using ethyl acetate (EtOAc). Extraction was carried out on days 4, 5, and 7 to vary the incubation time according to the stationary growth phase of the bacteria. The extraction method follows Ambarwati et al. (2020) with a bit of modification. The culture was extracted using EtOAc with a ratio of 1:1 v/v in a centrifuge tube. The mixture was shaken manually for 20 minutes and left for 15 minutes to separate the supernatant and water phase. The supernatant was poured into a container and evaporated with a fan and hair dryer to obtain EtOAc crude extracts (Riyanti et al., 2020a).

LC-HRMS measurement

A total of 45 EtOAc extracts were dissolved in MeOH to a final concentration of 10 mg/mL. Liquid Chromatography High-Resolution Mass Spectrometry (LC-HRMS) measurements were performed using a Micro-TOF-Q II mass spectrometer (Bruker, Billerica, MA, USA) with an ESI source combined with a Dionex Ultimate 3000 HPLC (Thermo Scientific, Darmstadt, Germany) using a 2.7 µm EC10/2 Nucleoshell C18 column (Macherey-Nagel, Düren, Germany) at 25°C. MS data were acquired in positive mode over 100 to 1000 m/z. Auto MS/MS fragmentation was achieved with increasing collision energy (35–50 kV with a gradient from 500 to 2000 m/z) at a frequency of 4 Hz for all ions with a threshold of 100. The injected volume was 2 µL with an

extract concentration of 1 mg/mL (Riyanti et al., 2020a).

Antibacterial assays

An antibacterial assay against Gram-positive *M. luteus* and Gram-negative *E. coli* test strains was performed using the disc diffusion method using sterile NA media. The concentration of the crude extract was 10 mg/mL dissolved in 10% DMSO. The test microbial inoculum was inoculated by swabbing the agar surface using a sterile cotton bud. Then, 10 µL of extract was dripped on a paper disk (7 mm diameter) and placed on the agar surface. Petri dishes were then wrapped and incubated for 1 hour at 4°C and then moved to 30°C for 24-48 hours (Riyanti et al., 2020a). The positive control, penicillin, was made in the same concentration as the extract, and DMSO 10% was used as a negative control. The diameter of the inhibition zone was observed and measured using a caliper with a millimeter scale.

Data analysis

MS/MS data will be converted from MassHunter (.d) data files to mzXML file format using MS Convert (Riyanti et al., 2020a). Data were analyzed using Global Natural Products Social Molecular Networking (GNPS; https://gnps.ucsd.edu/) by uploading data through the WinSCP application connecting to the GNPS account. Furthermore, the data were input into the default group, and GNPS analysis was carried out with a parent mass tolerance of 2.0 Da and an MS/MS fragment ion tolerance of 0.5 Da. Molecular networking data were analyzed and visualized using Cytoscape 3.10.0 software (Wang et al., 2016).

RESULTS

A pure isolate of *Sinomicrobium* sp. PAP.21 was precultured using Zobell Marine Broth 2216 medium, widely used to grow marine bacteria. Preculture of bacteria aims to activate bacteria that are in a dormant state (Manalu, 2017) and to optimize bacterial growth (Prihanto et al., 2018). Preculture results revealed bacterial growth that can be observed based on turbidity. Preculture with an incubation period of 2 days showed turbidity and was used for culture in 5 liquid culture media. In the culture, trace metals and vitamin B12 were added as a growth factor. Growth factors are elements which can not be synthesized by bacteria from nutrients in the media (Bonnet et al., 2019).

The culture was extracted by the LLE method to separate target compounds based on their relative solubility in two immiscible liquids (López-Rodríguez et al., 2022). LLE was applied to extract compounds from microbial cultures, the extract would contain fewer media components, allowing the purification step to be more straightforward until the isolation. The average weight of bacterial extracts obtained is shown in Table I.

Molecular networking analysis

Forty-five ethyl acetate extracts of *Sinomicrobium* sp. PAP.21 obtained from the OSMAC method were analyzed using molecular networking based on LC-HRMS data. The analysis was conducted in order to determine compounds possessing antibacterial activity. Molecular networking analysis was performed based on variations of media and incubation period to reveal the effect of different culture conditions on the production of secondary metabolites.

The results of molecular networking based on media variation formed 189 molecular networks. Meanwhile, 188 molecular networks were found in the variation of the incubation period. A total of 35 replicated compounds were obtained automatically (in comparison with the GNPS database), but only three compounds listed in Table II have no networking with media (Figures 1 and 2). Figure 1 shows a molecular network constructed by *Sinomicrobium* sp. PAP.21 in five different liquid media. Meanwhile, figure 2 displays molecular networking from different incubation periods and exhibits the compounds produced at any time.

The putative compounds that play an important role in the antibacterial activity are listed in Table II. Compounds produced by more than one medium may also affect the antibacterial activity in the A1BFe+C medium. However, the molecular networking analysis is only qualitative, and the amount of bioactive compound in the extract possessing an antibacterial effect cannot be determined.

Antibacterial activity

The *in vitro* antibacterial activity tests were carried out using the disc diffusion method against Gram-positive (*M. luteus*) and Gram-negative (*E. coli*) bacteria. The disc diffusion method is used for antimicrobial susceptibility testing, where antimicrobial agents diffuse into the media and inhibit the growth of test bacteria (Balouiri et al., 2016). The results of 45 ethyl acetate extracts of *Sinomicrobium* sp. PAP.21 against *M. luteus* obtained the diameter of the inhibition zone in the bacterial extracts cultured in A1BFe+Cmedia which showed intermediate

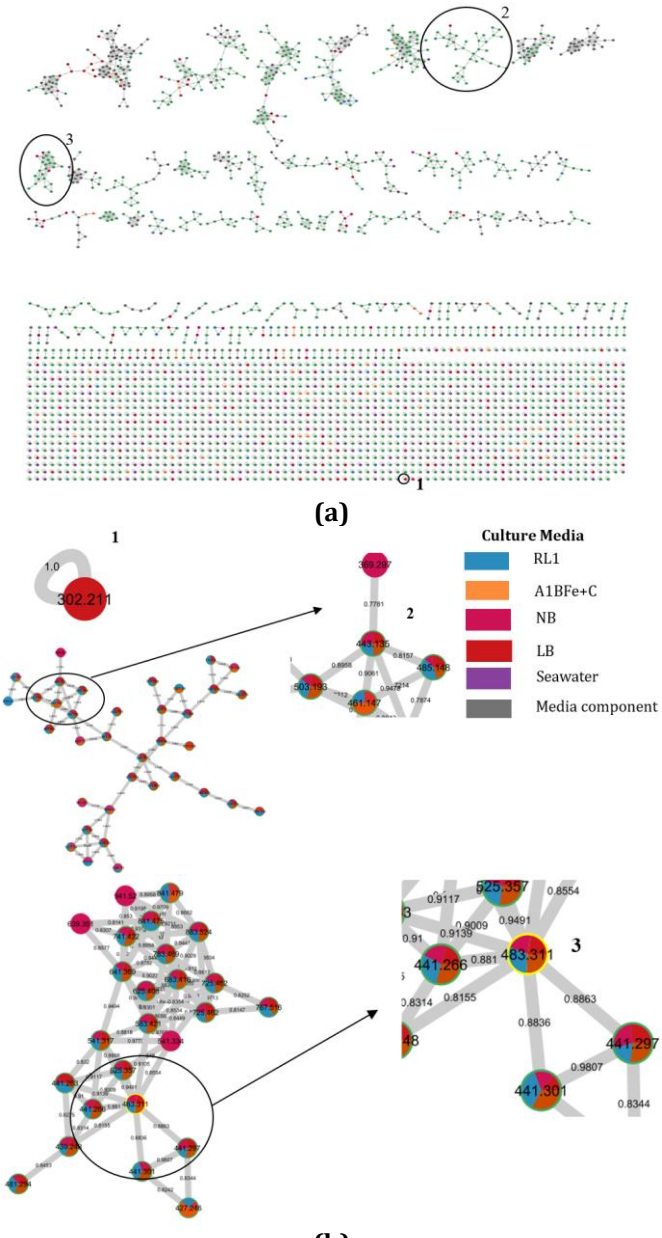
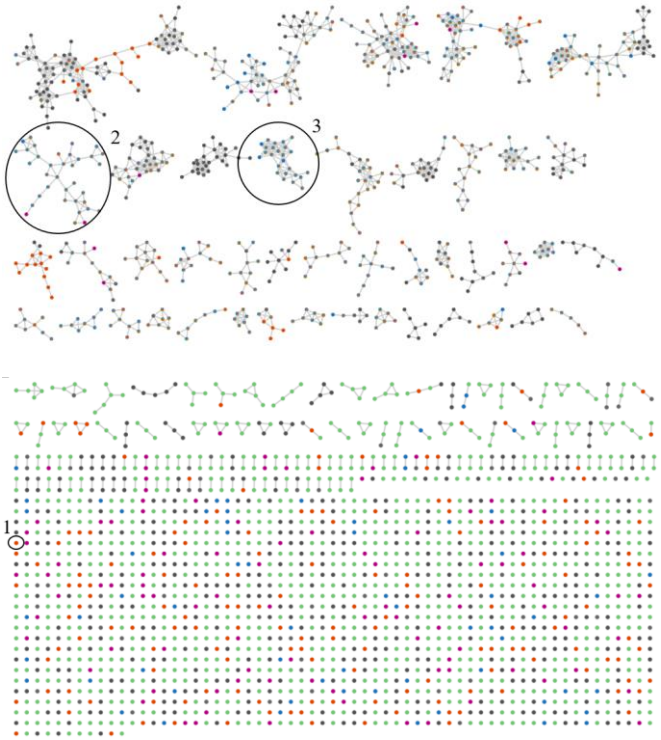


Figure 1. Molecular networking of *Sinomicrobium* sp. PAP.21 was produced by five different liquid media

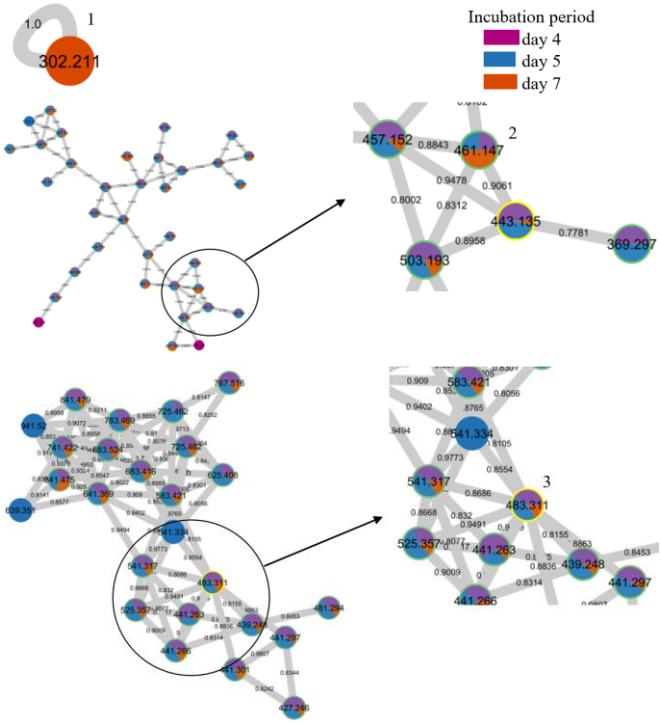
(a) Node's colors are based on each culture media. Blue: RL1 medium; orange: A1BFe + C medium; pink: NB medium; red: LB medium; purple: seawater medium; grey: all media
(b) Nodes highlighted in the colored box represent parent ions that are dereplicated as D-erythro-C18-Sphingosine (**1**), Picrolichenic acid (**2**), beta-D-Glucopyranose (**3**)

Table I. Extract weight in various liquid culture media and incubation period

Media	Average extract weight (mg) on three incubation periods		
	Day 4	Day 5	Day 7
RL1	12.53	68.03	72.93
A1BFe+C	63.73	35.70	79.83
NB	70.60	71.50	78.93
LB	43.40	80.03	107.03
Seawater	213.90	140.63	137.23



(a)

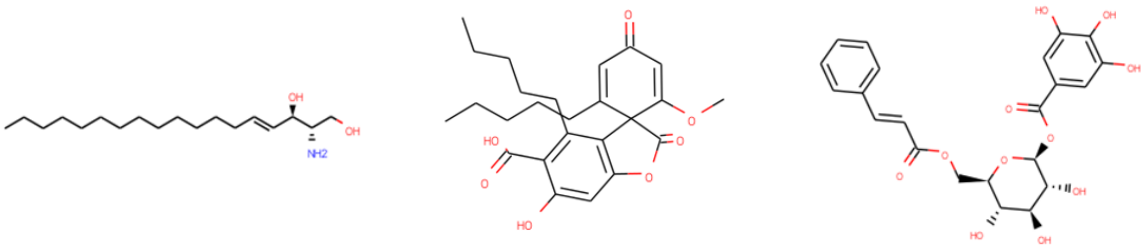


(b)

Figure 2. Molecular networking of *Sinomicrobium* sp. PAP.21 in 3 different incubation period

(a) Node's colors are based on each incubation period. Purple : day 4; blue : day 5; orange : day 7; grey: all media

(b) Nodes highlighted in the colored box represent parent ions that are dereplicated as D-erythro-C18-Sphingosine (**1**), Picrolichenic acid (**2**), beta-D-Glucopyranose (**3**)



(1) (2) (3)

Figure 3. Chemical structures of D-erythro-C18-Sphingosine (1), Picrolichenic acid (2), and beta-D-Glucopyranose (3)

Table II. Compounds with antibacterial activity

No	Mass-to-Charge Ratio (m/z)	Compound Name	Media	Incubation Period (days)	Biological Source	Bioactivity
1	302.211 [M+H]	D-erythro-C18-Sphingosine	LB	7	An endophytic fungus, *Acremonium* sp. (Khan et al., 2021)	Antibacterial activity against *Neisseria meningitidis*, *N. gonorrhoeae*, *S. aureus* and *E. coli* (Becam et al., 2017)
2	443.135 [M-H]	Picrolichenic acid	LB, NB, RL1, A1BFe+C	4, 5 and 7	Lichen (Ranković, 2019)	Antibacterial activity from *Pertusaria amara* extract against *S. aureus* (Judith et al., 2022)
3	483.311 [M+Na]	beta-D-Glucopyranose	LB, NB, RL1, A1BFe+C	4, 5 and 7	Endophytic bacterium *Pseudomonas fluorescens* (Nasab & Khodakaramian, 2022)	Antibacterial activity against *Bacillus subtilis*, *S. aureus*, and *Salmonella typhi* (Matin et al., 2013)

LB: Luria Bertani, NB: Nutrient Broth

Table III. Antibacterial activity against *Micrococcus luteus*

Sample (Medium_incubation period)	Inhibition diameter (mm) Mean ± SD	Activity category*
A1BFe+C_4	6.0 ± 0,9	Intermediate
A1BFe+ C_5	6.7 ± 0.4	Intermediate
A1BFe+ C_7	6.7 ± 0.1	Intermediate
Penicillin	35.6 ± 0.3	Very strong
DMSO 10%	0	Inactive

*According to Davis & Stout (1971)

activity (Table III). Herein, the extracts were only active against Gram-positive bacteria rather than Gram-negative bacteria.

DISCUSSION

Color differences can be observed in the culture results among various media, presumably caused by bacteria that produce colors or pigments. Pigments are secondary metabolites synthesized only when bacteria are grown under appropriate growth conditions, where pigmentation can be induced by the compositions of media used (Agarwal et al., 2023). Thus, different culture media will produce different colors. In RL1, A1BFe+C, LB, and NB media, yellow-colored cultures were yielded, as reported by Xu et al., (2013), and the genus *Sinomicrobium* afforded yellow pigments. However, cultures of this bacterium in seawater medium did not change its color, indicating the absence of pigments produced in this medium.

Most of the extracts cultured in the four different media showed an increasing weight in a time-dependent manner (Table 1). This is probably due to the longer incubation time and the more metabolites yielded by this bacterium. The highest extract weight was afforded in the seawater medium, while the lowest one was produced in the RL1 medium.

In molecular networking analysis, compound dereplication is recognized by automatic comparison using mass spectrometry profiles available in the database (Mohimani et al., 2018). The selected molecular networking is not connected to the media, indicating that bacterial cultures only produce the compound. Molecular networking can be formed because the cosine score is above 0.7 and has more than 6 matched peaks. When visualizing the data, a minimum cosine score of 0.7 is used to group different compound classes under the same molecular family (Purves et al., 2016).

Subsequently, the bioactivity of dereplicated compounds will be proved in this study to inhibit pathogenic bacteria through the *in vitro* antibacterial assay. Three compounds possess antibacterial activity based on the literature study as listed in Table II (Figure 3). These compounds will likely affect the results of the further *in vitro* antibacterial assay of the EtOAc extracts. Two of them do not originate from bacteria and are rarely reported as bacterial compounds. D-erythro-C18-Sphingosine belongs to the sphingolipids group of fatty acids. Picrolichenic acid is an aromatic polyketide in the depsides group. Beta-d-glucopyranose is included in the phenolic acids (C6-C1) group of cinnamic acids and derivatives or gallotannins. Those compounds are known to possess antibacterial activity against several pathogenic bacteria.

A study related to the genus *Sinomicrobium* was first published by Xu et al. (2013), which found a bacterial isolate, *S. oceani*. This bacterium was able to degrade alginate, so it can break down seaweed biomass (Jegatheesan et al., 2017). Another species, *S. pectinilyticum,* produced pectinase and α-amylase that can hydrolyse pectin and starch (Cheng et al., 2014). *S. oceani, S. pectinilyticum,* and *S. soli* featuring fatty acids iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{16:1}\omega6c/C_{16:1}\omega7c$). High levels of branched-chain fatty acids and 3-hydroxy C_{15}-C_{17} are typical of members of the *Flavobacteriaceae* family (Liu et al., 2019). Different from other species, *S. weinanense* contained fatty acid iso-$C_{15:0,}$ iso-$C_{17:0}$ 3OH, and summed feature 3, $C_{16:0}$ and iso-$C_{15:1}$ G (Wu et al., 2022), while *S. kalidii* possessed fatty acids including iso-$C_{17:0}$, iso-$C_{16:0}$ 3-OH, anteiso-$C_{17:0}$ and summed feature 6 ($C_{19:1}$ ω9c and/or $C_{19:1}$ ω11c) (Li et al., 2022).

Recently, Pringgenies et al. (2023) reported that hexadecanoic acid, methyl ester mostly found in six mangrove-derived bacteria with antibacterial and antifungal potentials. Up to now, only a few publications related to *Sinomicrobium* sp. PAP.21 that reported for their compounds and biological activity. On the other hand, based on our former study using antiSMASH analysis revealed that *Sinomicrobium* sp. PAP.21 encodes the BGCs siderophores desferrioxamine E, microviridin J, and pinensin (Riyanti et al., 2023). These compounds are known to have antibacterial properties.

Further study of such micro-fractionation can be conducted to trace the bioactive compounds in the subsequent fractions, following the isolation steps to obtain the pure active substances (Wibowo et al., 2019). However, molecular networking analysis is limited to compounds replicated and recorded in the database. There is a possibility that new compounds can be found to be active against pathogenic bacteria. Hence, micro-fractionation of the active extracts can be further investigated to determine the bioactive compounds (Riyanti et al., 2020b).

CONCLUSION

Based on the molecular networking analysis, *Sinomicrobium* sp. PAP.21 possessed three active compounds without networking with media and exhibited antibacterial activity from 45 EtOAc extracts of *Sinomicrobium* sp. PAP.21, the active extracts cultured on A1BFe+C medium were

capable of inhibiting the growth of *M. luteus*, even though they were inactive against *E. coli*.

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CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

REFERENCES

Agarwal, H., Bajpai, S., Mishra, A., Kohli, I., Varma, A., Fouillaud, M., Dufossé, L., & Joshi, N. C. (2023). Bacterial Pigments and Their Multifaceted Roles in Contemporary Biotechnology and Pharmacological Applications. *Microorganisms*, *11*(3), 1–19. https://doi.org/10.3390/microorganisms11030614.

Ambarwati, A., Wahyuono, S., Moeljopawiro, S., & Yuwono, T. (2020). Antimicrobial activity of ethyl acetate extracts of *Streptomyces* sp. CRB46 and the prediction of their bioactive compounds chemical structure. *Biodiversitas*, *21*(7), 3380–3390. https://doi.org/10.13057/biodiv/d210763

Antimicrobial Resistance Collaborators. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, *399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005.

Becam, J., Walter, T., Burgert, A., Schlegel, J., Sauer, M., Seibel, J., & Schubert-Unkmeir, A. (2017). Antibacterial activity of ceramide and ceramide analogs against pathogenic Neisseria. *Scientific Reports*, *7*(1), 1–12. https://doi.org/10.1038/s41598-017-18071-w.

Bonnet, M., Lagier, J. C., Raoult, D., & Khelaifia, S. (2019). Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. *New Microbes and New Infections*, *34*. https://doi.org/10.1016/j.nmni.2019.100622.

Brinkmann, S., Kurz, M., Patras, M. A., Hartwig, C., Marner, M., Leis, B., Billion, A., Kleiner, Y., Bauer, A., Toti, L., Pöverlein, C., Hammann, P. E., Vilcinskas, A., Glaeser, J., Spohn, M., & Schäberle, T. F. (2021). Genomic and Chemical Decryption of the Bacteroidetes Phylum for Its Potential to Biosynthesize Natural Products. *Microbiology Spectrum*, *10*(3), 1–27. https://doi.org/10.1128/spectrum.02479-21.

Cheng, B., Li, C., Lai, Q., Du, M., Shao, Z., Xu, P., & Yang, C. (2014). *Sinomicrobium pectinilyticum* sp. nov., A pectinase-producing bacterium isolated from alkaline and saline soil, and emended description of the genus *Sinomicrobium*. *International Journal of Systematic and Evolutionary Microbiology*, *64*, 2939–2943. https://doi.org/10.1099/ijs.0.061671-0.

Choi, E. J., Nam, S. J., Paul, L., Beatty, D., Kauffman, C. A., Jensen, P. R., & Fenical, W. (2015). Previously Uncultured Marine Bacteria Linked to Novel Alkaloid Production. *Chemistry and Biology*, *22*(9), 1270–1279. https://doi.org/10.1016/j.chembiol.2015.07.014.

Davis, W. W., & Stout, T. R. (1971). Disc plate method of microbiological antibiotic assay. II. Novel procedure offering improved accuracy. *Applied Microbiology*, *22*(4), 666–670. https://doi.org/10.1128/aem.22.4.666-670.1971.

Esposito, F. P., Giugliano, R., Sala, G. Della, Vitale, G. A., Buonocore, C., Ausuri, J., Galasso, C., Coppola, D., Franci, G., Galdiero, M., & de Pascale, D. (2021). Combining OSMAC approach and untargeted metabolomics for the identification of new glycolipids with potent antiviral activity produced by a marine Rhodococcus. *International Journal of Molecular Sciences*, *22*(16). https://doi.org/10.3390/ijms22169055.

Gavriilidou, A., Gutleben, J., Versluis, D., Forgiarini, F., Van Passel, M. W. J., Ingham, C. J., Smidt, H., & Sipkema, D. (2020). Comparative genomic analysis of Flavobacteriaceae: Insights into carbohydrate metabolism, gliding motility and secondary metabolite biosynthesis. *BMC Genomics*, *21*(1), 1–21. https://doi.org/10.1186/s12864-020-

06971-7.

Hamill, P. G., Stevenson, A., McMullan, P. E., Williams, J. P., Lewis, A. D. R., Sudharsan, S., Stevenson, K. E., Farnsworth, K. D., Khroustalyova, G., Takemoto, J. Y., Quinn, J. P., Rapoport, A., & Hallsworth, J. E. (2020). Microbial lag phase can be indicative of, or independent from, cellular stress. *Scientific Reports*, *10*(1), 1–20. https://doi.org/10.1038/s41598-020-62552-4.

Jegatheesan, A., Sudhakar, M. P., Poonam, C., Perumal, K., & Arunkumar, K. (2017). Isolation and characterization of alginate-degrading bacteria *Sinomicrobium oceani*. *Biomass Conversion and Biorefinery*, *7*(1), 51–58. https://doi.org/10.1007/s13399-016-0212-z.

Judith, T., Fourie, & Chianella. (2022). Antimicrobial properties of *English churchyard* lichens. *Access Microbiology*, 1–25.

Khan, M. S., Gao, J., Munir, I., Zhang, M., Liu, Y., Moe, T. S., Xue, J., & Zhang, X. (2021). Characterization of Endophytic Fungi, *Acremonium* sp., from *Lilium davidii* and Analysis of Its Antifungal and Plant Growth-Promoting Effects. *BioMed Research International*, *2021*. https://doi.org/10.1155/2021/9930210.

Li, L.F., Xu, L., Li, W.-H., & Sun, J.-Q. (2022). *Sinomicrobium kalidii* sp. nov., an indole-3-acetic acid-producing endophyte from a shoot of halophyte *Kalidium cuspidatum*. *International Journal of Systematic and Evolutionary Microbiology*, *72*(7). https://doi.org/https://doi.org/10.1099/ijsem.0.005452.

Li, M., Wang, K., Jia, C., Liu, T., Yang, S., Ou, H., & Zhao, J. (2021). Bacteroidetes bacteria, important players in the marine sponge larval development process. *IScience*, *24*(6), 102662. https://doi.org/10.1016/j.isci.2021.102662.

Liu, X., Lai, Q., Du, Y., Zhang, X., Zhong, H., & Shao, Z. (2019). *Sinomicrobium soli* sp. nov., isolated from arctic soil. *International Journal of Systematic and Evolutionary Microbiology*, *69*(4), 1070–1074. https://doi.org/10.1099/ijsem.0.003273.

López-Rodríguez, M., López-Rosales, L., Necci, G. D., Cerón-García, M. del C., Navarro-López, E., Gallardo-Rodríguez, J. J., Tristán, A. I., Abreu, A. C., & García-Camacho, F. (2022). The Isolation of Specialty Compounds from *Amphidinium carterae* Biomass by Two-Step Solid-Phase and Liquid-Liquid Extraction. *Toxins*, *14*, 1–19.

Manalu, R. T. (2017). Isolation and characterization of degrading hydrocarbons bacteria from Indonesia. *Sainstech Farma*, *10*(2), 23–28.

Matin, M. M., Bhuiyan, M. M. ., Debnath, D. C., & Manchur, M. A. (2013). Synthesis and comparative antimicrobial studies of some acylated d-glucofuranose and d-glucopyranose derivatives. *International Journal of Biosciences (IJB)*, *3*(8), 279–287. https://doi.org/10.12692/ijb/3.8.279-287.

Mohimani, H., Gurevich, A., Shlemov, A., Mikheenko, A., Korobeynikov, A., Cao, L., Shcherbin, E., Nothias, L. F., Dorrestein, P. C., & Pevzner, P. A. (2018). Dereplication of microbial metabolites through database search of mass spectra. *Nature Communications*, *9*(1), 1–12. https://doi.org/10.1038/s41467-018-06082-8.

Nasab, M. O., & Khodakaramian, G. (2022). The Inhibitory Activity of Some Endophytic Bacteria from *Satureja Khuzestanica* Leaves against Phythopathogenic Bacteria. *Biological Journal of Microorganism*, *11*(44), 145–158.

Pinedo-Rivilla, C., Aleu, J., & Durán-Patrón, R. (2022). Cryptic Metabolites from Marine-Derived Microorganisms Using OSMAC and Epigenetic Approaches. *Marine Drugs*, *20*(2), 1–34. https://doi.org/10.3390/md20020084.

Prihanto, A. A., Timur, H. D. L., Jaziri, A. A., Nurdiani, R., & Pradarameswari, K. A. (2018). Isolation and Identification endophytic bacteria mangrove *Sonneratia alba* gelatinase enzyme producer from the beach Sendang Biru, Malang, East Java. *Indonesia Journal of Halal*, *1*(1), 31–42. https://doi.org/10.14710/halal.v1i1.3114.

Pringgenies, D., Wilis, A. ., Feliatra, F., & Ariyanto, D. (2023). The antibacterial and antifungal potential of marine natural ingredients from the symbiont bacteria of mangrove. *Global Journal of Environmental Science and Management*, *9*(4), 1–14. https://doi.org/10.22035/gjesm.2023.04.

Purves, K., Macintyre, L., Brennan, D., Hreggviðsson, G., Kuttner, E., Ásgeirsdóttir, M. E., Young, L. C., Green, D. H., Edrada-Ebel, R., & Duncan, K. R. (2016). Using molecular networking for microbial secondary metabolite bioprospecting. *Metabolites*, *6*(1). https://doi.org/10.3390/metabo6010002.

Ranković, B. (2019). Lichen secondary metabolites:

Bioactive properties and pharmaceutical potential. In *Springer*. https://doi.org/10.1007/978-3-319-13374-4.

Riyanti, Balansa, W., Liu, Y., Sharma, A., Mihajlovic, S., Hartwig, C., Leis, B., Rieuwpassa, F. J., Ijong, F. G., Wägele, H., König, G. M., & Schäberle, T. F. (2020a). Selection of sponge-associated bacteria with high potential for the production of antibacterial compounds. *Scientific Reports*, *10*(1), 1–14. https://doi.org/10.1038/s41598-020-76256-2.

Riyanti, Marner, M., Hartwig, C., Patras, M. A., Wodi, S. I. M., Rieuwpassa, F. J., Ijong, F. G., Balansa, W., & Schäberle, T. F. (2020b). Sustainable Low-Volume Analysis of Environmental Samples by Semi-Automated Prioritization of Extracts for Natural Product Research (SeaPEPR). *Marine Drugs*, *18*(649), 1–15.

Riyanti, Zumkeller, C. M., Spohn, M., Mihajlovic, S., Schwengers, O., Goesmann, A., Riviani, R., Meinita, M. D. N., Schäberle, T. F., & Harwoko, H. (2023). Draft Genome Sequence of *Sinomicrobium* sp . Strain PAP.21, Isolated from a Coast Sample of Papua, Indonesia. *Microbiology Resource Announcements*, 20–22.

Schneider, Y. K., Jørgensen, S. M., Andersen, J. H., & Hansen, E. H. (2021). Qualitative and quantitative comparison of liquid–liquid phase extraction using ethyl acetate and liquid–solid phase extraction using poly-benzyl-resin for natural products. *Applied Sciences*, *11*(21). https://doi.org/10.3390/app112110241.

Spyere, A., Rowley, D. C., Jensen, P. R., & Fenical, W. (2003). New neoverrucosane diterpenoids produced by the marine gliding bacterium *Saprospira grandis*. *Journal of Natural Products*, *66*(6), 818–822. https://doi.org/10.1021/np0205351.

Tangerina, M. M. P., Furtado, L. C., Leite, V. M. B., Bauermeister, A., Velasco-Alzate, K., Jimenez, P. C., Garrido, L. M., Padilla, G., Lopes, N. P., Costa-Lotufo, L. V., & Pena Ferreira, M. J. (2020). Metabolomic study of marine *Streptomyces* sp.: Secondary metabolites and the production of potential anticancer compounds. *PLoS ONE*, *15*(12), 1–19. https://doi.org/10.1371/journal.pone.0244385.

Thawabteh, A. M., Swaileh, Z., Ammar, M., Jaghama, W., Yousef, M., Karaman, R., A. Bufo, S., & Scrano, L. (2023). Antifungal and Antibacterial Activities of Isolated Marine Compounds. *Toxins*, *15*(2), 1–21. https://doi.org/10.3390/toxins15020093.

Tortorella, E., Tedesco, P., Esposito, F. P., January, G. G., Fani, R., Jaspars, M., & De Pascale, D. (2018). Antibiotics from deep-sea microorganisms: Current discoveries and perspectives. *Marine Drugs*, *16*(10), 1–16. https://doi.org/10.3390/md16100355.

Wang, M., Carver, J., Phelan, V. *et al.* Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nat Biotechnol* **34**, 828–837 (2016). https://doi.org/10.1038/nbt.3597

Wang, F., Li, M., Huang, L., & Zhang, X. H. (2021). Cultivation of uncultured marine microorganisms. *Marine Life Science and Technology*, *3*(2), 117–120. https://doi.org/10.1007/s42995-021-00093-z.

Wibowo, J. T., Kellermann, M. Y., Versluis, D., Putra, M. Y., Murniasih, T., Mohr, K. I., Wink, J., Engelmann, M., Praditya, D. F., Steinmann, E., & Schupp, P. J. (2019). Biotechnological potential of bacteria isolated from the sea cucumber *Holothuria leucospilota* and *Stichopus vastus* from Lampung, Indonesia. *Marine Drugs*, *17*(11). https://doi.org/10.3390/md17110635.

Wu, P., Ren, H., Zhu, Q., Mei, Y., Liang, Y., & Chen, Z. (2022). *Sinomicrobium weinanense* sp. nov., a halophilic bacterium isolated from saline-alkali soil. *International Journal of Systematic and Evolutionary Microbiology*, *72*(4). https://doi.org/https://doi.org/10.1099/ijsem.0.005282.

Xu, Y., Tian, X. P., Liu, Y. J., Li, J., Kim, C. J., Yin, H., Li, W. J., & Zhang, S. (2013). *Sinomicrobium oceani* gen. nov., sp. nov., a member of the family Flavobacteriaceae isolated from marine sediment. *International Journal of Systematic and Evolutionary Microbiology*, *63*, 1045–1050. https://doi.org/10.1099/ijs.0.041889-0.