HAYATI
Journal of Biosciences

Vol. 29 No. 3, May 2022 343-352
DOI:10.4308/hjb.29.3.343-352
ISSN: 1978-3019
EISSN: 2086-4094

Taxonomy and Antimicrobial Activity of Gliding Bacterium from Indonesian Mangroves

Senlie Octaviana[1,2*], Tjandrawati Mozef[3], Joachim Wink[1]

[1]*Microbial Strain Collection, Helmholtz Center for Infection Research, Braunschweig, Germany*
[2]*Research Center for Biotechnology, Indonesian Institute of Sciences, Cibinong, Indonesia*
[3]*Research Center for Chemistry, Indonesian Institute of Sciences, Serpong, Indonesia*

ARTICLE INFO

Article history:
Received September 8, 2020
Received in revised form September 6, 2021
Accepted January 1, 2022

KEYWORDS:
Antimicrobial,
Indonesia,
mangrove,
gliding bacteria

ABSTRACT

The discovery of new antibiotic is needed, due to the increasing antimicrobial resistance in nature. Therefore, this study aims to isolate gliding bacteria and to ascertain their antimicrobial activity against pathogenic microbes. This research was conducted by isolating gliding bacteria from mangrove sediment in Muara Angke, North Jakarta, Indonesia. All of the strains were identified by 16S rRNA genes sequencing and those selected strain was characterized using polyphasic approach. The performance of crude extracts against to ten pathogenic microorganisms were detected using serial dilution test in 96-well plates. Mangrove gliding bacteria isolates designated 313MSO and 314MSO were showed high homology to *Ohtaekwangia kribbensis* with 96.20% and 99.12% similarity, respectively. The polyphasic approach led to the conclusion that the strain 313MSO was a new species of the genus *Ohtaekwangia*. Twenty-three crude extracts were obtained from cultivating the strain in twenty-three different media. The most of them inhibited the growth of *Staphylococcus aureus* Newman with the minimum concentration of 33.33-66.67 µg/ml. Four compounds (Marinoquinolines A-D) were obtained from HPLC-MS analysis. Furthermore, the strain 313MSO is presently being studied for in-depth identification of additional unknown metabolites detected in the crude extracts.

1. Introduction

Since Reichenbach in 1981 introduced the taxonomy of gliding bacteria, these bacteria have more attention for harboring different natural products. Gliding bacteria are Gram-negative bacteria, which have the ability to glide on surfaces and are grouped into the Flexibacteriae class, which includes two classification orders namely, *Cytophagales* and *Myxobacterales*. Nowadays, it are distinguished in the phylum level, as those without fruiting bodies belong to the phylum *Bacteriodetes*, while those possessing this feature belong to *Myxococcales*.

Over the past decades, members of phylum *Myxococcales* have proven to be promising source of new bioactive metabolites. Therefore, around six hundred derivatives and a hundred new carbon skeleton metabolites have been isolated and identified. These bioactive metabolites exhibit antibacterial, antifungal, antimalarial, antioxidative and antiviral activities (Gerth *et al.* 2003; Wenzel and Müller 2009; Landwehr *et al.* 2016; Mulwa and Stadler 2018). Meanwhile, inspite of the general outstanding in the production of diverse bioactive metabolite, the discovery of new antibiotic producers in nature is still needed.

Gliding bacteria are commonly found in various habitats (Dawid 2000). In 2011, *Ohtaekwangia* which a new genus of gliding bacterium, was found in Korea marine sand, belonging to the *Cythophagales* order, which was previously proposed as a novel species of *Ohtaekwangia koreensis* (3B-2^T) and *Ohtaekwangia kribbensis* (10AO^T) (Yoon *et al.* 2011). In the same year, Okanya *et al.* (2011) isolated Marinoquinolines A and five derivatives B-F from a strain, which is closely related to *Ohtaekwangia kribbensis*. These compounds reacted against pathogenic microorganism and also show cytotoxicity to growing mammalian cell lines.

Sangnoi *et al.* (2016) gave a report on novel genera of gliding bacteria, *Flavobacterium* and *Lysobacter* from a conservation site at Cheow Land Reservoir

* Corresponding Author
E-mail Address: senl001@brin.go.id

in southern Thailand. Moreover, all of these strains have antibacterial activities when tested with agar-well diffusion assay. The purpose of this study is to isolate and to identify gliding bacteria from Indonesian samples using 16S rRNA gene sequences. Initially, these bacteria were found as a contaminant in the agar plates for isolation of myxobacteria. Nevertheless, one of the new strains was selected for in-depth analyses and characterization using the polyphasic approach, and also evaluating their capability in the production of secondary metabolite, especially for antimicrobial activity.

2. Materials and Methods

2.1. Sampling Area

Samples from Mangrove sediments were taken onto sterile zip-log plastic bag from Taman Konservasi Muara Angke (6°105321'N 106°735578'E), Jakarta, Indonesia. All the samples were dried at 30°C and stored at room temperature.

2.2. Isolation of Gliding Bacterium

The strains were isolated using the methods of Dworkin *et al.* (2006) and placed on water-agar medium where their surfaces were carefully cross-streaked with living *Escherichia coli* K12. After five days, swarm colonies were observed using a dissecting microscope and transferred with a sterile needle into fresh water-agar with dead *Escherichia coli*. The isolates were typical gliding bacteria without fruiting bodies growing faster than myxobacteria. Pure cultures were then transferred into 20 ml CY/H liquid medium [per liter: 1.0 g defatted soy flour, 1.0 g glucose, 4.0 g starch (Cerestar), 1.5 g yeast extract, 1.5 g casitone, 1.0 g $CaCl_2 \cdot 2H_2O$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.008 g iron EDTA, 11.8 g HEPES, pH 7.3]. While 100 ml CY/H liquid medium of well-grown culture and 1.5 ml portion of them were conserved at -80°C.

2.3. Identification of Pure Cultures by 16S rRNA Sequences

The DNA from pure cultures were extracted using Invitek Spin Plant Mini Kit (Invisorb) following the manufacturer's instruction. The PCR reaction of 16S rRNA was performed in a Mastercycler Gradient (Eppendorf, Hamburg, Germany) following Mohr *et al.* (2016, 2017). The PCR products were then examined using electrophoresis on agarose (0.8%) and purified with NucleoSpin Gel and PCR Clean up Kit (Macherey-Nagel, Düren, Germany).

The pure PCR products were sent to the sequence service at HZI (Helmholtz Centre for Infection Research). The 16S rRNA gene sequences were assembled with the BioEdit programme (Hall 1999) and compared with the public database NCBI using BLAST search (Altschul *et al.* 1990). The phylogenies were inferred by the GGDC web server (Meier-Kolthoff *et al.* 2013) at http://ggdc.dsmz.de/ using the DSMZ phylogenomics pipeline of a single gene (Meier-Kolthoff *et al.* 2014). All of the sequences were aligned using MEGAX software. Maximum likelihood (ML) and maximum parsimony (MP) trees were inferred from the alignment with RAxML (Stamatakis 2014) and TNT (Goloboff *et al.* 2008), respectively. Based on the ML, rapid bootstrapping with the autoMRE criterion (Pattengale *et al.* 2010) and subsequent search for the best tree were used. While for MP, a thousand bootstrapping replicates were used in conjunction with tree-bisection-and-reconnection branch swapping and ten random sequence addition replicates. Lastly, the sequences were checked for a compositional bias using the X^2 test as implemented in PAUP* (Swofford 2002).

2.4. Morphological Identification

The morphology and cell size from agar plates were observed using microscope (AxioCam MRc9) at a magnification 1000x. Approximately ten to twenty randomly selected cells were measured (length) by using Axio-Vision Rel. 4.8 software followed by defining the Gram staining based on Sigma Aldrich manufacturer's instructions.

2.5. Physiological Identification

Different media namely R2A, VY/2, CY, and VY/4 ASW were selected and inoculated with 10 µl well-grown cultures. After been incubated at 30°C for five days, the plated were checked by visual inspection and the growth was characterized by measuring the swarming colonies. The optimal temperatures for their growth were determined after being incubated at 10°C, 20°C, 25°C, 28°C, 30°C, 34°C, 40°C, and 44°C. Moreover, the pH following 4.0, 5.0, 6.5, 7.5, 8.5, and 9.0 were observed for optimal growth. In the analysis of sodium tolerance, 10 µl well-grown cultures were inoculated on optimal conditions as mentioned above with different concentration of NaCl (0, 2, 5, 7.5, and 10%). Therefore, their entire physiological characteristic was observed by visual inspection.

2.6. Chemotaxonomy Identification

The flexirubin pigment played an important role within the members of Cytophagales and analyzed following Reichenbach (1981) instructions. Fifty mg of dry cell mass were prepared and fatty acid methyl ester extraction following the Microbial Identification System (MIDI) protocol. The fatty acids were then analysed using the GC-Chromatography, while its phospholipid was defined according to Carthwight (1993) instructions. The catalase and oxidase tests were observed following Sigma Aldrich protocol, when both of experiment tested positive they indicated bubble formation and discoloration to purple-blue, respectively. The characteristic of the enzymes were defined using APIZYM and APICAMPY, while the Gen III BIOLOG System was used for the assimilation substrates. Then, antibiotic resistances were checked using various antibiotics (50 mg/ml) and observed by visual inspection after five days.

2.7. Screening for Antimicrobial Activity

The selected pathogenic microorganism used in this antimicrobial study were Escherichia coli WT-BW 25113, *Escherichia coli* JW0451-02, *Acinetobacteri baumanii* DSM30008, *Pseudomonas aeruginosa* Pa14, *Staphylococcus aureus* Newmann, *Citrobacter freundii* DSM 30039, *Mycobacterium smegmatis* ATCC 700084, *Mucor himalis* DSM 2656, *Pichia anomala* DSM 6766, and *Candida albicans* DSM 1665. All were obtained from microbial strain collection group, HZI, Germany. The details for the culture conditions were mentioned in Supplementary 1.

The well-grown cultures of the gliding bacterium strain were prepared with 100 ml CY/H liquid medium and 10% of them was then inoculated in twenty-three different media (Supplementary 2) with XAD-adsorber resin. After five days, the cultures were filtered and were extracted with 70 ml acetone. After the solvent was evaporated, 1 ml MeOH was added and stored at -20°C.

The crude extracts were evaluated for antimicrobial activity in the serial dilution test using 96-well plates with ten different pathogenic microbes. After overnight incubation, the antimicrobial activity was evaluated by visual inspection. A clear zone indicated the crude extract inhibition of the pathogenic microorganism, while an overgrown of microbe indicated a negative inhibition activity.

The de-replication process was followed by using a fractionation to identify the active compounds. Therefore, the active crude extracts were analyzed by preparative HPLC, HPLC/MS and Brucker Data Analysis and were compared with the in-house database "myxobase" and/or DNP (dictionary of natural product). Then, the active compounds were identified as "new" or "known" compounds.

2.8. Nucleotide Sequences Accession Number

The GenBank/EMBL/DDBJ accession numbers for the partial 16S rRNA gene sequences of 313MSO and 314MSO strains were defined as MT591272 and MT591273, respectively.

3. Results

3.1. Strain Isolation

The two strains of gliding bacteria from Indonesian habitat were isolated (Table 1), purified, and grew fast after three days of inoculation. The 313MSO and 314MSO strains were isolated from Mangrove sediment, and compared to the partial 16S rRNA gene sequences using BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi). These strains were identified as *Ohtaekwangia* spp. with the similarity of 96.20 and 99.12%, respectively, while 313MSO strain had lower similarity (96.20%) than intraspecies. Therefore, it was selected for in-depth study.

A phylogenetic analysis of 16S rRNA gene sequences from strain 313MSO was shown in Figure 1. The input nucleotide matrix comprised 45 operational taxonomic units and 1449 characters, 713 of which were variable and 545 of which were parsimony-informative. The base-frequency check indicated no compositional bias ($p = 0.68$, $\alpha = 0.05$). The Maximum Likelihood (ML) analysis under the GTR+GAMMA model yielded a highest log likelihood of -16906.77, while the estimated alpha parameter was 0.27. The ML bootstrapping did not converge, hence a thousand replicates were conducted; and the average support was 63.43%. The Maximum Parsimony (MP) analysis yielded the best score of 3473 (consistency index 0.37, retention index 0.57), twenty-two best trees and MP bootstrapping average support was 70.86%.

The levels of partial 16S rRNA gene sequence similarity between strain 313MSO and other bacterial taxa are acceptable to differentiate this strain from known genera of the phylum *Bacteroidetes*. It should be classified in a new species within the genus *Ohtaekwangia.*

Table 1. Morphological properties of strain 313MSO

Characteristic	313MSO	3B-2T*	10A0-T*
Cell shape	Rod	Rod	Rod
Cell Size [µm]	1.0-5.0	1.0-5.0	1.5-7.5
NaCl Tolerances [w/v]			
0-1%	+	+	+
2.5-10%	-	-	-
Catalase	-	+	+
Oxidase	-	+	+
Flexirubin type pigment	-	+	+
Optimal growth	30°C	30°C	30°C
Optimal pH	6.5-7.5	6.5-7.5	6.5-7.5
Optimal medium	VY/2	R2A	R2A
Gram staining	negative	negative	negative
Antibiotic resistance			
Gentamycin [50 µg/ml]	+	-	+
Phospholipid	Phosphatidylinositol-mannosid	Phosphatodylethanolamine	Phosphatodylethanolamine

+ able, – unable, 3B-2T = *O. kribbiensis*, 10A0-T = *O. koreensis*

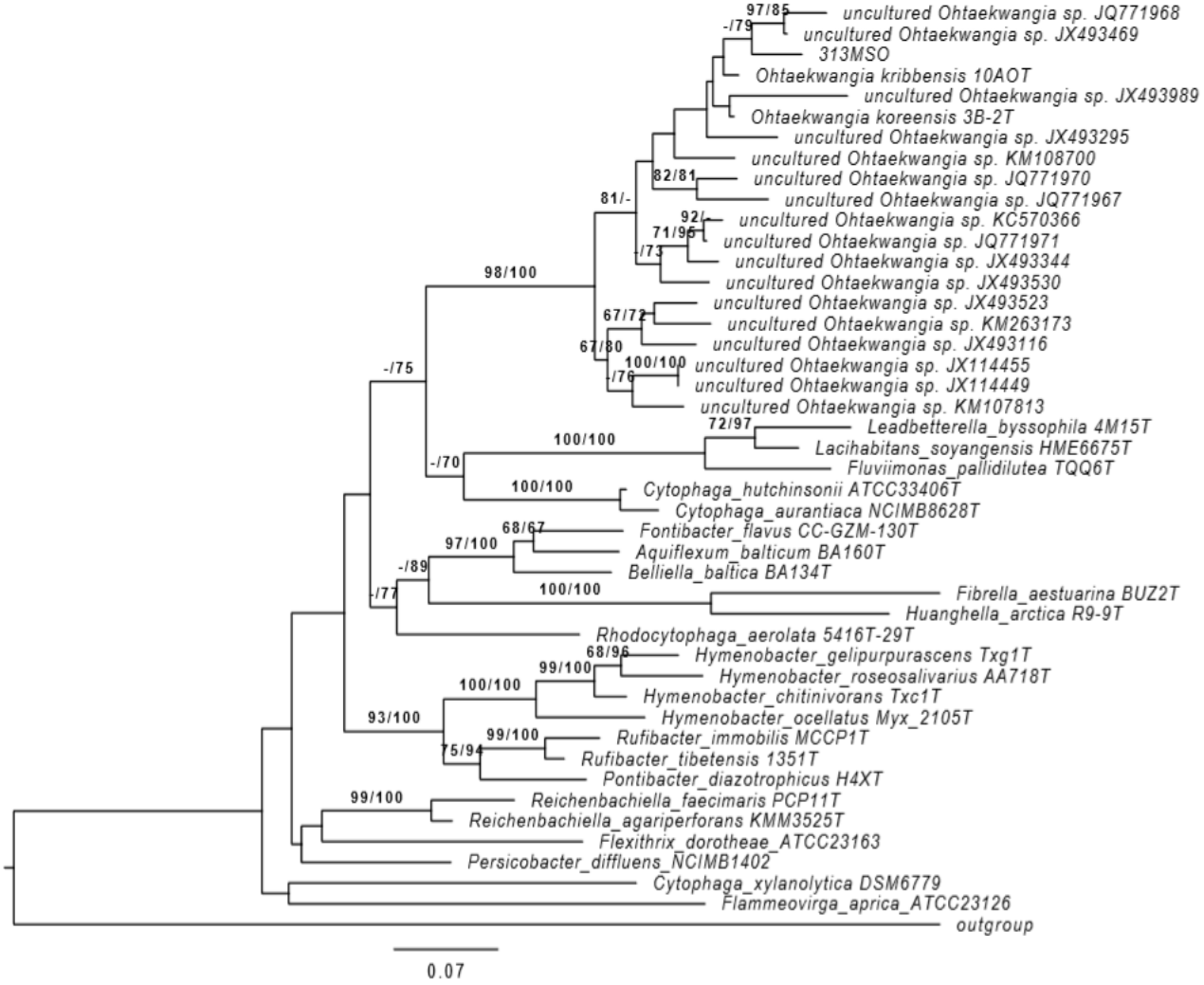

Figure 1. Maximum Likelihood (ML) tree inferred under the GTR+GAMMA model and rooted by outgroup-rooting. The branches are scaled in terms of the expected number of substitutions per site. The numbers above the branches are supporting values when larger than 60% from ML (left) and MP (right) bootstrapping

3.2. Characteristic of The New Strain

The gliding bacterial strain of 313MSO was characterized and compared with the strains of Ohtaekwangia kribbensis 3B-2^T and *Ohtaekwangia koreensis* 10AO^T. A typical colony bright yellow in colour with radial patterns were observed in Figure 2. As described in Table 3, the morphological characteristics of strain 313MSO were Gram-negative and rod-shaped with the optimum size of 1-5 µm. This strain showed the optimum growth in VY/2 and R2A medium. It preferably grew in pH ranging 6.5 to 7.5 at 30°C on agar plates. The yeast extract is better than carbohydrate for stimulating the growth of most of the members of *Cytophagales* order.

The 313MSO strain grew well in 0-1% NaCl and has no catalase and oxidase enzyme activities, and also flexirubin type pigments. However, the reference strains have these features. For antibiotic resistances, the strain showed resistance to gentamycin, however, susceptible to polymycin, kanamycin, ampicillin, and chloramphenicol (not included in Table 1). Although the strain had different morphological properties with references strains, it has no strength defined to the new species.

The biochemical properties of strain 313MSO were shown in Table 2. The following products were assimilated by this strain namely, acetoacetic acid, alpha-hyydroxy-butyric acid, D-lactic acid methyl ester, D-serine, glycerol, L-alanine, L-lactic acid, methyl pyruvate, myo-inositol, N-acetyl neuraminic acid, and sodium butyrate, however, were not produced by the reference strains. As mentioned in Table 3, the 313MSO strain showed no enzyme

Table 2. Biochemical properties of new strain

Characteristic	313MSO	3B-2T*	10A0-T*
Assimilation of (Biolog)			
3-Methyl glucose	-	+	-
Acetic acid	+	-	+
Acetoacetic acid	+	-	-
Alpha-hyydroxy-butyric acid	+	-	-
Alpha-keto-glutaric acid	-	-	+
Beta-hydroxy-D, L-butyric acid	-	-	+
Beta-methyl-D-dlucosidase	-	+	+
Bromo-succinic acid	-	-	+
Citric acid	-	-	+
D-aspartic acid	-	+	+
D-fructose-6-PO4	+	-	+
D-galactose	+	-	+
D-gluconic acid	+	-	+
D-lactic acid methyl ester	+	-	-
D-malic acid	-	-	+
D-mannose	+	-	+
D-saccharic acid	-	-	+
D-serine	+	-	-
Formic acid	-	-	+
Gentiobiose	+	-	+
Glucuornicamide	+	+	+
Glucuronic acid	-	-	+
Glycerol	+	-	-
Glycyl-L-proline	-	+	+
Guanidine HCl	-	-	+
L-alanine	+	-	-
L-arginine	-	-	+
L-fucose	+	+	+
L-galactonic acid lactone D-gluconic acid	-	+	+
L-glutamic acid	+	-	+
Linomycin	+	-	+
Lithium chloride	+	-	+

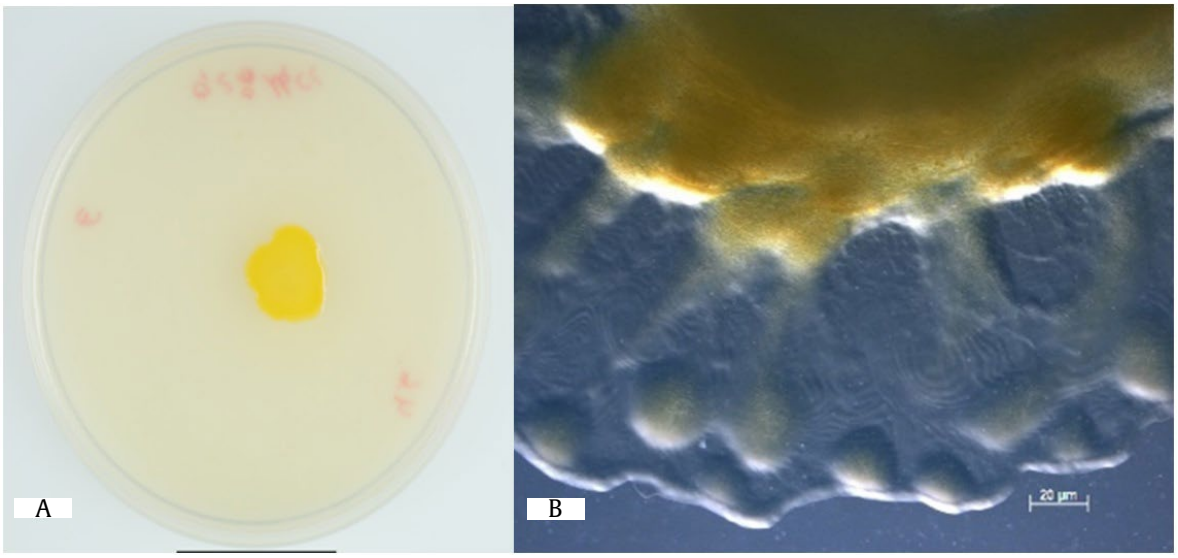

Figure 2. Morphological characteristic of gliding bacterium. Strain 313MSO was growth on VY/2 agar (A) and observed under optical microscope (B). The scale bars are 10-20 µm

Table 2. Continued

Characteristic	313MSO	3B-2T*	10A0-T*
L-lactic acid	+	-	-
L-malic acid	-	-	+
L-rhamnose	+	-	+
L-serine	+	-	+
Methyl pyruvate	+	-	-
Minocycline	+	-	+
Mucic acid	-	-	+
myo-inositol	+	-	-
N-acetyl neuraminic acid	+	-	-
N-acetyl-D-galactosamine	+	-	+
Nalidixic acid	+	-	+
p-hydroxy-phenylacetic acid	-	-	+
Potassium tellurite	+	-	+
Propionic acid	-	-	+
Quinic acid	-	+	+
Sodium butyrate	+	-	-
Sucrose	+	-	+
Tetrazolium violet	-	-	+
Tetrazolum blue	+	-	+
Troleandomycin	+	-	+
Vancomycin	+	-	+
APIZYM			
Trypsin	w	+	-
Naphtol-AS-B1-phosphohydrolase	+	+	-
Beta glucosidase	+	+	-
Alpha fucosidase	-	+	-
API CAMPY			
Esterase	-	+	-
HIP-purate	-	+	-
Gamma glutamyl transferase	+	-	-

+ able, – unable, w = weak, 3B-2T = *O. kribbiensis*, 10A0-T = *O. koreensis*

Table 3. Major fatty acid of the new strains

Fatty acid	313MSO	3B-2T*	10A0-T*
Straight-chain			
c15:0	-	-	2.1
c16:0	-	-	22.2
c18:0	1.59	-	-
Branched			
Iso c14:0	-	-	1.7
Iso c15:0	-	20.4	30.2
Iso c16:0	3.8	9.5	4
Iso c17:0	-	-	7.4
Unsaturated			
c16:1 ω7c	-	55.2	27.4
Unknown			
ECL 11.864	-	6.2	3.3
ECL 22.207	-	8.7	1.7

3B-2T = *O. kribbiensis*, 10A0-T = *O. koreensis*

activities for alpha fucosidase, esterase and HIP-purate, however, indicated close relationship to *Ohtaekwangia kribbiensis* 3B-2^T, based on APIZYM and APICAMPY tests.

The 313MSO strain also showed different characters of fatty acid properties compared to references strains. As mention in Table 3, the analyses of fatty acids were performed using MIDI protocol. The 313MSO strain had C18:0 straight-chain fatty acid and C16:0 iso brached fatty acid. Lastly, the strain 313MSO had different characters of the biochemical properties compared to the reference strains.

3.3. Antimicrobial Activity

The twenty-three crude extracts were examined for antimicrobial activity towards pathogenic microbes which included the five Gram-negative, two Gram-positive bacteria and three fungus activities (Figure 3). The antimicrobial assay (MIC) showed that most of the crude extracts exhibited growth inhibition against *Staphylococcus aureus* Newmann, including the strain 313MSO when cultured in P medium and 1/10 CY/H medium with the minimum concentration of 33.33 µg/ml. The 313MSO strain also showed the inhibition activity against *Escherichia coli* acrB JW0451-2, when cultured in 1/10 E medium and against *Pseudomonas aeruginosa* Pa14 with the minimum concentration of MIC 66.67 µg/ml, when cultivated in MA medium. However, all of the crude extracts had no inhibition towards *Candida albicans* DSM1665, *Acinetobacter baumannii* DSM30008, *Escherichia coli* WT BW25113, *Citrobacter freundii* DSM30039, *Mycobacterium smegmatis* ATCC700084, *Mucor himalis* DSM2656, and *Pichia anomala* DSM6766.

4. Discussion

Two gliding bacteria strains of 313MSO and 314MSO were isolated from mangrove sediment in North Jakarta, Indonesia. The differences in the gliding bacteria and myxobacterial strain were difficult to define, due to the unstable nature of the fruiting bodies in myxobacteria, which were lost during purification (Zhang *et al.* 2013). Furthermore, the comparison of 16S rRNA gene sequences was useful for identifying closely related strains. The 313MSO strain from mangrove showed the lowest similarity (96.20%) to this species.

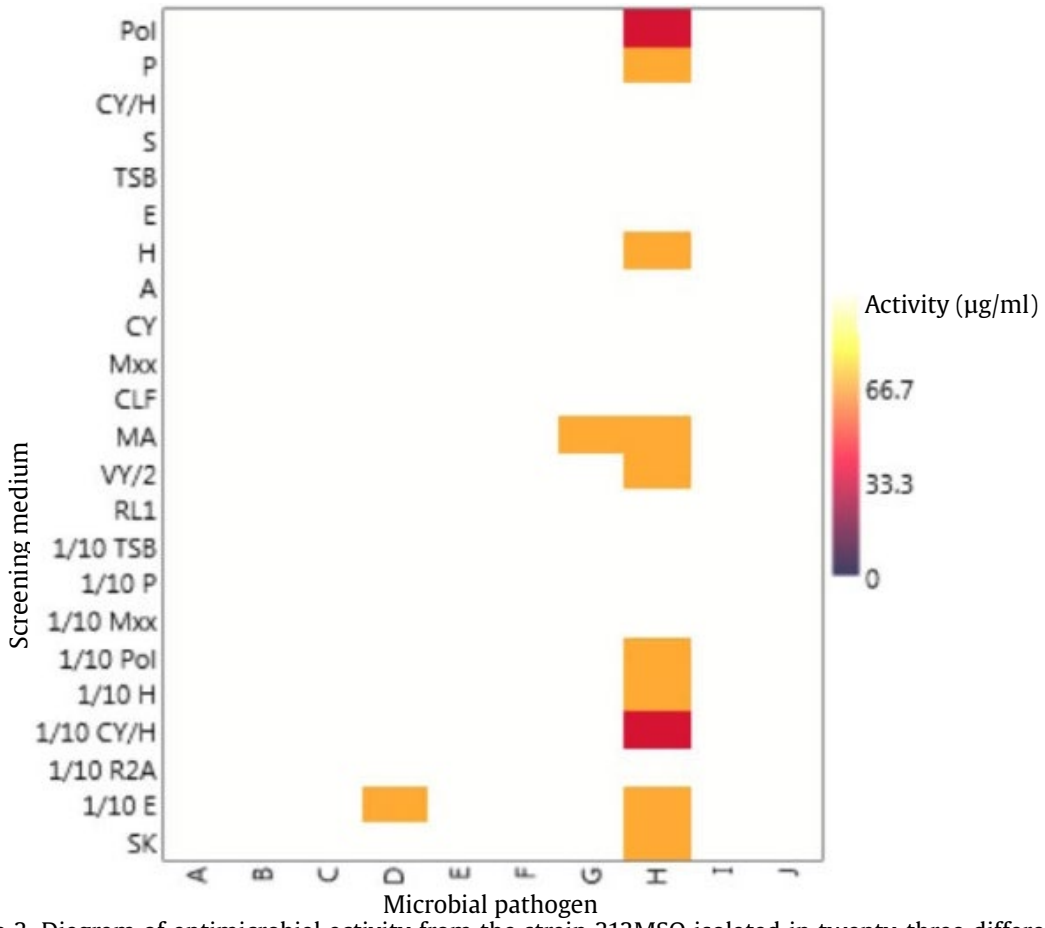

Figure 3. Diagram of antimicrobial activity from the strain 313MSO isolated in twenty-three different media that were tested against to ten different pathogenic microbes. The letters is designated by letters (A = *Candida albicans* DSM1665, B = *Acinetobacter baumannii* DSM30008, C = *Escherichia coli* WT BW25113, D = *Escherichia coli* acrB JW0451-2, E = *Citrobacter freundii* DSM30039, F = *Mycobacterium smegmatis* ATCC700084, G = *Pseudomonas aeruginosa* Pa14, H = *Staphylococcus aureus* Newmann, I = *Mucor himalis* DSM2656, J = *Pichia anomala* DSM6766). The different colour indicates the antimicrobial activity: the darker the red colour, the stronger inhibition activity, which correlates to the lower crude extract concentration

All of the gliding bacteria were isolated during a routine myxobacterial isolation project in the laboratory. The 313MSO strain was selected for in-depth study. It could optimally grow be grow in VY/2 medium, while reference strains grow in R2A medium (Table 1). A polyphasic study with the 16S rRNA gene sequence analysis of this strain showed different characters (Tables 1, 2, 3), also, the phylogenetic tree clearly exhibited separation from the reference strains (Figure 1). Therefore, it was proposed that novel should have similarity percentage below 98.65%. The determination of full genome sequences included G+C content, DNA-DNA hybridization and quinone production should be the subject of a further study.

This is the first report describing a novel taxonomy using the polyphasic approach and their potential for antimicrobial activity from unexplored Indonesian habitat, especially in the mangrove forest. These results provide new information about the ecology of a species of the gliding bacterium belonging to genus *Ohtaekwangia*. Moreover, the methodologies for the antimicrobial screening are useful for further studies of the economically important organisms.

The twenty-three crude extracts that were obtained from the 313MSO strain that were cultivated in different media (Figure 2) and most of them had specific inhibition towards *Staphylococcus aureus* Newmann with the minimum concentration 33.33-66.67 µg/ml. The 313MSO strain had stronger antibacterial activity towards pathogenic bacteria compared to the aquatic gliding bacteria with the minimum concentration 75 µg/ml (Sangnoi *et al.* 2016).

Base on de-replication with preparative HPLC and HPLC/MS, the active crude extracts from Pol and 1/10 CY/H medium with the minimum concentration MIC 33.33 µg/ml belonged to the fatty acid group. Moreover, Marinoquinolines A and five derivates (B-F) were also detected from both media. Despite the fact that

Okanya *et al.* (2011) isolated Marinoquinolines A and five derivates (B-F) using PWu25, which belong to *Ohtaekwangia* sp. in E broth medium, in the present study 313MSO strain, showed no antimicrobial activity in the same medium. Consequently, it is suggested that the strain 313MSO could be cultivated on other different media (optimization media for examples) and/or co-cultivated with microbial natural product producers for stimulating secondary metabolite. Moreover, all of the crude extracts may be analysed in other biological test models.

In conclusion, the mangrove is an ecosystem that harbours gliding bacteria, since it is an unusual environment between the marine and terrestrial habitat. The 313MSO strain was classified as a new species, when it showed different character from references strain within genera *Ohtaekwangia*. Their crude extracts showed specific antimicrobial activity towards Gram-positive pathogenic bacteria.

Conflicts of Interest

The authors declare that there is no conflict of interest on this research.

Acknowledgements

This research was supported with full funding by German Academic Exchange Service (DAAD)-GINAICO program for the PhD scholarship. We are grateful to our co-worker, Frederike ackert and Stefan lorenczyk for providing great assistance towards this research and also to Aileen Gollach, Klaus Peter Conrad, Birte Trunkwalter, and Stephanie Schulz for technical assistance, Dr. Gian Primahana for antimicrobial discussion.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *Journal Of Molecular Biology*. 215, 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2

Cartwright., 1993. Separation and analysis of phospholipids by thin layer chromatography. *Methods. Mol. Biol*. 19, 153-67. https://doi.org/10.1385/0-89603-236-1:153

Dawid, W., 2000. Biology and global distribution of myxobacteria in soils. *FEMS Microbiol Rev*. 24, 403–427. https://doi.org/10.1111/j.1574-6976.2000.tb00548.x

Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E., 2006. The prokaryotes-Delta and Epsilon Subclasses. Deeply Rooting Bacteria, seventh ed. Springer, New York. https://doi.org/10.1007/0-387-30747-8

Gerth, K., Pradella, S., Perlova, O., Beyer, S., Müller, R., 2003. Myxobacteria: proficient producers of novel natural products with various biological activities--past and future biotechnological aspects with the focus on the genus *Sorangium. J Biotechnol.* 106, 233-253. https://doi.org/10.1016/j.jbiotec.2003.07.015

Goloboff, P.A., Farris, J.S., Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. *Cladistics*. 24, 774-786. https://doi.org/10.1111/j.1096-0031.2008.00217.x

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series*. 41, 95-98.

Landwehr, W., et al. 2016. Actinobacteria and myxobacteria-two of the most important bacterial resources for novel antibiotics. *Microbiology and Immunology*. 398, 273-302. https://doi.org/10.1007/82_2016_503

Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., Göker, M., 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*. 14, 60. https://doi.org/10.1186/1471-2105-14-60

Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., Göker, M., 2014. Complete genome sequence of DSM 30083T, the type strain (U5/41^T) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Stand Genomic Sci.* 9, 2. https://doi.org/10.1186/1944-3277-9-2

Mohr, K.I., Stechling, M., Wink, J., Wilharm, E., Stadler, M., 2016. Comparison of myxobacterial diversity and evaluation of isolation success in two niches: Kiritimati Island and German Compost. *Microbiology Open*. 5, 268-278. https://doi.org/10.1002/mbo3.325

Mohr, K.I., Zindler, T., Wink, J., Wilharm, E., Stadler, M., 2017. Myxobacteria in high moor and fen: an astonishing diversity in a neglected extreme habitat. *Microbiology Open*. 6, e00464. https://doi.org/10.1002/mbo3.464

Mulwa, L., Stadler, M., 2018. Antiviral compounds from *Myxobacteria. Microorganism.* 6, 73. https://doi.org/10.3390/microorganisms6030073

Okanya, P.W., Mohr, K.I., Gerth, K., Jansen, R., Müller, R., 2011. Marinoquinolines A–F, Pyrroloquinolines from *Ohtaekwangia kribbensis* (Bacteroidetes). *J. Nat. Prod.* 74, 603-608. https://doi.org/10.1021/np100625a

Pattengale, N.D., Alipour, M., Bininda-Emonds, O.R.P., Moret, B.M.E., Stamatakis, A., 2010. How many bootstrap replicates are necessary. *J. Comput. Biol.* 17, 337–354. https://doi.org/10.1089/cmb.2009.0179

Reichenbach, H., 1981. Taxonomy of the gliding bacteria. *Ann. Rev. Microbiol.* 35, 339-634. https://doi.org/10.1146/annurev.mi.35.100181.002011

Sangnoi, Y., Anantapong, T., Kanjana-Opas, A., 2016. Antibacterial activity of aquatic gliding bacteria. *SpringerPlus.* 5, 116. https://doi.org/10.1186/s40064-016-1747-y

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England)*. 30, 1312-1313. https://doi.org/10.1093/bioinformatics/btu033

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0 b10. Sinauer Associates.

Wenzel, S.C., Müller, R., 2009. Myxobacteria-'microbial factories' for the production of bioactive secondary metabolites. *Mol. Biosyst*. 5, 567-574. https://doi.org/10.1039/b901287g

Yoon, J.H., Kang, S.J., Lee, S.Y., Lee, J.S., Park, S., 2011. *Ohtaekwangia koreensis* gen. nov., sp. nov. and *Ohtaekwangia kribbensis* sp. nov., isolated from marine sand, deep-branching members of the phylum *Bacteroidetes. J. of Systematic and Evolutionary Microbiology*. 61, 1066-1072. https://doi.org/10.1099/ijs.0.025874-0

Zhang, X., Yao, Q., Cai, Z., Xie, X., Zhu, H., 2013. Isolation and identification of myxobacteria from Saline-Alkaline Soils in Xinjiang, China. *Plos One*. 8, E70466. https://doi.org/10.1371/journal.pone.0070466

Supplemenatry 1. MIC Table

Pathogenic microbes	Medium	Inoculated in 20 ml med (µl)	Incubation (°C)	Antibiotic references
Candida albicans DSM 1665	Myc_1	90	30	Nystatin
Acinetobacter baumannii DSM 30008	Müller/Hinton	62	30	
Escherechia coli WT BW25113	Müller/Hinton	59	37	Gentamycin
Escherechia coli acrB JW0451-2	Müller/Hinton	77	37	Gentamycin
Citrobacter freundii DSM30039	Müller/Hinton	63	30	
Mycobacterium smegmatis ATCC700084	Middlebrook	150	37	Kanamycin
Pseudomonas aeruginosa PA14	Müller/Hinton	63	30 or 37	Gentamycin
Staphylococcus aureus Newmann	Müller/Hinton	43	30	Gentamycin
Mucor himalis DSM2656	Myc_1	150	30	Nystatin
Pichia anomala DSM6766	Myc_1	38	30	Nystatin

Supplemenatry 2. Medium for screening

Screening medium		Composition	Notes
A-medium	0.40%	glycerol (87% w/v)	pH 7.4
	0.40%	soy flour (degreased)	
	0.20%	yeast extract (marcor typ 9000)	
	0.10%	$MgSO_4 \cdot 7H_2O$	
	50 mM	HEPES (11.9 g/l)	
	8 mg/l	Fe-EDTA	
	0.80%	starch (cerestar)	
	0.10%	$CaCl_2 \cdot 2H_2O$	
CLF-medium	0.40%	fructose monohydrat	pH 7.0
	0.60%	glucose monohydrat	
	1.00%	skim milk	
	0.20%	yeast extract (marcor typ 9000)	
	0.10%	$CaCl_2 \cdot 2H_2O$	
	0.10%	$MgSO_4 \cdot 7H_2O$	
	50 mM	HEPES (11.9 g/l)	
CY-medium	0.30%	Castione	pH 7.2
	0.10%	yeast extract (marcor typ 9000)	
	0.10%	$CaCl_2 \cdot 2H_2O$	
	50 mM	HEPES (11.9 g/l)	
	1.60%	Agar (Difco)	
E-medium	0.40%	skim milk	pH 7.4
	0.40%	soy flour (degreased)	
	0.20%	yeast extract (marcor typ 9000)	
	1.00%	starch (cerestar)	
	0.10%	$MgSO_4 \cdot 7H_2O$	
	50 mM	HEPES (11.9 g/l)	
	8 mg/l	Fe-EDTA	
	0.50%	glycerol (87% w/v)	
H-medium	0.20%	soy flour (degreased)	pH 7.4
	0.20%	glucose monohydrat	
	0.80%	starch (cerestar)	
	0.20%	yeast extract (marcor typ 9000)	
	0.10%	$CaCl_2 \cdot 2H_2O$	
	0.10%	$MgSO_4 \cdot 7H_2O$	
	50 mM	HEPES (11.9 g/l)	
	8 mg/l	Fe-EDTA	

Supplemenatry 2. Continued

Screening medium		Composition	Notes
M-medium	1.00%	peptone (soy)	pH 7.2
	1.00%	maltose monohydrat	
	0.10%	$CaCl_2 \cdot 2H_2O$	
	0.10%	$MgSO_4 \cdot 7H_2O$	
	50 mM	HEPES (11.9 g/l)	
	8 mg/l	Fe-EDTA	
POL-medium	0.30%	Probion	pH 7.2
	0.30%	Starch (cerestar)	
	0.05%	$CaCl_2 \cdot 2H_2O$	
	0.20%	$MgSO_4 \cdot 7H_2O$	
	50 mM	HEPES (11.9 g/l)	
P-medium	0.20%	peptone (marcor M)	pH 7.5
	0.80%	starch (cerestar)	
	0.40%	Probion	
	0.20%	yeast extract (marcor typ 9000)	
	0.10%	$CaCl_2 \cdot 2H_2O$	
	0.10%	$MgSO_4 \cdot 7H_2O$	
	50 mM	HEPES (11.9 g/l)	
	8 mg/l	Fe-EDTA	
S-medium	0.40%	soy flour (degreased)	pH 7.4
	0.20%	glucose monohydrat	
	0.80%	starch (cerestar)	
	0.10%	$CaCl_2 \cdot 2H_2O$	
	0.10%	$MgSO_4 \cdot 7H_2O$	
	50 mM	HEPES (11.9 g/l)	
	8 mg/l	Fe-EDTA	
Mxx-medium	1.00%	Casein peptone	pH 7.0
	0.01%	$CaCl_2$ x $2H_2O$	
	0.03%	$MgSO_4$ x $7H_2O$	
	1 mg/l	$CoCl_2$	
	100 mM	HEPES (11.9 g/l)	
Modified MA medium	5%	peptone (marcor M)	pH 7.0
	1%	yeast extract (Difco)	
	9%	$MgSO_4 \cdot 7H_2O$	
	4%	$NaSO_4$	
	2%	$CaCl_2$	
SAP2 medium	1%	Tryptone	
	1%	yeast extract (Difco)	
VY/2 modified medium	5%	Baker's yeast*	
RL1 medium	3%	peptone (marcor M)	
	2%	yeast extract (Difco)	
	0.5%	KNO3	
	0.5 mg/l	vitamin B12	is sterilized by filtration
SK medium	5 g	Skim milk	
	3 g	yeast extract (Difco)	
	1 g	$MgSO_4$	