

## Bioactivity Profiles of Actinobacterium Strain BTA 1-131 (InaCC A1205) Isolated from Indonesian Sponge *Melophlus sarassinorum*

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### ABSTRACT

The phylum Actinobacteria is recognized as the most promising producer of many bioactive compounds. Among Actinobacteria, the genus *Streptomyces* is a prolific producer of many antibiotics, for example, Streptomycin. The present study investigates the bioactivity profiles of Actinobacterium BTA 1-131 (A1205) isolated from an Indonesian marine sponge, *Melophlus sarassinorum*. Molecular identification using the 16S rRNA gene sequencing showed that the isolate is similar to *Streptomyces kunmingensis* (98.4%). The isolate was cultivated in three fermentation media (ISP2, YS, and SYP) to select the best media for producing bioactive compounds. The compounds were extracted from solid and liquid cultures using methanol as a solvent. The crude methanolic extracts were tested for antibacterial and anticancer activities. The bioactivity screening indicated potential activity of the extracts against bacterial pathogen *Staphylococcus aureus* ATCC 13420, invasive breast cancer MDA-MB-231 and human colorectal adenocarcinoma cells CACO-2. Profiling using LC-MS/MS indicated the probable influence of fermentation media to induce the selective production of bioactive compounds. Although further dereplication and fractionation are essential for the specific identification of bioactive compounds, the present study demonstrates the potential of *Streptomyces* BTA 1-131 as a producer of tetracycline and/or staurosporine. Further genomic analysis, such as whole genome sequencing, will also be beneficial to enable comprehensive exploration of the bioactive potential of BTA 1-131 via a genome-mining approach.

**Keywords:** Actinobacteria, Bioactivity, Bioprospecting, Indonesia, Marine Sponge

### INTRODUCTION

Indonesia has been acknowledged as the wealthiest biodiversity region in the world (Huffard *et al.*, 2012). Located in the World Coral Triangle area, Indonesian water also offers enormous marine biodiversity, a potential resource for further exploration, marine bioprospecting, and marine drug discovery and development. Among

all marine organisms, Indonesian sponges have been reported as the most prolific sources for bioprospecting of new marine-derived bioactive compounds (Mehubub *et al.*, 2014). A recent study reviewed the discovery of natural products derived from Indonesian marine invertebrates, including sponges and tunicates (Izzati *et al.*, 2021). Several natural products have been derived

from Indonesian sponges; for example, the isolation of manzamines A from the sponge *Acanthostrongylophora ingens* (Furusato *et al.*, 2014), and isolation of crambescidins from the sponge *Clathria bulbotaxa* (Kasmiasi *et al.*, 2018).

Marine invertebrates and sponges (phylum Porifera) are the most prolific source of new natural products with pharmaceutical potential (Blunt *et al.*, 2017). However, the exploitation of raw material from marine invertebrates for bioactive compound production will impact their conservation, and the over-exploitation of sponge materials from their natural habitat could still disturb their ecological balance (Leal *et al.*, 2018; Maslin *et al.*, 2021). It is therefore essential to have an alternative sustainable production of promising compounds, as exemplified by cultivating sponge-associated microorganisms (Santos-Gandelman *et al.*, 2014; Santhi *et al.*, 2017).

Many sponge-derived compounds are suspected to be synthesized by their associated microorganisms (Brinkmann *et al.*, 2017), suggesting the importance of the sponge-associated microorganisms as the critical target for discovering and developing novel marine-derived bioactive compounds (Santos-Gandelman *et al.*, 2014; Blunt *et al.*, 2017). The phylum Actinobacteria, especially of the genus *Streptomyces*, is the most prominent producers of many bioactive compounds with pharmaceutical importance, including antibacterial and anticancer (Subramani & Aalbersberg, 2012; Subramani & Sipkema, 2019).

There have been studies on sponge-associated microorganisms in Indonesia though overall, comprehensive studies still need to be expanded. There are reports on the potential of sponge-associated microorganisms from Indonesia and their potential as compound producers, such as alkaloids (Waters *et al.*, 2014), as well as flavonoids, steroids, and tannins (Prastya *et al.*, 2019). Additionally, studies reported the potential of sponge-associated bacteria from Indonesia as producers of antimicrobial (Dita *et al.*, 2017) and antimalarial compounds (Waters *et al.*, 2014).

The present study aims to explore the bioactivity profiles of *Actinobacterium* BTA1-131 isolated from an Indonesian marine sponge, *Melophlus sarassinorum*. Studies reported that *M. sarassinorum* from Indonesia produces melophlins (Aoki *et al.*, 2000; Wang *et al.*, 2003; Arai *et al.*, 2016), a bioactive compound that showed cytotoxicity against the murine leukemia cell

L1210 (Xu *et al.*, 2006). The sponge has also been reported to produce melophluosides, a bioactive compound that is cytotoxic against HeLa cells (Sadahiro *et al.*, 2020). However, to our knowledge, the present study is the first to report the bioactivity profiles of sponge-associated actinobacteria isolated from *M. sarassinorum* of Indonesian origin.

## MATERIAL AND METHODS

### Rejuvenation of six actinomycetes isolates from a glycerol stock.

Six actinomycetes were rejuvenated from glycerol stock, namely BTA1-131, BLH 4-5, BLH 5-36, BLH 9-2, BLH 14-2, and BLB 13-2(3). The isolates were first cultivated on a solid YS medium with 2% NaCl and incubated at 28°C for six days. The six days old isolates were then re-cultivated onto three solid growth media and incubated at 28°C for another six days for a preliminary screening using an agar plug method (Balouiri *et al.*, 2016). The three media were ISP2 (4g/L yeast extract, 10g/L malt extract, and 4g/L glucose), YS (2g/L yeast extract and 10g/L starch), and SYP (4g/L yeast extract, 10g/L starch, and 2g/L peptone) all prepared with seawater with the addition of 20g/L of bacto agar. All media components were obtained from Merck, USA, and bacto agar were obtained from HiMedia.

The morphology of isolates in three solid media was observed using a scanning electron microscope (SEM). The SEM analysis was conducted with scientific and technical support from Advanced Characterization Laboratories Yogyakarta, National Research and Innovation Agency through E- Layanan Sains (ELSA) BRIN.

### Molecular identification using the 16S rRNA gene sequencing

The BTA1-131 isolate was identified using the 16S rRNA gene amplification and sequencing following a published protocol (Atikana *et al.*, 2021). The forward and reverse sequences were assembled using the BioEdit V.7.2.6 software. A comparison of the contig sequence to the NCBI GenBank database via BLAST (Basic Local Analysis Search Tool) search confirmed the identity of the isolate. The 16S nucleotide sequence of isolate BTA1-131 has been deposited to the public database GenBank NCBI with accession number MT280129. The pure isolate BTA1-131 has been deposited in the Indonesian Culture Collection (InaCC), National Research and Innovation Agency (BRIN) with accession number InaCC 1205.

**Preliminary screening of antibacterial activity**

The preliminary antibacterial screening was conducted using the agar plug method (Balouiri *et al.*, 2016) against three bacterial strains, namely *Bacillus subtilis* BRIN Collection, *Staphylococcus aureus* ATCC 13420, and *Escherichia coli* ATCC 9637. Before the assay, the three bacterial strains were incubated at 37°C overnight in Nutrient Broth (NB, Merck). After overnight incubation, the OD (Optical Densitometry) of the three bacterial strains was measured and adjusted to an equal Colony Forming Unit, CFU ( $1 \times 10^6$  CFU/mL) for antibacterial activity screening. The adjusted bacterial inoculum was then inoculated on Nutrient Agar (NA; Merck), which was then poured onto the disposable Petri dish and cooled down at RT until solid. Agar cylinders (5mm diameter) of the six days old isolates were overlaid on top of agar plates containing each of the bacterial pathogens. The antibacterial activity/inhibition is indicated as an apparent clear zone diameter surrounding the agar pieces, measured after 24h of incubation.

**Extraction of bioactive compounds from liquid and solid cultures**

Following a published protocol, the potentially active compounds were extracted using methanol as an extraction solvent (Carr *et al.*, 2010). Bacterial seed culture was prepared by inoculating the BTA1-131 into solid and liquid media (ISP2, YS, and SYP) and incubated at 28°C for six days before the extractions. The six days old isolate on solid medium (A10, A11, and A12) was sliced into small square pieces (approximately 1 cm × 1 cm) and collected in a 250 mL beaker. The pieces were soaked with 100 mL methanol and incubated overnight with the beaker wrapped in parafilm. In parallel, the six days old liquid cultures of BTA1-131 in 20 mL broth (C1, C2, and C3) were mixed with an equal volume of methanol and incubated overnight. The methanolic extracts were transferred into a 100 mL beaker and the organic solvent was removed/evaporated using a rotary evaporator. The viscous extract was left under a freeze-dryer to remove the remaining water content. The dried methanolic extracts were weighed and stored at 4°C before further bioactivity experiments.

**Screening for antibacterial activity of methanolic extracts and measurement of minimum inhibitory concentration (MIC)**

The antibacterial screening of methanolic extracts was conducted using the Kirby-Bauer

disk diffusion assay (Balouiri *et al.*, 2016) using the same bacterial pathogens in the agar plug assay. The three bacterial strains were cultured on NB and incubated overnight at 37°C. After overnight incubation, the OD of each bacterial strain was adjusted to an equal CFU ( $1 \times 10^6$  CFU/mL) and inoculated into NA, which was then poured onto a disposable Petri dish and cooled until solid (Warsito *et al.*, 2022). Sterile filter paper discs were placed on top of the bacterial lawn and 200 ng/μl concentration of the crude extracts was impregnated on the disc before incubating the plates at 37°C. The antibacterial activity was indicated as an apparent clear zone diameter surrounding the discs after 24h. The positive controls (K+) were Kanamycin (15 ug) for *B. subtilis*, Vancomycin (20 ug) for *S. aureus*, and Ampicillin (5 ug) for *E. coli*.

**Screening for anticancer activity and measurements of half-maximal inhibitory concentration (IC50)**

The invasive breast cancer MDA-MB-231 (ECACC 92020424) and cell lines and the human colorectal adenocarcinoma cells CACO-2 (ECACC 86010202) were purchased from the European Collection of Authenticated Cell Cultures (ECACC). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) growth medium supplemented with 10% of fetal bovine serum (v/v) (Sigma Aldrich, USA), 1% of penicillin/streptomycin (v/v) (Sigma Aldrich, USA) and 0.5% of fungizone (Sigma Aldrich, USA). The cells were kept at 37°C in a humidified incubator with 5% CO<sub>2</sub> and passaged every 3-4 days.

An MTT (3-(4, 5-dimethyl thiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay was conducted to measure the cell viability. The cells were seeded in a 96-well plate at a  $1.0 \times 10^4$  cells/well density and incubated for 24h. Medium-containing extract diluted in DMSO (max 0.5%) was added to the wells at a final concentration of 100, 10, and 1 μg/mL. Media containing 0.5% DMSO (negative control) and doxorubicin (positive control) were also applied at final concentrations of 5 μM and 0.5%, respectively. After 48h incubation, the spent medium was discarded, and cells were washed once with 1x Dulbecco Phosphate Buffer Saline (DPBS) (Sigma Aldrich, USA). Then, 100 μl of medium containing MTT solution (50 μg/100 μl) (Sigma Aldrich, USA) was added to each well and followed by four hours incubation. Finally, 100 μl of 10% SDS in 0.01 N HCl was added to each well and incubated overnight in the dark. The

absorbance of each well was measured at 570 nm using Infinite® 200 PRO microplate reader (TECAN, Switzerland). The percentage of cell viability as an indicator of cell survival after treatment with extracts was calculated using the following equation:

Cell viability (%) =

$$\frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of control} - \text{Absorbance of blank}} \times 100$$

Where the absorbance of the sample corresponds to the absorbance of each well per extract, the absorbance of control represents the average absorbance of the readings for negative control (0.5% DMSO). The absorbance of blank corresponds to the average absorbance of medium only without cells. The IC<sub>50</sub> was calculated using the software GraphPad Prism (version 9.3.1).

### Metabolite profiling

The metabolite profiling of BTA1-131 methanolic extract was conducted using LC-MS/MS according to published protocols (Kim *et al.*, 2020). Exion LC (Liquid Chromatography) (SCIEX, Framingham, MA, USA) coupled to a quadruple time-of-flight X500R mass spectrometer (SCIEX, Framingham, MA, USA) was used for LC-MS/MS analysis. Kinetex C18 column (50 mm × 2.1 mm, 2.6 μm) (Phenomenex, Torrance, CA (Central Asia), USA) was used for compound separation. The mobile phases were composed of 0.1% (v/v) formic acid in ultrapure water (A) and acetonitrile (B). Gradient elution was set at 30% solvent B at the start and linearly increased to 100% solvent B for 15 min with a flow rate of 0.2 mL/min.

The electrospray ionization (ESI) source of the MS (Mass Spectrometry) and MS/MS was set in positive mode and operated as follows: ion-spray voltage 5500 V; curtain gas 30 psi; ion source gas 1 50 psi; ion source gas 2 50 psi; declustering potential 80 V; temperature 550°C; collision energy 35 ± 15 V. The range of mass detected was from m/z 100~1500 Da for QTOF MS and m/z 50~1500 Da for MS/MS. The MS/MS spectra were obtained by the information-dependent acquisition (IDA). The parameters for the MS and MS/MS spectra in negative mode are nearly the same except for IonSpray voltage -4500 V; declustering potential -80 V; collision energy -35 ± 15 V. The acquired MS and MS/MS data were analyzed by SCIEX OS 1.6.1 software.

## RESULT AND DISCUSSION

The present study investigates the potential of actinobacterial isolates from Indonesian environments. Preliminary antibacterial activity testing using the agar plug assay was performed on the six actinobacteria, namely BTA1-131, BLH 4-5, BLH 5-36, BLH 9-2, BLH 14-2, and BLB 13-2(3) that have been grown on three solid media (ISP2, YS, SYP) against *B. subtilis*, *S. aureus*, and *E. coli*. Despite the differences in growth media, the preliminary screening showed three isolates (BLH 4-5, BLH 14-2, and BTA1-131) were consistently found to inhibit the growth of *S. aureus*. In addition, isolate BLH 4-5 also showed active inhibition against the growth of *B. subtilis*. However, no isolates among the six actinobacteria had activity against *E. coli*. The isolate BTA1-131 (InaCC A1205) showed the most promising candidate as producer of active compounds, thus this study progressed to focus on the potential of this isolate to produce compounds with antibacterial and anticancer activities.

There were no observed morphological differences between BTA1-131 grown in different fermentation media (Figure 1). Molecular identification using the 16S rRNA gene showed 98.4% similarity of BTA1-131 to *Streptomyces kunmingensis* strain NBRC 14463. The low similarity may indicate a possibility of this isolate as being a new species within the genus *Streptomyces*. An analysis of the whole genome sequencing of *Streptomyces* BTA1-131 is ongoing to confirm its novelty and its active metabolite-producing potential via the genome-mining approach.

Preliminary screening of BTA1-131 in agar plug assay showed potential growth inhibition of *B. subtilis* and *S. aureus*. Antibacterial screening of the BTA1-131 methanolic extracts (concentration 200ng/μL) against both *B. subtilis* and *S. aureus* confirmed its antibacterial activity, with the exception of BTA1-131 extract obtained from cultivation in SY liquid (Table I). Broth microdilution assay of the extracts further confirmed the antibacterial activity against *S. aureus* and *B. subtilis* with MICs of 100-300 ppm. However, there were no activity observed in any of the extracts against *E. coli*.

The present study also investigated the anticancer activity of BTA1-131 methanolic extracts against invasive breast cancer MDA-MB-231 and the human colorectal adenocarcinoma cells CACO-2. The anticancer screening indicated the bioactive potential of the methanolic extracts

against both the cell lines MDA-MB-231 and CACO-2. Due to limited samples, only  $IC_{50}$  of extracts from solid cultures were measured against the CACO-2 cells and the MDA-MB231. The lowest  $IC_{50}$  was observed in the BTA1-131 methanolic extracts derived from cultivation in a solid YS medium (Table II).

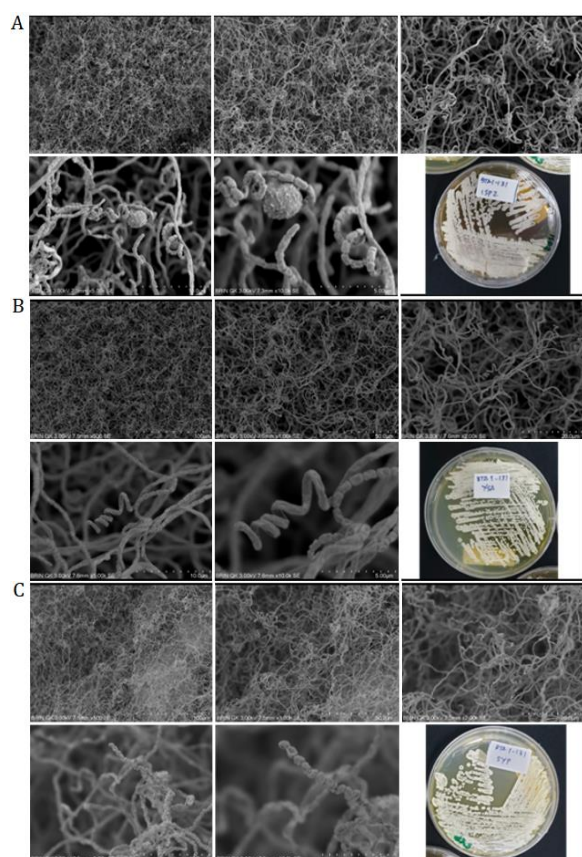


Figure 1. SEM images (1x to 10.000x) of BTA1-131 (InaCC A1205) morphology when grown on solid (a) ISP2, (b) YS, and (c) SYP media for six days.

The present study is the first to report the antimicrobial and anticancer activity of a bacterium isolated from the Indonesian marine sponge *M. sarassinorum*. Considering the reputation of marine actinomycetes as prolific producers of bioactive compounds, it is promising to look into the potential of BTA1-131 in producing compounds with biological activities. Other studies have reported several bacteria isolated from Indonesian sponges with demonstrated potential as bioactive compound producers. A bacterium isolated from the Indonesian sponge *Xestospongia testudinaria* (Cita *et al.*, 2016) and *Acanthella*

*cavernosa* (Murniasih *et al.*, 2016) showed potential antibacterial activity. Additionally, bacteria isolated from Indonesian sponge *Acanthostrongylophora ingens* showed a potential antimalarial activity (Waters *et al.* 2014) and bacteria isolated from sponge *Callyspongia aurizusa* demonstrated potential anti mycobacterium activity (Murniasih *et al.*, 2020). It is therefore likely that BTA1-131 isolated from *M. sarassinorum* of Indonesian origin would have similar potential to produce bioactive compounds with antibacterial and anticancer activity, as has been demonstrated in this study.

Molecular identification of BTA1-131 showed a 98.4% similarity to *Streptomyces kunmingensis* in the NCBI database. *S. kunmingensis* is a spore-forming bacteria that was first isolated from soil. *S. kunmingensis* has been reported to have the potential to produce anticancer compounds, endophenazine A and 5,6-dihydrouracil. Both compounds exhibited cytotoxicity against human breast adenocarcinoma cell line MCF-7 with  $IC_{50}$  values of  $20.23 \pm 1.37$  and  $28.98 \pm 1.58$   $\mu\text{g}/\text{mL}$ , respectively (Wei *et al.*, 2017). Further comparative genomics of the BTA1-131 to a type strain, for example, comparison with the genome of *S. kunmingensis* ATCC 35682 or NBRC 14463, can be implemented to confirm the species and the novelty of the actinobacterium strain BTA1-131.

Profiling of the potential compounds produced by the isolate BTA1-131 in three fermentation media was performed by LC-MS/MS analysis (Figure 2). The LC/MS profile of BTA1-131 methanolic extracts showed a relatively similar profile between the three media: ISP2 (ID.21836-1-BTA1), YS (ID.21836-2-BTA2), and SYP (ID.21836-3-BTA3). Gentibiose and lauryl diethanolamine were some of the known compounds detected in all the BTA1-131 methanolic extracts (Table III). However, due to the limitation of the database used in this study, many peaks were still considered unknown compounds (Table 3). On the other hand, several specific compounds were only observed in certain fermentation media; for example, tetracycline was only observed in the extracts from ISP2 medium, bensulide was only observed in the extracts from YS medium, and staurosporine was only observed in the extracts of SYP medium (Table III). The results indicated a probable influence of the fermentation medium in the ability to produce certain compounds, thus potentially influencing its bioactivity.

Table I. Antibacterial activity of the crude methanol extracts of BTA1-131 (concentrations of 200 ng/ $\mu$ L). The diameter of inhibition was measured including the diameter of the disc.

Sample code	Fermentation Media	Average diameter of inhibition (mm)		
		<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>
		ATCC 6051	ATCC 13420	ATCC 9637
A10	ISP2 solid	16.1 $\pm$ 0.6	12.1 $\pm$ 0.5	-
A11	YS solid	18.3 $\pm$ 0.7	13.7 $\pm$ 0.2	-
A12	SYP solid	18.8 $\pm$ 0.3	14.4 $\pm$ 0.1	-
C1	ISP2 liquid	15.2 $\pm$ 0.1	11.1 $\pm$ 0.9	-
C2	YS liquid	18.8 $\pm$ 1.2	12.9 $\pm$ 0.1	-
C3	SYP liquid	8.5 $\pm$ 0.3	-	-
K+	-	11.9 $\pm$ 0.7	12.8 $\pm$ 0.2	8.7 $\pm$ 0.2

Table II. Anticancer activity of the crude methanol extracts of BTA1-131 against the invasive breast cancer MDA-MB-231 and the human colorectal adenocarcinoma cells CACO-2

Sample code	Fermentation Media	IC <sub>50</sub> ( $\mu$ g/mL)			
		MDA-MB231		CACO-2	
		R2	IC <sub>50</sub>	R2	IC <sub>50</sub>
A10	ISP2 solid	0.92	11.9	0.88	52.59
A11	YS solid	0.93	10	0.98	36.86
A12	SYP solid	0.98	7.2	0.95	44.81

The LC-MS/MS analysis of BTA1-131 methanolic extracts in this study showed its potential as a producer of gentiobiose, lauryl diethanolamine, tetracycline, bensulide, and staurosporine. Gentiobiose, a rare disaccharide, is a product of glucose caramelization (Sugisawa & Hiroshi, 1966) that serves as a growth substrate and is a constituent of the cell wall and deoxyribonucleic acid in microorganisms (NCATS, 2022). Lauryldiethanolamine is a surfactant used for antistatic agents and ingredients for cosmetics, such as shampoo and hair conditioning (Wang *et al.*, 2013). Tetracycline is a relatively cheap antibiotic commonly used against both Gram-positive and Gram-negative bacteria and to treat chlamydia, mycoplasma, and rickettsia (Chopra & Roberts, 2001). Bensulide is a pesticide/herbicide commonly used to control weeds in agriculture and vegetable crops (Antonius, 2009). Staurosporine is a bioactive compound with the potential as an antifungal, antibacterial, and immunosuppressant and was also isolated from *Streptomyces staurosporeus* (Park *et al.*, 2013).

Our study provided further evidence of the antibacterial and anticancer activity of BTA1-131 methanolic extracts. This study also demonstrated that cultivation in three different fermentation media could yield varying compounds, highlighting

the potential of sponge-associated actinobacterium from Indonesia to produce an array of different compounds having different bioactivity. Although no differences were observed in the overall bioactivity of BTA1-131, the LC-MS/MS profiling indicated that different fermentation media led to the production of different bioactive compounds, as exemplified by tetracycline production only from using ISP2 medium and staurosporine production in SYP medium. These results highlight the importance of selecting appropriate fermentation media to produce specific metabolite or bioactive compounds to target specific bioactivity.

The present study underlined the influence of different fermentation media on the production of actinobacterial bioactive compounds, which is one of many techniques that have been implemented to activate cryptic bacterial metabolic pathways. Our approach is similar to the one strain many compounds (OSMAC) approach, which highlights the potential of a single bacterial strain to produce different molecules under different environmental conditions (Romano *et al.*, 2018). Modifying cultivation conditions may trigger the activation of the previously silent/cryptic gene clusters and may lead to new natural products being discovered (Pinedo-Rivilla *et al.*, 2022).

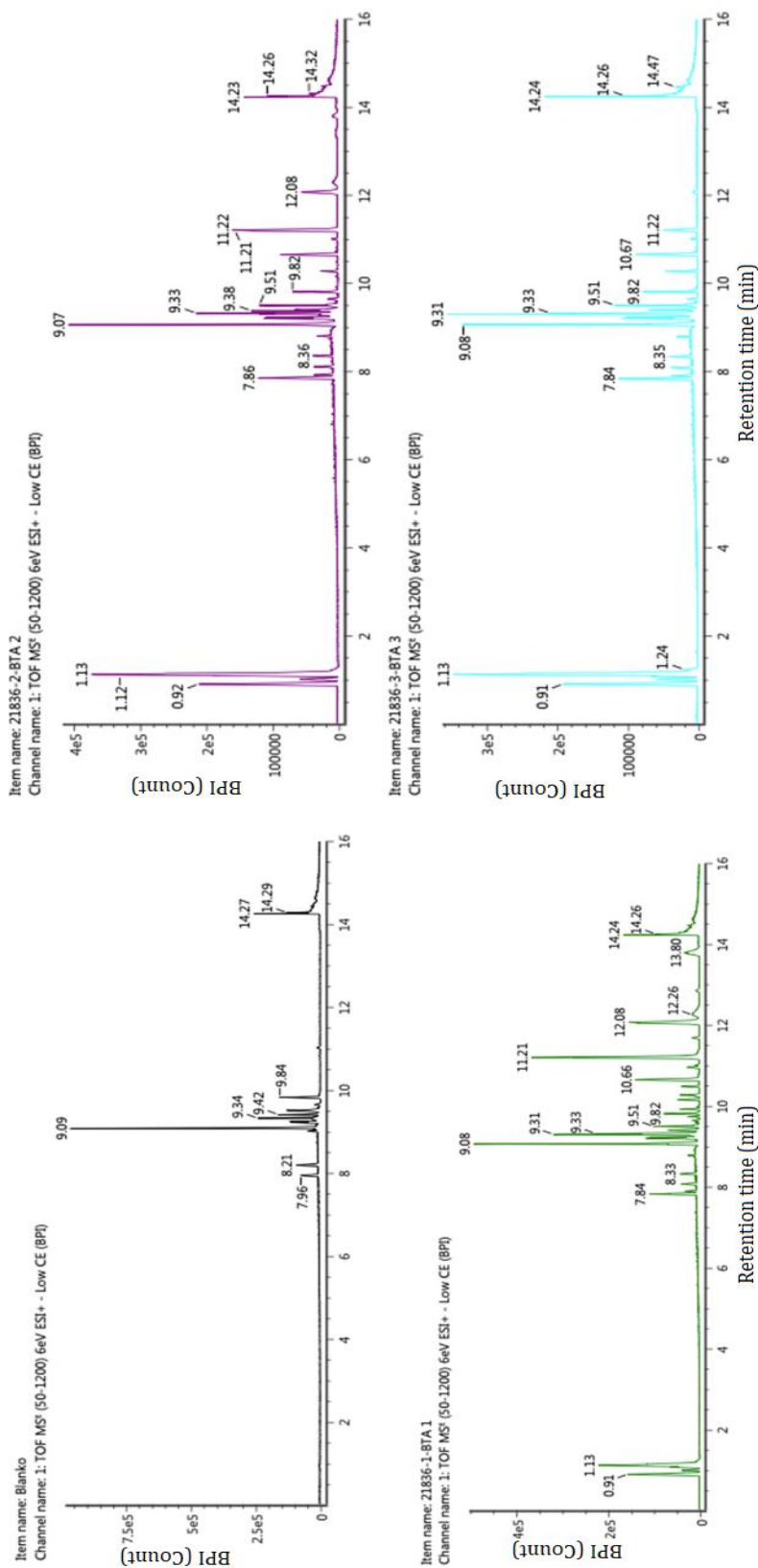


Figure 2. The LC-MS/MS profiling of BTA1-131 methanolic extracts, grown in ISP2 (ID.21836-1-BTA1, green), YS (ID.21836-2-BTA2, red) and SYP (ID.21836-3-BTA3, ocean blue), compared to methanol only as negative control (black).

Table III. The LC/MS profile of BTA1-131 methanolic extracts grown in three media (ISP2, YS, and SYP)

Retention time (min)	m/z (data)/reference	Neutral mass (Da)/reference	Chemical structure	Compounds	Activity (ref)	References
<b>BTA1-131-ISP2</b>						
1.14	365.1161/ 365.105103	342.11621/ 342.11622	$C_{12}H_{22}O_{11}$	Gentiobiose	glucose	Sugisawa & Hiroshi 1966
7.84	274.2730/ 274.274	273.26678/ 273.2668	$C_{16}H_{35}NO_2$	Lauryldiethanolamine	surfactant	Wang <i>et al.</i> 2013
10.29	467.1019;	444.11152/ 444.1533	$C_{22}H_{24}N_2O_8$	Tetracycline	antibiotic	Chopra & Roberts 2001
11.22	610.1847	609.17791	Candidate Mass $C_{20}H_{15}N_{23}O_2$	unknown		
12.08	684.2051	683.19808	candidate mass $C_{16}H_{13}N_{33}O$	unknown		
9.31	628.3239	627.31742	Candidate mass $C_{19}H_{37}N_{19}O_6$	unknown		
<b>BTA1-131-YS</b>						
1.13	365.1044	342.11621	$C_{12}H_{22}O_{11}$	Gentiobiose	glucose	Sugisawa & Hiroshi 1966
7.87	274.2732	273.26678	$C_{16}H_{35}NO_2$	Lauryldiethanolamine	surfactant	Wang <i>et al.</i> 2013
11.22	610.1852	609.17791	Candidate Mass $C_{20}H_{15}N_{23}O_2$	unknown		
9.31	628.3236	627.31742	Candidate mass $C_{19}H_{37}N_{19}O_6$	unknown		
9.38	398.1052/ 398.0678	397.09687/ 397.0605	$C_{14}H_{24}NO_4PS_3$	bensulide	herbicide	Antonius 2009
12.08	684.2038	683.19808	candidate mass $C_{16}H_{13}N_{33}O$	unknown		
<b>BTA1-131-SYP</b>						
1.14	365.1049	342.11621	$C_{12}H_{22}O_{11}$	Gentiobiose	glucose	Sugisawa & Hiroshi 1966
7.85	274.2730	273.26678	$C_{16}H_{35}NO_2$	Lauryldiethanolamine	surfactant	Wang <i>et al.</i> 2013
7.92	318.2994	317.29299				
10.28	467.1021	466.09587/ 466	$C_{28}H_{26}N_4O_3$	Alkaloid AM-2282, stauorosporine	antibiotic, anticancer, antifungal	Park <i>et al.</i> 2013
9.31	628.3234	627.31742	Candidate mass $C_{19}H_{37}N_{19}O_6$	unknown		
11.22	610.1855	609.17791	Candidate Mass $C_{20}H_{15}N_{23}O_2$	unknown		

Different bioactive compounds observed in the LC-MS/MS profiling of the present study confirmed the importance of fermentation media selection in producing bioactive compounds with a particular target activity.

Studies on *M. sarassinorum* from Indonesia indicated its ability to produce bioactive compounds. Examples of which are melophlins that showed cytotoxicity against the murine leukemia cell L1210 (Aoki *et al.*, 2000; Wang *et al.*, 2003; Xu *et al.*, 2006; Arai *et al.*, 2016) and melophluosides that showed cytotoxicity against HeLa cells (Sadahiro *et al.*, 2020). In addition, a multi-omic

profiling study of *M. sarassinorum* from Guam showed that the sponge also produces the bioactive compound sarasinosides (Mohanty *et al.*, 2020). However, these compounds were currently not detected in the metabolite profiles of BTA1-131 using LC-MS/MS, probably due to the untargeted liquid chromatography approach. A more targeted method, for example, High-Performance Liquid Chromatography (HPLC) using standards of melophlins, melophuositides, and sarasinosides, can be applied in the future to confirm whether these compounds were also detected in their sponge-associated microorganisms.



## CONCLUSION

The present study has shown preliminary evidence of the bioactivity of BTA1-131 (InaCC A1205) isolated from an Indonesian sponge, *M. sarassinorum*. Methanolic extracts obtained from the solid and the liquid cultures in all three media showed antibacterial activity against *B. subtilis* and *S. aureus*, as well as anticancer activity against the invasive breast cancer cell lines MDA-MB-231 and the human colorectal adenocarcinoma cells CACO-2. LC-MS/MS analysis showed differential metabolite profiles that highlighted the essentiality of fermentation media selection in inducing the production of specific bioactive compounds.

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