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Bioprospecting secondary metabolites from *Streptomyces* sp. HPM-9-01/B9-A from South Kalimantan as a potential antibiotic candidate

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ABSTRAK

Aktinobakteri dari marga Streptomyces *secara luas dikenal sebagai salah satu reservoir utama antibiotik. Bakteri ini banyak ditemukan di berbagai lingkungan, termasuk tanah, perairan tawar, dan ekosistem laut. Dalam penelitian ini, dilakukan isolasi, karakterisasi, serta pendekatan analisis meliputi teknik morfologi, genomik, dan metabolomik untuk menyelidiki potensi isolat HPM-9-01/B9-A, yang diperoleh dari hutan pinus di Mentaos, Kalimantan Selatan, sebagai kandidat penghasil antibiotik. Karakterisasi morfologi yang dikombinasikan dengan sekuensing 16S rRNA mengidentifikasi* strain *tersebut sebagai* Streptomyces albulus*. Uji antibakteri menunjukkan aktivitas penghambatan terhadap lima bakteri patogen, dengan zona hambat kategori kuat sebesar 16,75 ± 2,50 mm terhadap* Salmonella typhimurium*. Analisis LC-MS, yang dilanjutkan dengan identifikasi metabolit sekunder menggunakan MassLynx dan ChemSpider, mengungkap tiga senyawa utama: $C_{10}H_{16}N_7O_2$, $C_{12}H_{18}NO_3$, dan $C_{17}H_{21}N_4O_5$. Senyawa-senyawa ini diprediksi memiliki potensi antibakteri sekaligus aktivitas neuroaktif. Secara keseluruhan, temuan ini menyoroti potensi menjanjikan isolat* Streptomyces *sp. HPM-9-01/B9-A sebagai sumber senyawa antibiotik baru.*

Kata kunci: aktinobakteria, Streptomyces* sp.*, Kalimantan Selatan, kandidat antibiotik

ABSTRACT

Actinobacteria of the genus *Streptomyces* are widely recognised as a major reservoir of antibiotics. These bacteria occur abundantly in diverse environments, including soil, freshwater, and marine ecosystems. In this study, we employed isolation, characterisation, and analytical approaches encompassing morphological, genomic, and metabolomic techniques to investigate the potential of isolate HPM-9-01/B9-A, obtained from a pine forest in Mentaos, South Kalimantan, as a candidate for antibiotic production. Morphological characterisation combined with 16S rRNA sequencing identified the strain as *Streptomyces albulus*. Antibacterial assays demonstrated inhibitory activity against five pathogenic bacteria, with a strong inhibition zone of 16.75 ± 2.50 mm observed for *Salmonella typhimurium*. LC-MS analysis, supported by secondary metabolite identification using MassLynx and ChemSpider, revealed three major compounds: $C_{10}H_{16}N_7O_2$, $C_{12}H_{18}NO_3$, and $C_{17}H_{21}N_4O_5$. These metabolites are predicted to possess antibacterial and neuroactive properties. Collectively, these findings highlight the promising potential of *Streptomyces* sp. isolate HPM-9-01/B9-A as a source of novel antibiotic compounds.

Key Words: actinobacteria, *Streptomyces* sp., South Kalimantan, antibiotic candidate

INTRODUCTION

The prevalence of antibiotic resistance represents a grave health concern, both domestically in Indonesia and on a global scale (Aslam, 2018). This situation is gaining attention due to the increase in the number of deaths each year, both in developed and developing countries. The ineffectiveness of antibiotics prescribed by medical practitioners can be attributed to a number of factors. These include the misuse of antibiotics not in accordance with prescription, self-purchasing without the requisite prescription, and the increasingly widespread use of various types of antibiotics in farming and aquaculture. Actinobacteria, notably *Streptomyces* sp., constitute the predominant source of antibiotics, a fact that has persisted since their initial identification in 1928 (Simeis, 2021; Mast, 2019). This type of bacteria has been found to be abundant in various ecosystems, including soil, particularly in alkaline soil (Barka, 2015), and in water, both fresh and salt water. Given the significant potential for identifying antibiotic candidates from these bacteria, further exploration of the secondary metabolite profile that leads to the production pathway of new types of antibiotics is imperative. Despite the prevalence of Actinobacteria in the current antibiotics landscape, there is a paucity of research focusing on this bacterial type.

An exploration of Actinobacteria from soil samples from South Kalimantan was able to isolate a variety of Actinobacteria isolates such as *Kitasatospora* sp. (Ariyanti et al., 2026) and *Streptomyces* sp. which was widely known as Actinobacteria species potential for production of secondary metabolites for antibiotics. Thus, in this study, a series of isolation, characterisation and analysis including morphology, genomic, and metabolomic approach will be conducted to explore the potential of Actinobacteria isolate of HPM-9-01/B9-A for its candidate antibiotic producer.

METHODOLOGY

This study aims to characterise the Actinobacteria isolate which was obtained from a soil sample of pine forest in Mentaos, South Kalimantan, at species level using genomic approach, confirmed by 16S rRNA sequencing, following with antimicrobial test against pathogenic bacteria, and identification of potential secondary metabolites using metabolomic approach. The characterisation is aimed to know the potency of the isolate in producing bioactive compounds based on its genetic for antibiotic resource candidate. The stages of the research conducted are outlined as follows.

Sample collection

The sample of Actinobacteria isolate was obtained from soil, collected from pine forest, Mentaos, South Kalimantan, S 03º26.066’ E 114 º50.020’ (**FIGURE 1**). The sampling collection was conducted on April 24th-27th, 2024. The forest area has urban forest characteristics, wet soil condition with the surface covered by pine leaves and fruit litter, some areas covered with moss. In-situ observation was performed coverage soil profile (pH, temperature, humidity, and environment, surrounding the sample location).

Research procedures

Morphological characterisation of the isolate

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Morphological characterisation of the isolates was performed by observing the isolated colony, i.e colour, shape, mycelium type and pigment production. Gram staining following with observation under the microscope are performed to identified Gram type of bacteria (positive or negative), and microscopic profile of the isolate.

Genomic identification of the isolate

The 16S rRNA sequencing was carried out to identify the Actinobacteria isolate at the species level. DNA extraction, PCR amplification and 16S rRNA sequencing were performed using Oxford Nanophore Technology (ONT) platform, following Ariyanti et al. (2026).

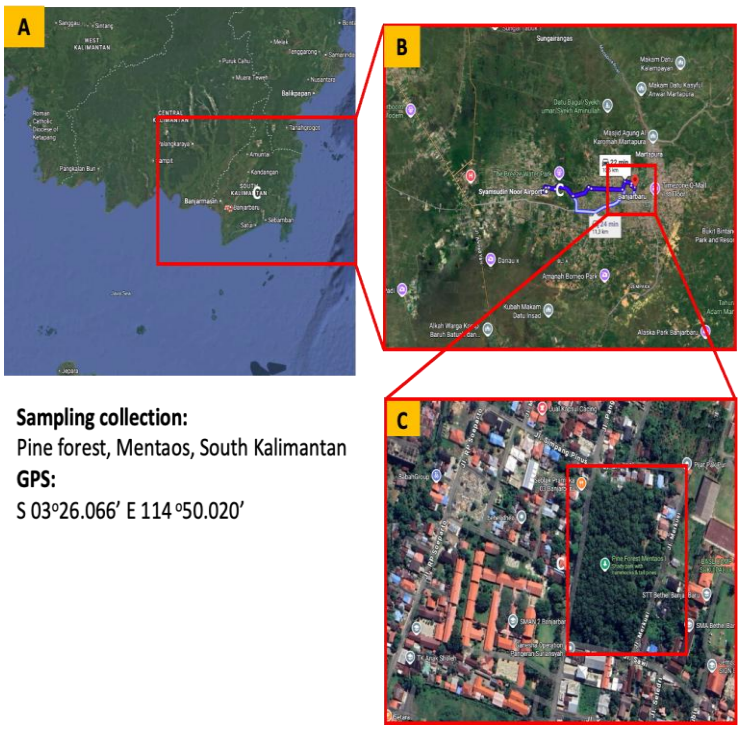

FIGURE 1. Map location and GPS information of soil sample collection of HPM-9-01/B9-A from pine forest, Mentaos, South Kalimantan.

Antimicrobial activity test against pathogens

Pure colonies of the isolates were tested for their antimicrobial ability against five pathogenic bacteria: *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus*, *Salmonella typhi*, and *Bacillus cereus,* using the diffusion method (Ardiansah et al., 2025). Antimicrobial activity represented by production of clear zones were measured based on the diameter to determine the inhibition zone (antimicrobial activity spectrum). The measurement results categorised into 4 clusters: very strong (>20 mm), strong (10-20 mm), middle (5-10 mm) or low (<5 mm), (Davis and Stout, 1971).

Identification of secondary metabolite compounds

The sample was prepared by inoculated the Actinobacteria isolate into 100 ml of ISP-2 medium supplemented with nystatin (50 µg/mL), ampicillin (25 µg/mL) and nalidixic acid (25 µg/mL), following with incubation in a room temperature (28°C) with agitation 180 rpm for 7 days. The culture was harvested by centrifugation at 5000 rpm for 10 minutes in temperature

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4°C. The supernatant was then filtered using a syringe filter NY 0.02 µm and wrapped. The supernatant was sent for LC-MS (Mangurana, et al., 2019). The chromatogram from LC-MS results were analysed using Masslynx software and the name of the compounds was confirmed using Chemspider (www.chemspider.com) (Lipinski et al., 2021).

Data analysis techniques

In this study, all data were analysed descriptively based on the designated type of analysis platform.

RESULTS AND DISCUSSION

Morphology confirmation of potential actinobacteria isolate

Actinobacteria isolate HPM-9-01/B9-A originated from soil samples of pine forest, Mentaos, South Kalimantan. Based on X-Ray Fluorescence (XRF) analysis, the mineral profile shows that the soils was dominated by mineral elements Cr (Chromium), Sb (Antimony), Zn (Zinc), and Sn (Tin), while other mineral elements are found in small amounts (**TABLE 1**).

TABLE 1. Results of soil mineral examination of soil sample collection of HPM-9-01/B9-A using XRF method.

Soil Mineral	Concentration
Cadmium (Cd)	N.D (<2.2 ppm)
Chromium (Cr)	455 ppm
Antimony (Sb)	34.5 ppm
Zinc (Zn)	6.9 ppm
Tin (Sn)	8.2 ppm

Morphology characterisation of isolate after grown at purification medium ISP-2 (www.Actinobase.org; Wink, 2024) shows that the colony of HPM-9-01/B9-A was coccus, white colour, convex elevation, forming aerial mycelium at with produce light brown to grey powdery substrate above the colony (**FIGURE 2B-C**). Meanwhile, Gram staining test shows final colour purple with the filament profile under microscope belong to Gram positive bacteria (**FIGURE 2A**).

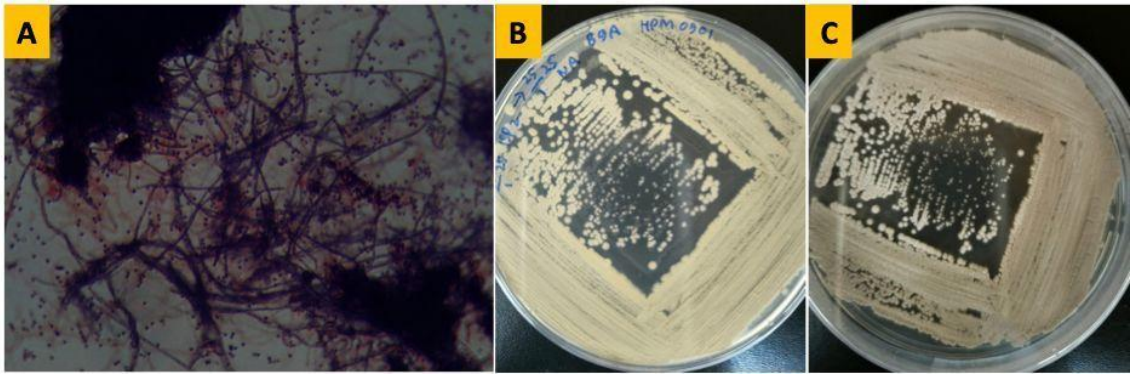

FIGURE 2. Morphology characterisation of *Actinobacteria* HPM-9-01/B9-A isolate from pine forest, Mentaos, South Kalimantan. (A) Gram staining test, showing the filaments with final colour purple, refers to gram positive bacteria; (B-C) showed morphology colony of the isolate.

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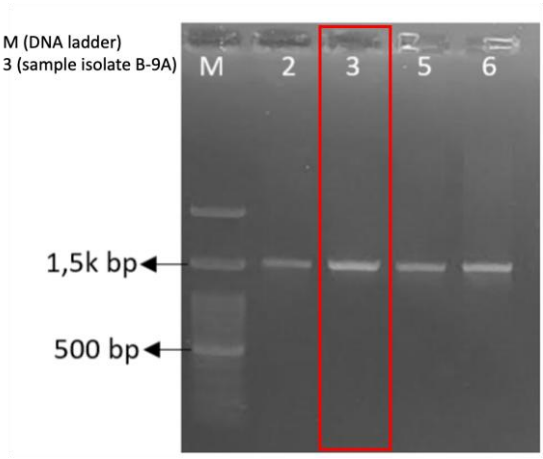

FIGURE 3. A 16S rRNA PCR amplification results following with sequencing analysis using ONT platform of *Actinobacteria* isolate HPM-9-01/B9-A from pine forest, Mentaos, South Kalimantan. Gel electrophoresis results confirm 1500 bp amplicon (sample no.3 showed in a box with red lines).

Confirmation using genomic approach by 16S rRNA PCR amplification showed a 1500 bp amplicon (**FIGURE 3**). A 16S rRNA sequencing using Oxford Nanophore Technology (ONT) platform following with BLASTn analysis (https://blast.ncbi.nlm.nih.gov) results to the confirmation of the isolate HPM-9-01/B9-A at species level belong to *Streptomyces albulus* NK660 (**SUPPLEMENTARY FILE 1**).

Antibacterial Activity Test Against Pathogenic Bacteria

Antibacterial activity tests against pathogenic bacteria were conducted using the disc diffusion method. Five pathogenic bacteria were used, *E. coli* (ATCC 25922), *S. typhimurium* (ATCC 14208), *Staphylococcus aureus*, *S. typhi*, and *B. cereus*. Negative control using distilled water and positive control using ampicillin (Suloi, 2022; Mahdalena, 2019; Hashary, 2021; Kumala, 2025). The observation results of the inhibition zone (clear zone) that appeared in the antibacterial activity test of *Streptomyces* sp. HPM-9-01/B9-A against the pathogenic bacteria tested are summarised in **TABLE 2** and **SUPPLEMENTARY FILE 2**.

TABLE 2 Results of inhibition zone observation in the antibacterial activity test of *Streptomyces* sp. HPM-9-01/B9-A isolate against five pathogenic bacteria.

No.	Pathogenic bacteria	Average of clear zone (mm)	Inhibitory zone category*
1	*Escherichia coli* (ATCC 25922)	0 ± 0	Not identified
2	*Salmonella* Typhimurium (ATCC 14028)	16.75 ± 2.50	Strong
3	*Staphylococcus aureus*	0 ± 0	Not identified
4	*Salmonella typhi*	3.75 ± 0.96	Weak
5	*Bacillus cereus*	4.25 ± 2.22	Weak
6	Positive control (Ampicillin)	28.25 ± 5.12	Strong
7	Negative control (aquadest)	0 ± 0	Not identified

*Source: Davis and Stout (1971)

Based on the results shown in **TABLE 2** above, the antibacterial activity of *Streptomyces* sp., HPM-9-01/B9-A isolates against five type of pathogenic bacteria showed strong inhibition against *Salmonella* Typhimurium (ATCC 14028) with average inhibition zone of 16.75 ± 2.5 mm. Meanwhile, weak inhibition shows against two pathogenic bacteria *Salmonella typhi* and *Bacillus cereus*, with the average inhibition 3.75 ± 0.96 mm and 4.25 ± 2.22, respectively. The

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clear inhibition zone formed by *Streptomyces* sp, HPM-9-01/B9-A isolates indicates significant antibacterial activity against *S. typhimurium*, but lower than the positive control (ampicillin), which is a broad-spectrum antibiotic. Inhibition zones >14 mm are generally considered to indicate moderate to strong activity according to several standard antibacterial test references (CLSI, 2019; Balouiri, 2016).

Profile of secondary metabolites (SMs) using Liquid Chromatography-Mass Spectrophotometry (LC-MS) analysis

Metabolomic assay approach using LC-MS methods was carried out to identify potential SMs produced by the *Streptomyces* sp, HPM-9-01/B9-A isolate. The chromatogram (**FIGURE 4**) shows 16 SMs candidates were captured, with 5 SMs identified using Masslynx following with structure and chemical identification using chemspider (www.chemspider.com) (**TABLE 3**).

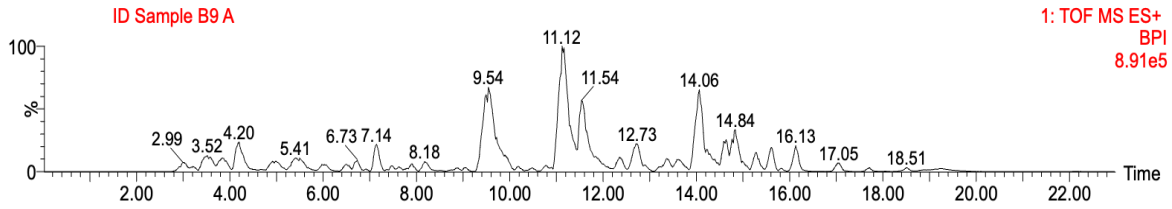

FIGURE 4. Chromatogram of the LC-MS results of HPM-9-01/B9-A isolate.

TABLE 3. Information of structure and chemical identification of SMs of *Streptomyces* sp. HPM-9-01/B9-A isolate using Chemspider (www.chemspider.com).

RT	% Area*	Formula*	Name of Compound**
3.02	1.32	$CH_{16}N_{21}O_{10}$	Not found
3.52	2.56	$CH_{14}N_{16}O_4SCl$	Not found
4.20	3.56	$C_4H_{14}N_7$	Not found
5.45	1.82	$C_8H_{16}NO$	(2,2,5,5-Tetramethyl-1-purrolidinyl)oxidanyl
6.71	1.24	$C_{11}H_{20}N_3O_2$	3-(2-Methyl-2-propanyl)-2,4-dioxo-1,3-diaza-5-azoniaspiro[4.5]decane
7.14	2.04	$CH_{16}N_{15}O$	Not found
8.20	0.74	C_3HN_{10}	Not found
9.54	16.38	$C_{12}H_{18}NO_3$	4-Hydroxybenzoylcholine
11.15	19.44	$C_{10}H_{16}N_7O_2$	1-[(4-Methyl-1,2,5-oxadiazol-3(2H)-ylidene)(nitroso)methyl]-3,5,7-triaza-1-azoniatricyclo[3.3.1.1~3,7~]decane
11.56	11.33	$C_{11}H_{24}N_{31}O_4$	Not found
12.35	0.93	$C_2H_{22}N_{17}O$	Not found
14.06	11.79	$C_{17}H_{21}N_4O_5$	3,7-Diacetyl-5-(4-nitrobenzoyl)-3,7-diaza-1-azoniabicyclo[3.3.1]nonane
14.82	8.10	$C_5H_{30}N_{17}$	Not found
16.13	2.12	$C_{29}H_{39}O_8$	Not found
17.03	0.86	$CH_{23}N_32O_2$	Not found
18.51	0.37	$C_{16}H_{50}N_{15}O$	Not found

*: analysis using Masslynx
**: analysis using Chemspider (www.chemspider.com)

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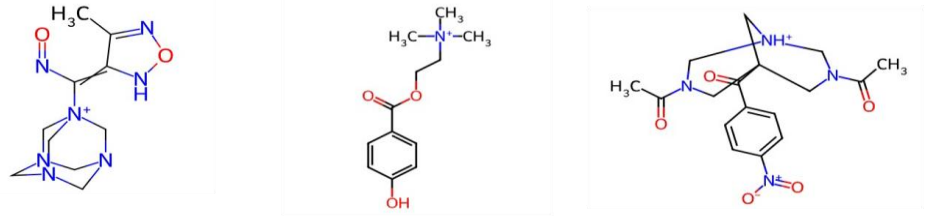

FIGURE 5. Chemical structure of top three potential secondary metabolites of *Streptomyces* sp. HPM-9-01/B9-A isolate with the highest % area. (A) $C_{10}H_{16}N_7O_2$ (1-[(4-Methyl-1,2,5-oxadiazol-3(2H)-ylidene)(nitroso)methyl]-3,5,7-triaza-1-azoniatricyclo[3.3.1.1~3,7~]decane); (B) $C_{12}H_{18}NO_3$ (4-Hydroxybenzoylcholine); and (C) $C_{17}H_{21}N_4O_5$ (3,7-Diacetyl-5-(4-nitrobenzoyl)-3,7-diaza-1-azoniabicyclo[3.3.1]nonane).

From this analysis, three compounds: $C_{10}H_{16}N_7O_2$, $C_{12}H_{18}NO_3$ (4-hydroxybenzoylcholine) and $C_{17}H_{21}N_4O_5$ (3,7-diacetyl-5-(4-nitrobenzoyl)-3,7-diaza-1-azoniabicyclo[3.3.1]nonane) was identified as the potential SMs with highest % area, with chemical structure described (**FIGURE 5**).

The 4-Hydroxybenzoylcholine has cholinesterase-inhibiting and antibacterial potential, with its activity likely contributing to the inhibition zone. Compound $C_{17}H_{21}N_4O_5$ (3,7-Diacetyl-5-(4-nitrobenzoyl)bicyclo(3.3.1)nonane) is projected to possess nitrobenzoyl group-mediated antibacterial and neuroactive potential, with activity presumed to be active against *S. typhimurium*. Both are correlated with the pathogenic activity of *S. typhimurium*. The structure of the first candidate compound, 4-Hydroxybenzoylcholine ($C_{12}H_{18}NO_3$), indicates that this compound is a choline ester with a benzoyl moiety containing an aromatic hydroxyl group. The combination of choline and phenolic groups indicates potential cholinergic activity or specific interactions with bacterial enzymes. Meanwhile, the structure of the second compound, 3,7-Diacetyl-5-(4-nitrobenzoyl)-3,7-diaza-1-azoniabicyclo(3.3.1)nonane, includes a bicyclo(3.3.1) nonane ($C_{17}H_{21}N_4O_5$), a relatively rare type of compound in natural metabolites, with two nitrogen atoms (diaza) and nitro-benzoyl substituents suggesting a possible antibacterial mechanism based on aromatic NO_2 groups. Thus, these structures not only support the LC-MS results and the 16.75 ± 2.50 mm zone of inhibition bioassay against *Salmonella typhimurium*, but also reinforce that isolate B9A originates from the genus *Streptomyces*, which is a producer of natural antibiotics, thereby holding potential for further development as a candidate for a new antibiotic.

Streptomyces isolates are known to be a major source of antibiotics, with more than 70% of clinically used antibiotics originating from this genus (Barka, 2015). Based on identification using a genomic approach with 16S rRNA sequencing, HPM-9-01/B9-A isolate shares 100% similarity with *Streptomyces* sp. *albulus* NK660 (BLASTn analysis results: https://blast.ncbi.nlm.nih.gov). According to Geng (2014), this isolate is known as a producer of ε-PL, a homopolyamino acid L-lysine, which has broad antimicrobial activity, including against various gram-positive and gram-negative bacteria. Given the genetic similarity of HPM-9-01/B9-A to *Streptomyces* sp. *albulus* NK660, there is a high probability that HPM-9-01/B9-A also possesses ε-PL biosynthetic genes, or at least the potential for similar production. Furthermore, the genome of *S. albulus* NK660 contains biosynthetic clusters that enable the production of secondary metabolites (Gu, 2014). Furthermore, metabolic and genetic

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manipulation of *S. albulus* has shown that regulatory changes can unlock/potentially enhance the expression of alternative metabolite clusters. For example, constitutive expression of genes in the DAP (diaminopimelate) pathway increases ε-PL production (Hasebe, 2023). Environmental conditions such as acidic pH, stress, and ATP supply (Wang, 2020) can influence the ε-PL production response and gene expression for other metabolites in *S. albulus* NK660, thus similar optimisation in isolate HPM-9-01/B9-A has the potential to increase its antibacterial activity or produce new metabolites. Thus, based on this genetic similarity, HPM-9-01/B9-A has high potential to produce ε-PL or other related secondary compounds. Future prospect of this research coverage a genomic approach (whole genome sequencing analysis of isolate following with genome mining for identification of biosynthetic gene clusters: PKS, NRPS, and others related to antibiotic), combine with metabolomic approach (optimisation of culture conditions for specific SMs-related antibiotic production) can be attributed to new potential of antibiotic candidates. Therefore, these findings indicate the important potential of the *Streptomyces* sp. isolate HPM-9-01/B9-A to be developed as a source of new antimicrobial compounds, especially against gram-negative bacteria such as *S.* Typhimurium, which often show resistance to conventional antibiotics.

CONCLUSIONS

An Actinobacteria isolate HPM-9-01/B9-A originated from pine forest Mentaos in South Kalimantan was successfully confirmed at species level as *Streptomyces albulus* NK660. This isolate showed antibacterial activity against two common pathogens, with the highest inhibition zone (16.75 ± 2.50 mm), categorised as strong inhibition zone against *S. typhimurium*. It has potential for antibiotics materials showed by the production of secondary metabolites identified: $C_{10}H_{16}N_7O_2$; $C_{12}H_{18}NO_3$; and $C_{17}H_{21}N_4O_5$ that is projected has antibacterial and neuroactive potential.

AUTHOR CONTRIBUTIONS

D.A., M.F., A.B.K.: project conception; D.A., M.F., A.B.K., M.H.: methodology, data analyses; D.A., M.F.: original manuscript draft; D.A., M.F., A.B.K., N.I., A.R.W.: manuscript review and editing.

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CONFLICTS OF INTEREST STATEMENT

There are no conflicts to declare.

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ETHICAL COMPLIANCE

This research involved the isolation and characterisation of *Streptomyces* sp. (Actinobacteria) from environmental samples. As the study did not include human participants, vertebrate animals, or sensitive data, ethical clearance was not required.

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SUPPLEMENTARY FILES

SUPPLEMENTARY FILES 1. 16S rRNA sequencing analysis using Oxford Nanophore Technology (ONT) platform.

>B9A-HPM0901
GCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCTCCTTCGGGAG
GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGG
AAACGGGGTCTAATACCGGATATGACACGGGGTCGCATGATCTCCGTGTGGAAAGCTCCGGCGGT
GAAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGGT
AGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGG
CCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAGAGTGACGGTACCTGCAGAAGAAGCG
CCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGG
CGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGC
ATTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGC
GCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGC
GAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAG
GTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTAC
GGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAGCGGAGCATGTGGCTTA
ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCGTGGAGACACGGT
CCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCTTTCGGGGTGATGGGGACTCA
CAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATG
TCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAAT
CTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTTGCTAG
TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTC
ACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAATCGTCGAAGGTGG
GACTGGCGATTGGGACGAAGT

SUPPLEMENTARY FILES 2. Figure of inhibition zone in the antibacterial activity test of HPM-9-01/B9-A isolate against five pathogenic bacteria: (1) *Escherichia coli* (ATCC 25922), (2) *Salmonella typhimurium* (ATCC 14028), (3) *Staphylococcus aureus*, (4), *Salmonella typhi*, and (5) *Bacillus cereus*.

1. HPM-9-01/B9-A isolate against *Escherichia coli* (ATCC 25922)

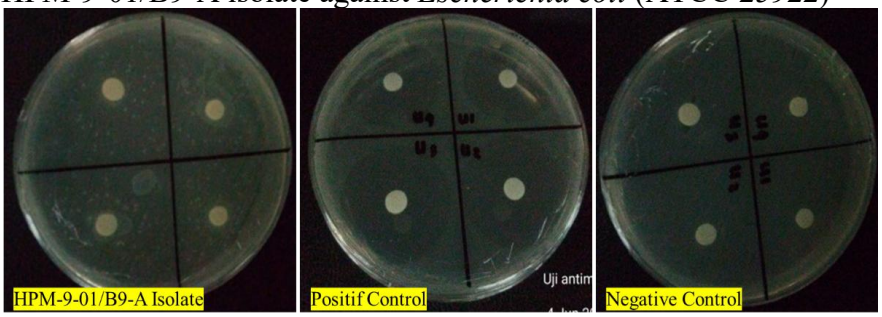

FIGURE S1. The HPM-9-01/B9-A isolate does not show an inhibition zone (left figure).

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2. HPM-9-01/B9-A isolate against *Salmonella* Typhimurium (ATCC 14028)

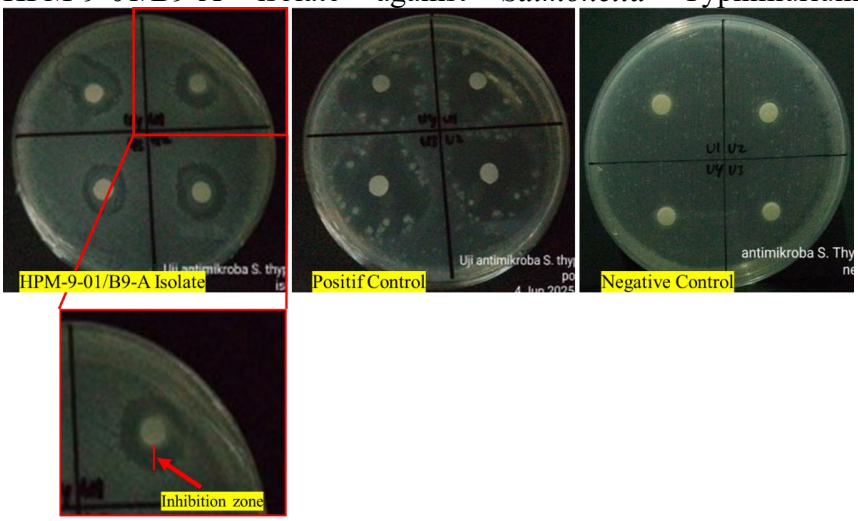

FIGURE S2. The HPM-9-01/B9-A isolate shows an inhibition zone categorised as 'strong' (left figure).

3. HPM-9-01/B9-A isolate against *Staphylococcus aureus*

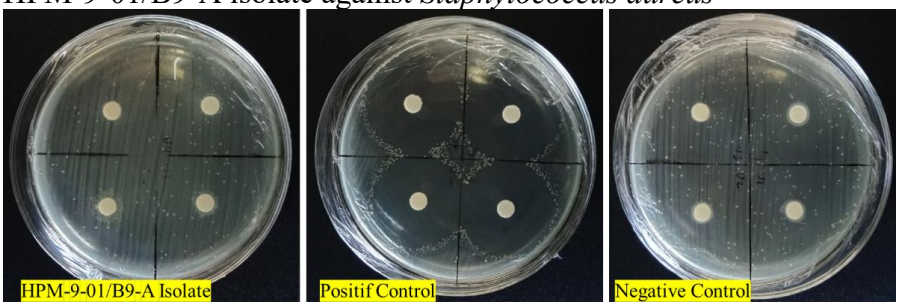

FIGURE S3. The HPM-9-01/B9-A isolate does not show an inhibition zone (left figure).

4. HPM-9-01/B9-A isolate against *Salmonella typhi*

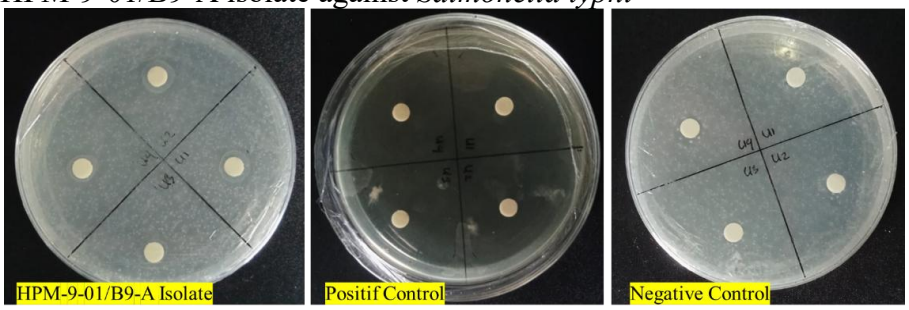

FIGURE S4. The HPM-9-01/B9-A isolate shows an inhibition zone categorised as 'weak' (left figure).

5. HPM-9-01/B9-A isolate against *Bacillus cereus*

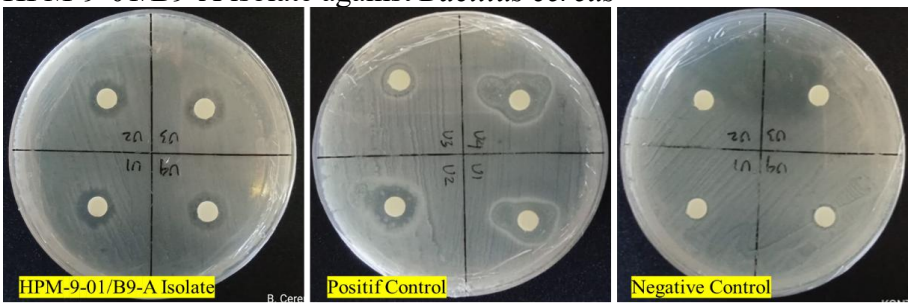

FIGURE S5. The HPM-9-01/B9-A isolate shows an inhibition zone categorised as 'weak' (left figure).

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