The Marine Actinobacterium *Streptomyces* sp. BTA 1-131 as a Potential Producer of Anti-Nontuberculous Mycobacterial (Anti-NTM)

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ABSTRACT

Nontuberculous mycobacteria (NTM) are environmental microorganisms, also known as opportunistic pathogens, found in patients with pulmonary tuberculosis. The emergence of antibiotic resistance is increased by prolonged antibiotic treatment for NTM infections. Therefore, alternative sources of new antibiotics are essential for the treatment of NTM infections. A marine actinobacterium, *Streptomyces* sp. BTA 1-131, isolated from a marine sponge, *Melophlus sarasinorum*, has been reported as a potential source of antibacterials and anticancer agents. The present study aimed to investigate the potential of *Streptomyces* sp. BTA 1-131 against two NTMs: *Mycobacterium smegmatis* and *Mycobacterium fortuitum*. *Streptomyces* sp. BTA 1-131 was fermented in three cultivation media (SYP, ISP2, and YS), and the secondary metabolites were extracted using methanol. The bioactivity screening showed inhibition of all methanolic extracts against the growth of *M. smegmatis* and *M. fortuitum*. The methanolic extract, which could inhibit both mycobacteria, was a crude extract derived from SYP liquid medium fermentation. The isolated compounds in this study were preliminarily identified using thin-layer chromatography (TLC). The TLC results showed different potential compounds in the crude extracts of *Streptomyces* sp. BTA 1-131 also highlighted the impact of the fermentation medium on the production of metabolites from *Streptomyces*. This study also added knowledge about the importance of the Indonesian marine actinobacterium *Streptomyces* sp. BTA 1-131 as a promising producer of anti-NTM compounds.
Keywords: Marine, Nontuberculous mycobacteria, Antimycobacterial, Antibiotic.

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Introduction

Nontuberculous mycobacteria (NTMs) are considered mycobacteria other than *Mycobacterium tuberculosis* and *Mycobacterium leprae*. The NTMs are environmental microorganisms found in water, soil, and dust but have emerged as human pathogens in recent years. There are more than 200 different types of NTM microorganisms discovered, which are divided into two groups based on their growth: rapidly-growing mycobacteria (RGM) and slowly-growing mycobacteria (SGM) (Tarashi et al., 2022). The NTMs are called opportunistic pathogens because they have been reported to cause infection in patients with immunocompromised, skin, and soft tissue infections (SSTI), and pre-existing pulmonary diseases such as tuberculosis (TB), cystic fibrosis (CF), bronchiectasis, chronic obstructive pulmonary disease (PD), and non-CF lung cancer (Wu et al., 2018; Ratnatunga et al., 2020; Wang et al., 2022).

Numerous cases of NTM outbreaks have been reported in various healthcare facilities, and the prevalence of NTM infections is increasing worldwide. Since 2013, a global outbreak of *Mycobacterium chimaera* infections, one of the SGM groups, has also been reported in cardiac surgery patients and immunocompromised (Hasse et al., 2020; Schreiber et al., 2021). In another study, 527 of 933 (56%) patients with pulmonary and extrapulmonary diseases met the microbiological criteria for NTM disease established by the American Thoracic Society and the Infectious Diseases Society of America. The number of NTM isolates that infected each patient exceeded three types, with *Mycobacterium avium* complex being the most predominant in pulmonary disorders and RGM being the most common for extrapulmonary diseases (Cassidy et al., 2009). Saptawati et al. (2022) indicated *Mycobacterium fortuitum* as the most common RGM isolates (51%) among 94 NTM isolates of patient sputum in three TB referral centers in Java, Indonesia. In addition to respiratory infections, *M. fortuitum* has also been observed in intravascular infections, surgical infections, and pseudoinfections (Desai et al., 2018). The other type of RGM, *Mycobacterium smegmatis*, has been reported in cosmetic surgical procedures. Patients with persistent SSTI following cosmetic surgery should be made aware of the possibility of *M. smegmatis* infection by medical professionals (Sparks et al., 2023).

Clinicians faced numerous challenges while diagnosing and treating nosocomial NTM, one of them being the therapy of antimycobacterial combination in therapeutic management. Healthcare providers found it challenging to identify and manage *M. smegmatis* infections in patients because the disease is uncommon in standard clinical practice. In patients with pre-existing lung disease, infection with NTM causes mostly TB-like PD. According to research in Germany, the mortality rate for NTM-PD was 22.4%, with expenditures four times higher (Diel et al., 2017). Effective therapy for NTM-PD patients is complex due to various factors such as high cost, time consumption, low toxicity, and high resistance levels. Antibiotic resistance can be caused by prolonged antibiotic treatment and inappropriate treatment for NTM infections due to an inaccurate diagnosis of the disease. Intrinsic NTM characteristics such as impermeable cell walls, thickness, and granuloma formation may reduce antimicrobial influx and protein production that targets antibiotics, thus leading to antibiotic resistance (Tarashi et al., 2022). Current antibiotics have limited capacity to treat NTM infections, especially those resistant to certain antibiotics. Therefore, exploring potential new/novel sources is essential, especially to target the NTMs. Indonesia has been acknowledged to have tremendous potential for its marine biodiversity, including the potential bioactive compounds derived from marine sponges and their associated microbes (Atikana et al., 2023a).

The research conducted by Sedjati et al. (2020) discovered that marine sponges-associated fungus *Trichoderma longibrachiatum* produces bioactive substances that inhibit *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Bacillus cereus*. Another study also reported the potential of actinomycetes associated with marine sponges to develop numerous secondary metabolites that possess antibacterial properties (Mutalib et al., 2020). Marine microbes, especially bacteria associated with sponges, can be a source of natural chemicals that may also contribute to developing anti-NTM compounds (Aucklo & Wu, 2016; Wibowo et al., 2023). Among all bacteria, actinomycetes are reported to have contributed to as much as 64% of all natural antibiotic classes, including anti-NTM compounds (Hutchings et al., 2019; Mamada et al., 2022). Therefore, exploring marine bacteria, especially the actinomycetes, can also contribute to developing anti-NTM compounds.

Current antimycobacterial compounds, including erythromycin (macrolide), tuberactinomycin (viomycin), and kanamycin (aminoglycosides), were derived from non-marine bacteria (Hutchings et al., 2019). These antimycobacterial compounds inhibit mycobacterial protein synthesis in different ways. Macrolides bind to the bacterial 50S ribosomal subunit (Linh et al., 2018), aminoglycosides bind to the bacterial 30S ribosomal subunit (Krause et al., 2016), and viomycin blocks the elongation factor G (EF-G)-catalyzed translocation of messenger RNA onto the ribosome (Holm et al., 2016). A study reported that amikacin, a kanamycin derivative, has been used to treat pulmonary patients with NTM infections (Daley et al., 2020). There have been several claims that marine microorganisms could produce antibiotics, but only limited efforts have been made to find anti-NTM. Our previous study reported the potential of marine actinobacteria from Indonesian water to inhibit the growth of *S. aureus* and *B. subtilis*. The study also reported the potential of *Streptomyces* sp. BTA 1-131, isolated from the marine sponge *Melophlus sarasinorum*, as a producer of antibacterial and anticancer compounds (Atikana et al., 2023b). The present study aimed to investigate the potential of sponge-associated *Streptomyces* sp. BTA1-131 inhibits the growth of NTM, i.e., *M. smegmatis* and *M. fortuitum*. These two mycobacteria, *M. smegmatis* and *M. fortuitum*, were selected as representatives of

the RGM NTM microorganisms. To the best of our knowledge, this study is the first to report the potential of the sponge-associated *Streptomyces* from Indonesia as a producer of anti-NTM agents.

Material and Methods

Preparation of marine actinobacterial crude extracts

Streptomyces sp. BTA 1-131 used in this study belongs to the Research Centre for Applied Microbiology, National Research and Innovation Agency (BRIN) collection. The strain was isolated from the marine sponge *Melophlus sarasinorum* from Lembeh Strait, Bitung, Indonesia (Atikana et al., 2023b)—the preparation of crude extracts of *Streptomyces* sp. BTA 1-131 in this study followed previously published protocols (Carr et al., 2010; Atikana et al., 2023b). The strain BTA 1-131 was cultivated in three different liquid fermentation media containing a combination of organic nitrogen sources and carbohydrates essential for optimizing microbial fermentation processes: ISP2, SYP, and YS. The ISP2 medium had a composition of (g/L) glucose, 4; malt extract, 10; and yeast extract, 4. The SYP medium had a composition of (g/L) starch, 10; yeast extract, 4; and peptone, 2. Meanwhile, the YS medium had a composition of (g/L) yeast extract, 2 and starch, 10. All were prepared with seawater. All media components were obtained from Merck, USA, and the bacto agar was obtained from HiMedia. The present study employed the methanolic extract derived from various cultivation media, namely ISP2 liquid (ISP2-L), SYP liquid (SYP-L), and YS liquid (YS-L).

BTA 1-131 strain was rejuvenated from glycerol stocks to media YS agar with 2% NaCl and incubated for 6 days at 28°C (Figure 1). Bacterial seed culture was prepared by inoculating the six-day-old *Streptomyces* sp. BTA1-131 into the liquid fermentation media (Figure 1). The six-day-old seed culture was transferred to 100 mL of liquid media and then incubated for metabolite production for 14 days at 28°C, with the agitation of 150 rpm. The 14 day-old-culture was mixed with 100 mL of methanol and incubated overnight. The extracts were then transferred to a beaker, and the organic solvent was removed using a rotary evaporator. The remaining water content was removed by using a freeze-dryer. The dried extracts were stored at 4°C before further bioactivity experiments.

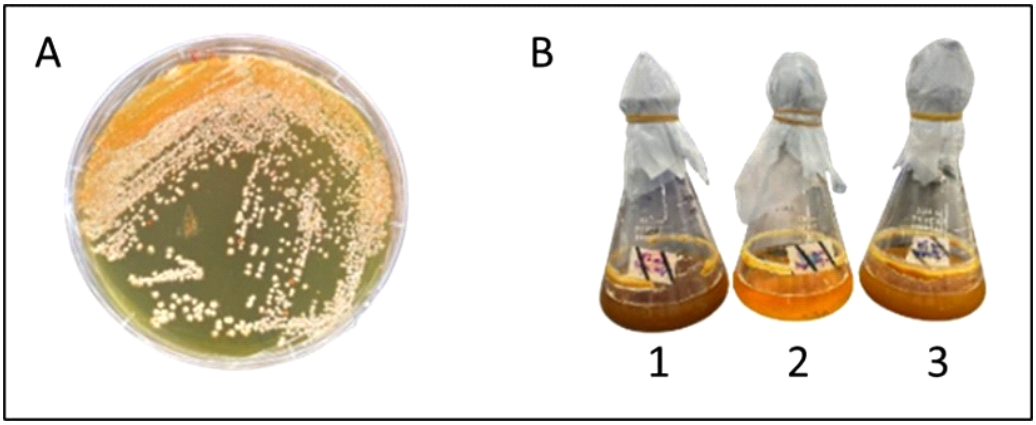

Figure 1. The 14-day-old *Streptomyces* sp. BTA 1-131 grown on YS agar plate (A) and 14-day-old broth culture in media ISP2 (B1), YS (B2) and SYP (B3)

Preliminary screening of anti-mycobacterium using disc diffusion assay against M. smegmatis

In the preliminary screening, the methanolic extracts of *Streptomyces* sp. BTA 1-131 (ISP2-L, SYP-L, and YS-L) were tested against the growth of *M. smegmatis* ATCC 700084. *M. smegmatis* was obtained from the Research Centre for Applied Microbiology, BRIN. Prior to the bioactivity screening, *M. smegmatis* was precultured in Mueller Hinton Broth (Merck). The bioactivity screening of the extracts used the Kirby Bauer disc diffusion assay and was prepared following the published protocols (Atikana et al., 2023b; Warsito et al., 2023). All crude extracts were dissolved in 1% DMSO (diluted in sterile aquadest) and sonicated for 20 min. Sterile filter paper discs were placed on top of the bacterial lawn, and a final 0.5 mg/disc of crude extracts was impregnated in the sterile paper disc before incubating the plates at 37°C. The antibacterial activity was indicated by an apparent clear zone surrounding the discs after 24h of incubation. After overnight incubation, the diameter of inhibition was measured in millimeters (mm). In addition to the extracts, 1% DMSO was used as a solvent/negative control, and the antibiotic kanamycin (30 µg, Oxoid) was used as the drug/positive control.

The bioactivity of methanolic crude extracts was evaluated against M. fortuitum *by quantification of the reduction percentage using broth microdilution*

The inhibitory effect of the methanolic extracts of *Streptomyces* sp. BTA 1-131 (ISP2-L, SYP-L, and YS-L) against *M. fortuitum* ATCC 6841 was assessed following the published protocols by Rakhmawatie et al., (2019) and Clinical and Laboratory Standard Institute [CLSI] (2011) with a slight modification by quantifying the number of viable cells. The method was used for high-throughput screening to identify planktonic cell growth using resazurin sodium salt dye (Sigma Aldrich) as a biological stain by broth microdilution. The experiment was carried out on a 96-well polystyrene round-bottom microtiter plate (IWAKI). The test was performed on *M. fortuitum* ATCC 6841. *M. fortuitum* was obtained from the Department of Microbiology, Faculty of Medicine, Public Health, and Nursing (FKKMK) of Gadjah Mada University. The medium for broth microdilution was Middlebrook 7H9 broth (Becton Dickinson), with other supplements such as 0.1% Bacto Casitone (Becton Dickinson), 0.5% glycerol (Merck), and 10% OADC (oleic acid, albumin, dextrose, catalase) (Becton Dickinson). The reduction percentage is the number of planktonic cells that could reduce resazurin indicator dye (blue non-fluorescent) to fluorescent pink resofurin. The number of living cells was analyzed quantitatively by measuring an absorbance-based microplate reader (SH-1000 Corona Electric). The absorbance of oxidized and reduced forms of resazurin were 600 nm and 570 nm, respectively. Samples were measured for the reduction to have a percentage of three *Streptomyces* sp. BTA 1-131 extracts. In the test well, the final concentration of mycobacteria inoculum was 2.5 x 10^5 CFU/mL. The drug control was amikacin (amikacin disulfate salt, Sigma Aldrich) with a concentration range of 8–0.125 µg/mL using a 2-fold serial dilution. All crude extracts were dissolved in 1% DMSO (diluted in sterile aquadest) and sonicated for 20 min. The final concentration of each extract in each test well was 0.781 mg/mL. The 96-well plates were incubated at 37°C for 4 days. After that, 25 µL of 0.01% resazurin was added to each well. Plates were incubated for 24 hours at 37°C. The experiments were conducted in triplicate. The percentage reduction was calculated using the formula (Lee, 2017) below:

$$Reduction\ percentage = \frac{\varepsilon ox\ A600nm\ \times A570nm\ tx - \varepsilon ox\ A570nm\ \times A600nm\ tx}{\varepsilon red\ A570nm\ \times A600nm\ t0 - \varepsilon red\ 600nm\ \times A570nm\ t0}$$

Where

å is the Molar Ext. coefficients for resazurin

A is the measured absorbance at a given wavelength

t0 is the first measurement

tx is the measurement at four days of incubation

The molar extinction coefficient of resazurin in the oxidized and reduced forms is shown in Table 1.

Table 1. The molar extinction coefficient for resazurin

Wavelength	Reduced Resazurin εred	Oxidized Resazurin εox
570 nm	155,677	80,586
600 nm	14,652	117,216

Detection of Chromatographic Profile of Crude Extracts Streptomyces *sp. BTA 1-131 by TLC*

The crude extracts were applied on the TLC plate as much as 5 µL. The stationary phase used in this present study was TLC aluminium Silica gel 60 F254 (Merck). The TLC plates were developed in chloroform (Merck): methanol (Merck) with a ratio of 5:3 (v/v), chloroform: ethyl acetate: methanol with a ratio of 5:4:1 (v/v) and chloroform: ethyl acetate: methanol with a ratio of 8.5:0.5:1 (v/v) to illustrate the profile of extracts. The plates were observed under ultraviolet (UV) light in a chamber using 254 and 365 nm wavelengths.

Results and Discussion

This study reported the potential of *Streptomyces* sp. BTA 1-131 to produce anti-NTM compounds. The preliminary screening of *Streptomyces* sp. BTA1-131, as a potential producer of anti-NTM, was observed by assessing the bioactivity of crude extracts from three media, SYP-L, ISP2-L, and YS-L, against *M. smegmatis* ATCC 70084. The screening observed the highest activity in the methanolic extract derived from a SYP-L cultivation medium, followed by YS-L and ISP2-L cultivation media, respectively (Figure 2). Although the extract derived from SYP-L showed lower inhibition compared to the antibiotic kanamycin, it showed a good diameter of inhibition (> 20 mm) as a crude extract (Figure 2).

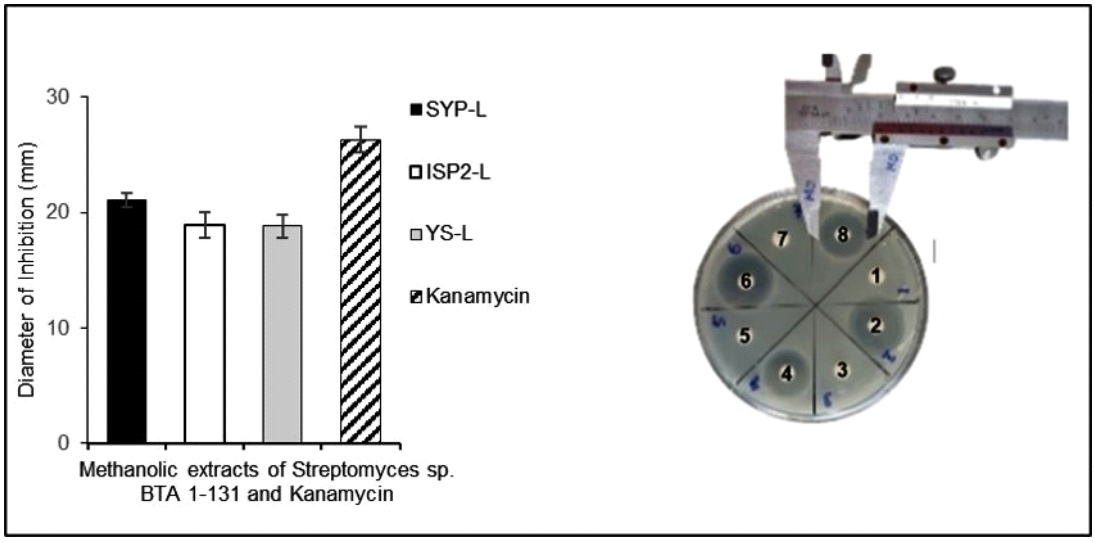

Figure 2. Preliminary screening of methanolic extracts of *Streptomyces* sp BTA1-131 (0.5 mg/disc) against *Mycobacterium smegmatis* ATCC 700084 (A) and the diameter zone of inhibitions (B). The methanolic extracts of *Streptomyces* sp. BTA 1-131 were derived from the three-fermentation media SYP-liquid (SYP-L/disc 6), ISP liquid (ISP-L/disc 4), and YS liquid (YS-L/disc 8). Discs 3, 5, and 7 were media only. The antibiotic kanamycin (30 µg/disc) was used as a positive control (disc 2), and the DMSO (1%) was used as a negative control (disc 1).

The preliminary screening in this study used the disc diffusion method. This method was selected due to its simplicity and rapid to apply. The preliminary screening showed the potential activity of *Streptomyces* sp. BTA1-131 against *M. smegmatis* ATCC 700084. *M. smegmatis* was selected in this study because it was commonly used as a model organism for research on TB and other types of mycobacteria (Sparks et al., 2023). An Initial study and evaluation of *M. smegmatis* is a starting point for further research on other NTM, i.e., *M. fortuitum* ATCC 6841. *M. fortuitum* was selected as it is the most prevalent species of RGM associated with various human infections. In this study, the potential anti-NTM activity of *Streptomyces* sp. BTA 1-131 extracts (SYP-L, ISP2-L, and YS-L) against *M. fortuitum* ATCC 6841 were investigated by determining the percent reduction value of resazurin (Figure 3). This study used the broth microdilution method to determine the ability of the strain BTA 1-131 to inhibit the growth of *M. fortuitum* ATCC 6841. The microdilution method can detect inhibition using a low amount of extracts. The broth microdilution method is more quantitative than the disc diffusion method because it performs dilutions accurately and dispenses the aliquot into the well with broth medium (Wanger, 2009). This method used resazurin, also called Alamar Blue, to indicate cell viability and cytotoxic activity. The color changes from non-fluorescent blue to pink fluorescent (resofurin), reflecting its metabolic activity (Costa et al., 2021). This observation was applied as a qualitative analysis to describe the activities rapidly. However, the number of viable cells in each well could not be determined by observing the color change. The optical density (OD) value of each test well was used quantitatively to evaluate the growth density of a mycobacterium after being exposed to a sample of marine bacterial extracts. The percent reduction of three extracts is shown in Figure 3A.

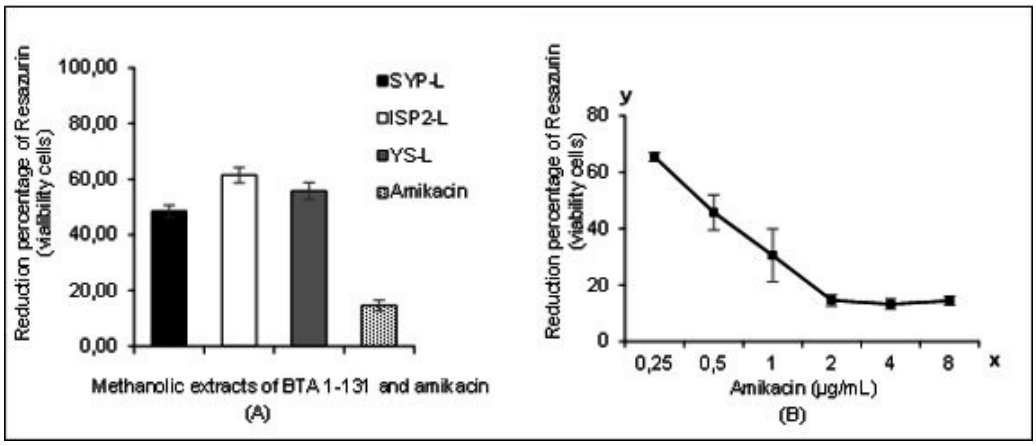

Figure 3. Reduction percentage of the methanolic extracts of *Streptomyces* sp. BTA 1-131 at 0.781 mg/mL and amikacin 2 µg/mL (A) and curve of amikacin reduction percentage at 0.25 – 8 µg/mL (B). The bioactivity was tested against *M. fortuitum* ATCC 6841. The methanolic extracts were derived from SYP-liquid (SYP-L), ISP-liquid ISP-L), and YS-liquid (YS-L) cultivation.

The percent reduction value of the methanolic extracts derived from three cultivation media against *M. fortuitum*, which was 48.45 to 61.54%, indicated that almost half of mycobacterial growth in the sample well was inhibited. The lower the percent reduction value, the fewer the number of living cells reduced resazurin to resofurin. Among these extracts, the methanolic extract of *Streptomyces* sp. BTA 1-131 derived from the SYP-L cultivation medium had the lowest percent reduction (48.45%), followed by YS-L and ISP2-L cultivation media. This study found that the methanolic extract of *Streptomyces* sp. BTA 1-131 derived from the SYP-L cultivation medium demonstrated more capability to inhibit *M. fortuitum* growth than extracts from other cultivation media (Figure 3A). In contrast, our previous study reported that the methanolic extract derived from the YS-L cultivation medium had the highest inhibition diameter against *S. aureus* ATCC 13420 and *B. subtilis* ATCC 6051. However, the methanolic extracts derived from the SYP-L medium did not inhibit the growth of *S. aureus* ATCC 13420 and showed weak activity against *B. subtilis* ATCC 6051 (Atikana et al., 2023b). This study also demonstrated the impact that different fermentation media might produce different metabolites in the crude extracts, among which compounds offered potential not only as antibacterial but also as antimycobacterials. The bioactivity of the methanolic extract from *Streptomyces* sp. BTA 1-131, derived from SYP-L, was comparable to amikacin at 0.25 to 0.5 µg/mL concentrations, exhibiting a percent reduction in *M. fortuitum* of 45.75 to 65.43%. As a drug control, amikacin exhibited a percent reduction value of 14.58% at a Minimum Inhibitory Concentration (MIC) value of 2 µg/mL, aligning with the qualitative test. The MIC was established at 2 µg/mL, indicated by the absence of color change from blue (resazurin) to pink (resofurin). This result is supported by previous work by Raaijmakers et al. (2021), which indicated that amikacin's MIC_{50} and MIC_{90} against *M. fortuitum* were d" 2 µg/mL.

The differences in the metabolites that might be produced in each of the extracts were determined by using TLC analysis to obtain a chromatogram profile. The chromatogram profile demonstrated the correlation between metabolites produced by *Streptomyces* sp. BTA 1-131 under different cultivation media and the biological activity obtained. Chromatogram patterns were identified using three different types of mobile phases. The methanolic extracts derived from the SYP-L and ISP2-L showed a similar chromatogram pattern in TLC plates. It differed for the YS-L medium extracts (Figure 4). The separation using chloroform: methanol (5:3) mobile phase was able to find one different compound (Rf 0.5) owned by extract derived from YS-L (Figure 4A). The Rf value of each compound in each extract is shown in Table 2. The methanolic extracts of *Streptomyces* sp. BTA 1-131 derived from SYP-L and ISP-L contained many compounds with blue-bright fluorescence in UV 365nm (on TLC plates A, C, and E). Chromatogram patterns indicate differences in compound composition, which could affect biological activity. The results correspond to the differences in biological activity of the extracts found in this study. This finding aligned with our previous study, which identified unique compounds in the LC/MS profiles of three methanolic extracts of *Streptomyces* sp. BTA 1-131. Alkaloid staurosporine, notable for its anticancer and antimicrobial properties, was found in the methanolic extract derived from the SYP medium cultivation. Furthermore, the methanolic extract of the ISP2 medium appeared to contain the antibiotic tetracycline, whereas the methanolic extract of the YS medium may contain the herbicide

bensulide. However, gentiobiose and lauryl diethanolamine are likely present in all methanolic extracts of *Streptomyces* sp. BTA 1-131 (Atikana et al., 2023b). The secondary metabolites derived from sponge-associated *Streptomyces* BTA 1-131, identified in the LC/MS Profile, have not been previously reported as antimycobacterial agents.

Unfortunately, this study showed that the TLC analysis's mobile phase could not effectively separate the extracts' compounds due to polar compounds eluting poorly. These polar compounds were observed in all methanolic extracts and marked with yellow squares on the TLC plates C-F (Figure 4). The main limitation of our current research is the absence of exploration into the antimycobacterial compound, and numerous compounds remain uncharacterized as the standard reagents for detecting a certain group of compounds (e.g., Dragendoff's reagent, vanillin-sulphuric acid reagent) were not used. Moreover, some metabolites remain uncharacterized by the LC/MS profile in the previous study. Therefore, further investigation is crucial to confirm those metabolites and identify unknown metabolites in the methanolic extracts derived from *Streptomyces* BTA 1-131 using LC-HRMS/MS.

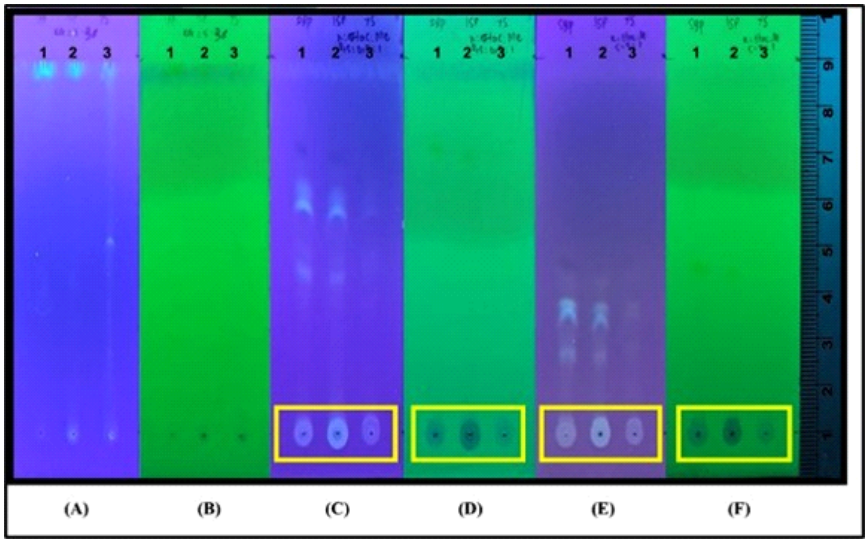

Figure 4. Chromatographic profile of three methanolic extracts of *Streptomyces* sp. BTA 1-131 on TLC aluminium Silica gel 60 F254 (left to right were extracts obtained on SYP liquid (1), ISP liquid (2), and YS liquid (3) cultivation media). Mobile phases: (TLC plates of A and B) chloroform: methanol (5:3); (TLC plates of C and D) chloroform: ethyl acetate: methanol (5:4:1); (TLC plates of E and F) chloroform: ethyl acetate: methanol (8.5:0.5:1). TLC plates A, C and E were a chromatogram pattern at UV 365nm. The TLC plates B, D and F were at UV 254nm.

Table 2. TLC profile of three methanolic extracts of *Streptomyces* sp. BTA 1-131 in different mobile phases

TLC plates and mobile phases	Total spots and Rf Value								
	Extract derived from SYP-L			Extract derived from ISP-L			Extract derived from YS-L		
	Spot	UV 365 nm	UV 254 nm	Spot	UV 365 nm	UV 254 nm	Spot	UV 365 nm	UV 254 nm
TLC plates A and B chloroform: methanol (5:3)	-	-	-	-	-	-	1	0.5	-
TLC plates C and D chloroform: ethyl acetate: methanol (5:4:1)	1	0.53	-	1	0.53	-	-	-	-
	2	0.69	-	2	0.69	-	-	-	-
	3	0.74	-	3	0.74	-	-	-	-
TLC plates E and F chloroform: ethyl acetate: methanol (8.5:0.5:1)	1	0.31	-	1	0.31	-	-	-	-
	2	0.43	-	2	0.43	-	-	-	-
	3	0.58	-	3	0.58	-	-	-	-

The current study adds to the knowledge of the potential of methanolic extract derived from *Streptomyces* sp. BTA 1-131 to inhibit the growth of two NTMs, *M. smegmatis* ATCC 700084 and *M. fortuitum* ATCC 6841, despite the suboptimal activity in *M. fortuitum* ATCC 6841 (<50%). These findings may be attributed to the crude methanolic extract which probably contained many compounds.

Notwithstanding these limitations, it is still promising to obtain anti-NTM compounds from the strain BTA 1-131 through fermentation optimization and purification of the crude extracts, for example, using high-performance liquid chromatography equipped with TLC-bioautography. Different approaches can be implemented to optimize the fermentation process, such as one strain of many compounds (OSMAC) and/or a co-cultivation method. The OSMAC is one of the strategies to influence the production of secondary metabolites by introducing different parameters in the fermentation process to produce target compounds, for example, to induce anti-NTM compounds. This study had implemented the OSMAC strategy, where *Streptomyces* sp. BTA 1-131 was cultivated in three different media (SYP, ISP2, and YS). It has also been reported that the methods could activate the metabolite pathways and trigger silent biosynthetic gene clusters to produce different bioactive molecules. Another promising approach is co-cultivation methods, where microbes are cultivated with different microbial strains. Microbial co-cultivation was also reported to affect the production of microbial secondary metabolites (Romano et al., 2018). The interactions between one strain and the other(s) in a culture medium might be friendly, hostile, or competitive. A beneficial outcome of co-cultivating two or more strains is typically an increased synthesis of recognized compounds or an accumulation of cryptic compounds not discovered in axenic culture. This impact could be attributed to the generation of enzymes that activate metabolite precursors or the possibility that strain may induce epigenetic changes in the producing strain (Pan et al., 2019).

Conclusion

The present study reported the potential of sponge-associated *Streptomyces* sp. BTA 1-131 to inhibit the growth of both NTM, *M. smegmatis* ATCC 700084 and *M. fortuitum* ATCC 6841. The most effective inhibition against *M. smegmatis* and *M. fortuitum was* observed in the strain BTA 1-131 methanolic extract cultivated in SYP liquid media. Distinct secondary metabolites were exhibited by the strain BTA 1-131 cultivated in three different liquid media, as observed in the TLC profiles. The detection of chromatogram patterns of methanolic extracts of *Streptomyces* sp. BTA 1-131 in three mobile phases showed differences in compound composition, potentially influencing the biological activity. This study highlights the potential of sponge-associated *Streptomyces* sp. BTA 1-131 to produce anti-NTM metabolites. Further research is required to optimize strain BTA 1-131 fermentation, especially the production of anti-NTM compounds in both SYP and ISP2 fermentation media.

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