Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v54i4.3372

Research Article

Isolation and Characterization of Antibacterial Actinomycetes from BRIS Soil in Setiu, Terengganu, Targeting ESKAPE Infections

Amirah Ahmad[1], Hamidah Idris[1]*, Mohd Fakharul Zaman Raja Yahya[2,3]*

1. Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak, Malaysia
2. Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
3. Integrative Pharmacogenomics Institute, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

*Corresponding author: fakharulzaman@uitm.edu.my

ABSTRACT

Antibiotic resistance poses a significant challenge to global health, necessitating the discovery of novel antimicrobial agents. Actinomycetes are prolific producers of bioactive compounds, contributing to over two-thirds of clinically utilized antibiotics. While actinomycetes are widely recognized for their antibiotic production, little is known about those from BRIS soil in Setiu, Terengganu, and their antibacterial efficacy against ESKAPE pathogens remains unexplored. This study evaluated the antibacterial activity of actinomycetes isolated from BRIS soil in Setiu, Terengganu, specifically against ESKAPE pathogens. The isolates were characterized by color groups, extracted using ethyl acetate and methanol, and screened for antibacterial activity using a gel plug assay. A selected actinomycete isolate was further analyzed by gas chromatography-mass spectrometry (GC-MS), well diffusion (WD), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays, along with molecular identification. The results showed that isolate BA71 exhibited the largest inhibition zones with both methanol and ethyl acetate extracts compared to the other isolates tested. GC-MS analysis of the ethyl acetate extract from isolate BA71 identified nine bioactive compounds, including stigmasta-5,24(28)-dien-3-ol, gamma sitosterol, and lupeol. The WD, MIC, and MBC assays further confirmed the isolate's bactericidal and bacteriostatic properties. Phylogenetic analysis based on 16S rRNA gene sequences revealed a close relationship between isolate BA71 and *Streptomyces malaysiense* MUSC 136T, with 100% similarity. These findings highlight the potential of BRIS soil-derived Streptomyces in the discovery of novel antibiotics, contributing to the ongoing search for effective treatments against ESKAPE pathogens.

Key words: Actinomycetes, antibacterial activity, BRIS soil, ESKAPE pathogens

INTRODUCTION

Antibiotic resistance among pathogens has become a major global health and economic threat, recognized by the World Health Organization (WHO) as one of the top ten dangers to global health (Kumar 2021). The development of antibiotic resistance is driven by certain bacterial modifications that occur in response to selection pressures among bacteria (Safini *et al*., 2024). Despite numerous alternative strategies to control pathogens, such as the use of phytochemicals and synthetic compounds (Man *et al*., 2022; Kamaruzzaman *et al*., 2022; Johari *et al*., 2023; Hamdan *et al*., 2024), antibiotic resistance remains a persistent and growing challenge (Yahya *et al*., 2025). These alternative approaches have shown potential in targeting resistant strains; however, the resilience and adaptability of pathogens continue to undermine efforts, underscoring the need for novel and more effective interventions in combating antibiotic resistance. To address this challenge, the discovery of novel antibiotics with mechanisms of action distinct from existing macrolides, such as erythromycin (Isa *et al*., 2022), is essential for overcoming resistance in pathogenic bacteria. Actinomycetes are among the most extensively studied organisms due to their potent ability to produce bioactive compounds, accounting for approximately 75% of antibiotics with a wide range and diversity of mechanisms (Selim *et al*., 2021). The genus *Streptomyces*, in particular, is prolific in secondary metabolite production, responsible for over two-thirds of clinically used antibiotics, including daptomycin, amphotericin, doxorubicin, vancomycin, and rapamycin (Bae *et al*., 2013; Barka *et al*., 2016). Given this high potential, researchers continue to explore actinomycete isolates from diverse habitats, including oceans (Akhter *et al*., 2018), polar regions (Sivasankar *et al*., 2018), arid and saline soils (Binayke *et al*., 2018; El Karkouri *et al*., 2019), as well as soils from marine environments, deserts, mountains, and mines (Nafis *et al*., 2019). Actinomycetes from these unique habitats often exhibit significant biosynthetic potential and can produce potent novel metabolites (Hei *et al*., 2021). However, there is a high probability of rediscovering known compounds due to the widespread focus on actinomycetes in drug development. To avoid redundancy, it is recommended that scientists explore previously unexplored environments to isolate rare actinomycetes, thereby increasing the likelihood of discovering novel compounds beneficial for antibiotic development (Ezeobiora *et al*., 2022).

Soil is one of the primary sources for isolating actinomycetes, with most bioactive compounds to date having been discovered from terrestrial environments (Dimri *et al*., 2020). However, over time, the rate of novel compound discovery from this environment

Article History
Accepted: 24 September 2025
First version online: 21 December 2025
Cite This Article:
Ahmad, A., Idris, H., Yahya, M.F.Z.R. 2025. Isolation and characterization of antibacterial actinomycetes from bris soil in Setiu, Terengganu, targeting ESKAPE infections. Malaysian Applied Biology, 54(4): 98-105. https://doi.org/10.55230/mabjournal.v54i4.3372

has declined, while the re-isolation of known compounds has increased (Lam, 2006). To address this, Beach Ridges Interspersed with Swales (BRIS) soil was selected in this study as a source for isolating high-potential actinomycetes that may produce novel bioactive compounds. BRIS soil is considered problematic due to its poor physical and chemical properties—characterized by aridity, nutrient deficiency, low water retention, and unsuitability for agriculture (Mustapha *et al*., 2017). These challenging conditions make BRIS soil an intriguing environment for isolating actinomycetes with the potential to produce novel compounds. Although BRIS soil is characterized by poor fertility, low water-holding capacity, and high sand content, actinomycetes exhibit remarkable adaptability through several survival mechanisms, including production of heat- and drought-resistant spores that endure harsh conditions (Selim *et al*. 2021).

Although actinomycetes are well-known for their antibiotic-producing capabilities, research on those isolated specifically from BRIS soil in Setiu, Terengganu, remains limited. Additionally, the antibacterial potential of these isolates against clinically challenging ESKAPE pathogens has not been fully explored. Therefore, the objective of this study was to evaluate the antibacterial efficacy of actinomycetes isolated from BRIS soil in Setiu, Terengganu, against ESKAPE pathogens.

MATERIALS AND METHODS

Isolation of actinomycetes

The actinomycetes used in this study were isolated from BRIS soil samples collected in Setiu, Terengganu (coordinates: 5.51282, 102.81485). Samples were obtained from four series of BRIS soil: Rhu Tapai, Rudua, Jambu, and Baging. At each of two collection spots, soil was sampled at four depths: 0–20 cm, 20–50 cm, 50–70 cm, and 70–100 cm. The stratified sampling of BRIS soil at four depth intervals is methodologically justified and reflects known variations in physicochemical and biological properties with soil depth. For example, subsurface layers (20–50 cm) are a transition zone with fluctuating moisture and nutrient gradients, favoring facultative oligotrophic microbes like actinomycetes. The collection was conducted under the supervision and guidance of experts. The soil samples were stored in sterile Falcon tubes, kept on ice, and transported to the Microbiology Laboratory at the Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Perak, Malaysia. Selective isolation of actinomycetes was performed using standard plating techniques, and the isolates were maintained on yeast malt agar (ISP 2).

Characterization of actinomycetes

A total of 20 pure actinomycete isolates were selected and inoculated onto oatmeal agar (ISP 3) to induce spore production and peptone-yeast extract-iron agar (ISP 6) to observe melanin production. Through visual inspection, where melanin-producing actinomycetes typically form brown to black pigments around colonies after 3–7 days of incubation. This is due to the oxidation of tyrosine/phenolic compounds by tyrosinase enzymes, forming eumelanin. The plates were then incubated at 28°C for 14 days and 4 days, respectively. Colonies grown on ISP 3 were observed for aerial spore mass color, substrate mycelium pigmentation, and the color of diffusible pigments, which were then compared to the National Bureau of Standards (NBS) color name charts. The isolates were grouped into multi- and single-member color categories.

Preparation of test microorganisms

The ESKAPE pathogens, specifically *Enterococcus raffinosus* (ATCC 49464), methicillin-resistant *Staphylococcus aureus* (ATCC 35591), *Klebsiella pneumoniae* (ATCC 700603), carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* (ATCC 27853), and *Enterobacter aerogenes* (ATCC 51697), were obtained from the Faculty of Medicine, Universiti Kebangsaan Malaysia, and used as test microorganisms to assess the antibacterial activity of the extracts. These organisms were cultivated in Mueller-Hinton broth (MHB) at 37°C overnight (Fiedler, 2014). The resulting microbial suspensions were then adjusted to a standard density using the 0.5 McFarland standard to prepare the bacterial inoculum.

Primary antibacterial screening using gel plug assay

Primary screening of the antibacterial activity of the isolates was conducted using a plug assay. Five millimeter plugs were cut from ISP 2 agar inoculated with pure actinomycete isolates, using a sterile cork borer. The plugs were then arranged on sterile, empty plates. Test organisms grown in Mueller-Hinton broth (MHB) were mixed into molten Mueller-Hinton agar (maintained at 50°C) and poured over the plates containing actinomycete plugs. The plates were allowed to solidify and were incubated overnight at 37°C. The inhibition zones formed around the plugs were recorded in millimeters (mm).

Extraction of bioactive metabolites produced by Isolate BA71

The cultures were prepared in ISP 2 and incubated in a shaker at 180 rpm at 28°C for 10 days. ISP 2 provides more balanced nutrition for general maintenance. It supports robust growth of diverse actinomycetes without promoting excessive sporulation. After incubation, the cultures were centrifuged at 3000 rpm for 20 min to separate the cell mass from the supernatant. The supernatant was mixed with ethyl acetate in a 1:1 ratio and incubated overnight at room temperature. The mixtures were centrifuged at 2500 rpm for 5 min, and the upper layer (ethyl acetate layer) was removed using a micropipette and transferred into new microcentrifuge tubes. The ethyl acetate extracts were dried by evaporating using a heat block at 50°C for 4 to 6 hr. Once dry, the extracts were dissolved in 2% (v/v) DMSO with a 1mg/mL concentration prior to antibacterial activity screening against ESKAPE pathogens. Separated biomass was mixed with 1mL of methanol and incubated overnight at room temperature. The mixtures were centrifuged at 14000 rpm for 10 min. The supernatant was collected and dried by evaporating using a heating block at 60°C. Once dry, the methanol extracts were dissolved in 2% (v/v) DMSO with a 1 mg/mL concentration prior to antibacterial activity screening against ESKAPE pathogens.

GC-MS analysis of isolate BA71 ethyl acetate extract

The ethyl acetate extract of isolate BA71 was dissolved in LC-grade methanol at a concentration of 1 mg/mL for GC-MS analysis. The analysis was conducted using an autosampler method on a gas chromatography instrument equipped with a capillary column. The column oven temperature was initially set at 100°C for 5 min, then increased to 240°C at a rate of 5°C per min, and finally raised to 300°C at a rate of 5°C per min for 5 min. The injector temperature was maintained at 220°C, while the interface temperature was set at 200°C. Helium, used as the carrier gas, was maintained at a flow rate of 1 mL/min. A split ratio of 1:10 was used, optimized based on the expected metabolite concentration range. The mass spectrometer operated in scan mode over a mass range of 40–600 m/z for spectral readings. The resulting data were processed with specialized software to identify metabolites, which were then compared against the customized reference mass spectral library (NIST, Gaithersburg, MD, USA) (Ibnouf *et al*., 2022).

Secondary antibacterial screening using the well diffusion assay

The pathogen inoculum was evenly spread onto Mueller-Hinton agar (MHA) plates using a sterile swab, and 7 mm diameter wells were created using sterile micropipette tips. Each well was filled with 50 µL of either the ethyl acetate or methanol extract. Tetracycline served as the positive control, while 2% (v/v) DMSO, the solvent for the extracts, was used as the negative control. Following overnight incubation at 37°C, the inhibition zones were measured and recorded.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays

The ethyl acetate extracts of isolate BA71 were also tested for minimum inhibitory concentration (MIC) against MRSA and *E. raffinosus*. MIC was determined using the microtiter broth dilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines with 96-well plates. Each well was filled with 100 µL of Mueller-Hinton broth inoculated with the test organisms, and 100 µL of varying extract concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/mL) was added to the plates. Sterile broth and streptomycin served as the negative and positive controls, respectively. Each plate was mixed well and incubated overnight at 37°C. The experiment was conducted in triplicate. Absorbance was measured at 570 nm using a UV spectrophotometer, and the optical density (OD) readings were recorded (Manoharan, 2018; Mogana *et al*., 2020). Minimum bactericidal concentration (MBC) testing involved transferring 10 µL from the MIC wells to Mueller-Hinton agar (MHA) plates, followed by incubation at 37°C for 18–24 hr (Ozturk & Ercisli, 2006).

DNA extraction and amplification of the 16S rRNA gene

Genomic DNA was extracted using the GeneDireX DNA Extraction Kit (GeneDireX Inc., USA) following the manufacturer's protocol. The extracted DNA was stored at −20°C. Total genomic DNA from each isolate was used as a template for amplification of the 16S rRNA gene. The 16S rRNA gene was then amplified by polymerase chain reaction (PCR) using the universal forward primer 27f (5'–AGA GTT TGA TCC TGG CTC AG–3') and reverse primer 1525r (5'–AAG GAG GTG ATC CAG CC–3'), along with the DNA sample, dNTPs, DNA polymerase, and $MgCl_2$. The mixtures were placed in a thermal cycler for 25 to 35 cycles, with an initial denaturation at 94°C for 2 to 5 min, followed by denaturation at 94°C for 20 to 40 sec, annealing for 1 min at an appropriate temperature, extension at 72°C for 2 min, and a final extension at 72°C for 5 min. The PCR products were stored at −20°C until further use. Amplification products were analyzed by electrophoresis in a 2% (w/v) agarose gel stained with GelGreen.

Purification of PCR products

PCR products were purified and sequenced by New Gene Sdn. Bhd., Malaysia, following the manufacturer's protocol, which employed Sanger sequencing.

Phylogenetic analysis of isolate BA71

Partial 16S rRNA gene sequences obtained were compared with full sequences available in the GenBank database using BLAST, and pairwise sequence similarities were determined using the EzTaxon web server (http://eztaxon-e.ezbiocloud.net) (Kim *et al*., 2012). Based on the sequence data, phylogenetic analysis was then performed using MEGA 11.0 (Tamura *et al*., 2021) across computing platforms with Neighbour-Joining, Maximum Likelihood, and Maximum Parsimony algorithms. Bootstrap values, based on 1000 replicates, were generated according to the evolutionary distance model of Jukes & Cantor.

RESULTS AND DISCUSSION

Characteristics of actinomycete isolates

Table 1 summarizes morphological characteristics of various actinomycete isolates based on aerial spore mass color, substrate mycelium color, and diffusible pigment color. Aerial spore mass colors ranged from light olive brown to yellowish white and light reddish brown. Color variations are typical in actinomycetes and can be indicators of specific strain characteristics or metabolic profiles. Substrate mycelium colors observed here included greenish yellow, dark greenish yellow, moderate olive, and others. These colors can reflect the interaction between the mycelium and the culture medium and may provide insights into the nutritional requirements of each isolate. Diffusible pigment colors such as deep yellow and light olive brown were observed. The presence or absence of diffusible pigments can serve as a distinguishing feature, with certain pigments linked to secondary metabolite production or specific strain traits. Isolate BA83 exhibited melanin production in the ISP6 column. Melanin is a characteristic pigment that can protect from UV light and other environmental stressors, and it may be used as a marker for identifying certain actinomycetes. The characteristics of actinomycetes observed in this study align with the morphological features of Actinobacteria reported in previous studies (Vijayakumar *et al*., 2007; Li *et al*., 2016).

Table 1. Characteristics of actinomycete isolates from BRIS soil in Setiu, Terengganu

No.	ISP3 Characteristics			ISP6 (melanin production)	Isolate codes and source			
	Aerial spore mass colour	Substrate mycelia colour	Colour of diffusible pigment		Rhu tapai	Baging	Jambu	Rudua
1.	94 (Light Olive Brown)	99 (Strong Greenish Yellow)	85 (Deep Yellow)	-				BA167
2.	90 (Grayish Yellow)	86 (Light Yellow)	-	-	BA70			
3.	120 (Moderate Yellow Green)	103 (Dark Greenish Yellow)	94 (Light Olive Brown)	-	BA26			
4.	136 (Moderate Yellowish Green)	107 (Moderate Olive)	85 (Deep Yellow)	-	BA28			
5.	106 (Light Olive)	55 (Strong Brown)	85 (Deep Yellow)	-	BA8, BA41, BA59			
6.	98 (Brilliant Greenish Yellow)	86 (Light Yellow)	105 (Grayish Greenish Yellow)	-				BA165, BA166, BA170
7.	86 (Light Yellow)	86 (Light Yellow)	-	-				BA176
8.	148 (Very Pale Green)	87 (Moderate Yellow)	-	-			BA148, BA153	BA159
9.	91 (Dark Grayish Yellow)	69 (Deep Orange Yellow)	85 (Deep Yellow)	-		BA71		
10.	85 (Deep Yellow)	87 (Moderate Yellow)	82 (Vivid Yellow)	+		BA83		
11.	92 (Yellowish white)	86 (Light yellow)	-	-				BA156
12.	90 (Grayish Yellow)	90 (Grayish Yellow)	-	-				BA177
13.	42 (Light Reddish Brown)	40 Strong Reddish Brown	-	-				BA163
14.	94 (Light Olive Brown)	99 (Strong Greenish Yellow)	85 (Deep Yellow)	-				BA167

Antibacterial potential of actinomycetes

The methanol and ethyl acetate extracts of selected isolates demonstrated inhibitory activity only against *E. raffinosus* and MRSA (Figure 1). This result indicates that the extract may preferentially target Gram-positive bacteria due to better penetration through the single peptidoglycan layer. For methanol extracts, seven isolates showed antibacterial activity, with the largest inhibition zones formed by isolates BA156 and BA167 against *E. raffinosus* (26 mm). The largest inhibition zone against MRSA was produced by isolates BA71 and BA153, each with a zone of 20 mm. For ethyl acetate extracts, nine isolates showed antibacterial activity against *E. raffinosus* and seven against MRSA. The largest inhibition zones formed against *E. raffinosus* and MRSA were 28 mm and 24 mm, respectively, both produced by isolate BA71. The present study demonstrates the antibacterial efficacy of an ethyl acetate extract from a BRIS soil actinomycete. Separately, Dar & Ahmad (2024) reported on actinomycetes isolated from Northern Indian soil. They revealed that an ethyl acetate fraction from *Streptomyces rameus*-IMA46 inhibited biofilm formation at sub-MIC concentrations. Among all isolates tested, isolate BA71 produced consistently large inhibition zones (≥20 mm) against both pathogens, warranting its selection for further analysis using GC-MS, well diffusion assays, microplate assays, and molecular identification.

Bioactive compounds of ethyl acetate extract of isolate BA71

Nine high-quality bioactive compounds were detected in the ethyl acetate extract of isolate BA71 namely pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- detected at 24.64 min, L-Proline, N-pivaloyl-, ethyl ester at 24.82 min, diethyltrisulphide at 25.00 min, γ-Sitosterol at 33.14 min, stigmasta-5,24(28)-dien-3-ol, (3β,24Z)- at 35.49 min, lupeol at 38.96 min, 26,26-Dimethyl-5,23-ergostadien-3β-ol at 39.22 min, 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester at 41.58 min, and dl-α-Tocopherol at 42.84 min (Table 2). Previous studies have identified the bioactivities of some of these compounds. For instance, the stigmasta compound has been reported to exhibit antibacterial activity against multiple antibiotic-resistant *Helicobacter pylori* (Wang *et al*., 2011). γ-Sitosterol, the major compound in *Cissus quadrangularis*, is also known for its antibacterial efficacy against *S. aureus* and *Escherichia coli* (Paul *et al*., 2024). Additionally, lupeol has shown moderate antibacterial activity against MRSA (Rosandy *et al*., 2021).

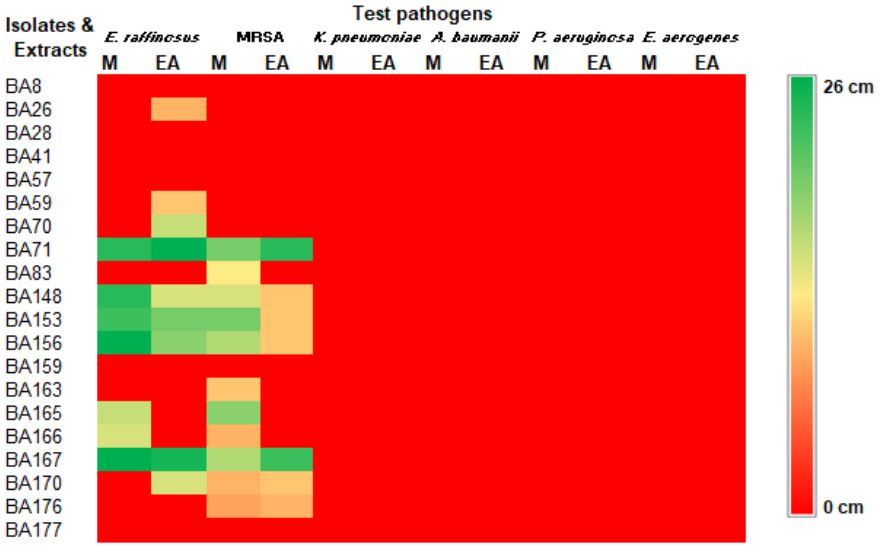

Fig. 1. Heat map showing antibacterial efficacy of actinomycete isolates against ESKAPE pathogens based on gel plug assay. M: methanol extract. EA: ethyl acetate extract.

Table 2. Bioactive compounds identified in the ethyl acetate extract of isolate BA71

Peak area (%)	Retention time (min)	Compounds	Molecular weight
28.87	35.49	Stigmasta-5,24(28)-dien-3-ol	412.69
4.02	33.14	Gamma sitosterol	414.71
3.03	42.84	Alpha tocopherol	416.68
2.79	38.96	Lupeol	426.72
0.92	24.64	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	210.27
0.54	25.00	Diethyltrisulphide	154.32
0.24	39.22	26,26-Dimethyl-5,23-ergostadien-3. beta. -ol	426.7
0.15	41.58	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	399.49
0.01	24.82	L-Proline, N-pivaloyl-, ethyl ester	227.3

Antibacterial potential of ethyl acetate extract of isolate BA71

The ethyl acetate extract of isolate BA71 produced large inhibition zones against *E. raffinosus* and MRSA (Table 3). The ethyl acetate extract of isolate BA71 exhibited an MIC and MBC of 31.25 µg/mL against *E. raffinosus* (Table 4). For MRSA (ATCC 35591), the MIC was 31.25 µg/mL, while the MBC was 125 µg/mL. The MBC/MIC ratio against *E. raffinosus* and MRSA was 1 and 4, respectively. Actinomycete extracts are considered natural products. The antibacterial activity of natural products is deemed significant when MIC values are lower than 100 µg/mL, moderate when 100≤MIC≤625 µg/mL, and weak when MIC values are above 625 µg/mL (Ríos & Recio, 2005; Kuete, 2010). The equal MIC and MBC values against *E. raffinosus* suggest that the extract has a bactericidal effect. Conversely, the MBC/MIC ratio of 4 for MRSA indicates that the extract primarily acts as bacteriostatic at lower concentrations (Makade *et al*., 2024).

Table 3. Antibacterial efficacy of ethyl acetate of isolate BA71 against ESKAPE pathogens based on the well diffusion assay

Test pathogens	*E. raffinosus*	MRSA	*K. pneumoniae*	*A. baumanii*	*P. aeruginosa*	*E. aerogenes*
Inhibition zone (mm)	28	24	-	-	-	-

Table 4. MIC and MBC values of ethyl acetate extract of isolate BA71 ethyl

Pathogen	Ethyl acetate			Streptomycin		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
E. raffinosus (ATCC 49464)	31.25	31.25	1.0	31.25	31.25	1.0
MRSA (ATCC 35591)	31.25	125	4.0	31.25	31.25	1.0

Identity of isolate BA71

The PCR product demonstrated successful amplification of the 16S rRNA gene from isolate BA71 (Figure 2). Isolate BA71 exhibited 100% nucleotide similarity with *Streptomyces malaysiense*, with no sequence differences (0/1420 base pairs differing) (Table 5). This level of identity strongly indicates that isolate BA71 is the same species as *S. malaysiense*. Isolate BA71 was also found to cluster closely with *S. malaysiense*, with a high bootstrap support value of 95%. (Figure 3). Bootstrap values provide

confidence in the branching pattern, with values above 70% generally considered robust in phylogenetic studies. This high bootstrap support indicates a close evolutionary relationship between isolate BA71 and *S. malaysiense*, suggesting they belong to the same species. In a study by Dar and Ahmad (2024), soil actinomycete isolate IMA-46 from Northern Indian was identified as *Streptomyces rameus* through 16S rRNA gene sequence analysis. The ethyl acetate fraction of this strain demonstrated inhibitory effects against *S. aureus* ATCC 3160, *E. coli* ATCC 25922, *P. aeruginosa* PAO1, and *K. pneumoniae* ATCC 1705. Separately, Abduljaba and Salih (2022) isolated antimicrobial-producing soil actinomycetes from four regions in Iraq (Mosul, Dohuk, Sulaymaniyah & Erbil), which were also identified as Streptomyces species through phylogenetic analysis. Collectively, Streptomyces species are a common soil actinomycete with antibacterial properties. The antibacterial efficacy of BRIS soil-derived *S. malaysiense* observed herein may be linked to microbial membrane damage (Mazumdar & Thakur, 2024).

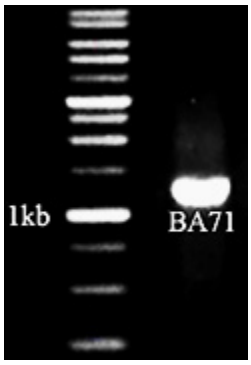

Fig. 2. The amplified 16S rRNA gene product of isolate BA71.

Table 5. Nucleotide similarities (%) and differences derived from 16S rRNA gene sequences among BA71 and *Streptomyces* species

No.		1	2	3	4	5	6
1	Isolate BA71	---	0/1420	6/1420	7/1420	14/1420	131/1069
2	*S. malaysiense* MUSC 136^T (LBDA02000093)	100	---	6/1428	7/1447	14/1447	131/1096
3	*S. phaeoluteichromatogenes* NRRL 5799^T (AJ391814)	99.58	99.58	---	4/1428	12/1428	132/1077
4	*S. misionensis* DSM 40306^T (FNTD01000004)	99.51	99.52	99.72	---	11/1447	133/1096
5	*S. cupreus* PSKA01^T (JACMSF010000162)	99.01	99.03	99.16	99.24	---	132/1096
6	*N. dassonvillei subsp. crassaminis* D1^T (LR606207)	87.75	88.05	87.74	87.86	87.96	---

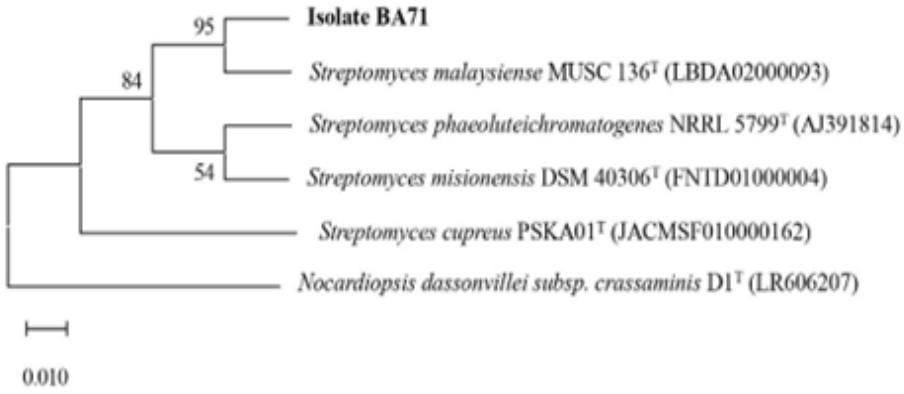

Fig. 3. Phylogenetic tree of isolate BA71.

CONCLUSION

This study successfully isolated and characterized actinomycetes from BRIS soil in Setiu, Terengganu, demonstrating their antibacterial properties. The ethyl acetate extract of Streptomyces sp. BA71 exhibited strong antibacterial activity against *E. raffinosus* and MRSA, with GC-MS analysis revealing the presence of several known antibacterial bioactive compounds. These findings suggest that BRIS soil holds promise for discovering novel actinomycetes that could contribute to the development of new antibiotics. Further exploration of these unique habitats may lead to breakthroughs in combating ESKAPE pathogens.

ACKNOWLEDGEMENTS

This research has been carried out under the Fundamental Research Grants Scheme (FRGS/1/2021/STG03/UPSI/03/1) provided by the Ministry of Education of Malaysia. The authors would like to extend their gratitude to Universiti Pendidikan Sultan Idris (UPSI), which helped manage the grants.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abduljaba, M.H. & Salih, T.S. 2022. Antimicrobial activity of ten local actinobacterial strains against ESKAPE, *Bacillus subtilis* and *Pseudomonas baetica* pathogens. South Asian Journal of Research in Microbiology, 13(4): 1-10. https://doi.org/10.9734/sajrm/2022/v13i4253

Akhter, N., Liu, Y., Auckloo, B.N., Shi, Y., Wang, K., Chen, J., Wu, X. & Wu, B. 2018. Stress-driven discovery of new angucycline-type antibiotics from a marine *Streptomyces pratensis* NA-ZhouS1. Marine Drugs, 16(9): 331. https://doi.org/10.3390/md16090331

Bae, M., Kim, H., Shin, Y., Kim, B., Lee, S., Oh, K.B., Shin, J. & Oh, D.C. 2013. Separacenes A-D, novel polyene polyols from the marine actinomycete, *Streptomyces* sp. Marine Drugs, 11(8): 2882-2893. https://doi.org/10.3390/md11082882

Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.P., Clément, C., Ouhdouch, Y. & van Wezel, G.P. 2016. Taxonomy, physiology, and natural products of actinobacteria. Microbiology and Molecular Biology Reviews, 80(1): 1-43. https://doi.org/10.1128/MMBR.00019-15

Binayke, A., Ghorbel, S., Hmidet, N., Raut, A., Gunjal, A., Uzgare, A., Patil, N., Waghmode, M. & Nawani, N. 2018. Analysis of diversity of actinomycetes from arid and saline soils at Rajasthan, India. Environmental Sustainability, 1(1): 61-70. https://doi.org/10.1007/s42398-018-0003-5

Dar, M.S. & Ahmad, I. 2024. Screening and evaluation of antibacterial active strains of Actinomycetes isolated from Northern Indian soil for biofilm inhibition against selected ESKAPE pathogens. Journal of Umm Al-Qura University for Applied Sciences, 10: 562-577. https://doi.org/10.1007/s43994-024-00164-8

Dimri, A.G., Chauhan, A. & Aggarwal, M.L. 2020. Antibiotic potential of actinomycetes from different environments against human pathogens and microorganisms of industrial importance: a review. Science Archives, 1(1): 32-48. https://doi.org/10.47587/SA.2020.1102

El Karkouri, A., Assou, S.A. & El Hassouni, M. 2019. Isolation and screening of actinomycetes producing antimicrobial substances from an extreme Moroccan biotope. Pan African Medical Journal, 33: 329. https://doi.org/10.11604/pamj.2019.33.329.19018

Ezeobiora, C.E., Igbokwe, N.H., Amin, D.H., Enwuru, N.V., Okpalanwa, C.F. & Mendie, U.E. 2022. Uncovering the biodiversity and biosynthetic potentials of rare actinomycetes. Future Journal of Pharmaceutical Sciences, 8(1): 23. https://doi.org/10.1186/s43094-022-00410-y

Fiedler, H.P. 2014. Screening for bioactivity. In: Microbial Diversity and Bioprospecting. A.T. Bull (Ed.). ASM Press, Washington. pp. 324-335. https://doi.org/10.1128/9781555817770.ch30

Hamdan, H.F., Ross, E.E.R., Jalil, M.T.M., Hashim, M.A. & Yahya, M.F.Z.R. 2024. Antibiofilm efficacy and mode of action of *Etlingera elatior* extracts against *Staphylococcus aureus*. Malaysian Applied Biology, 53(1): 27-34. https://doi.org/10.55230/mabjournal.v53i1.2808

Hei, Y., Zhang, H., Tan, N., Zhou, Y., Wei, X., Hu, C., Liu, Y., Wang, L., Qi, J. & Gao, J.M. 2021. Antimicrobial activity and biosynthetic potential of cultivable actinomycetes associated with lichen symbiosis from Qinghai-Tibet Plateau. Microbiological Research, 244: 126652. https://doi.org/10.1016/j.micres.2020.126652

Ibnouf, E.O., Aldawsari, M.F. & Ali Waggiallah, H. 2022. Isolation and extraction of some compounds that act as antimicrobials from actinomycetes. Saudi Journal of Biological Sciences, 29(8): 103352. https://doi.org/10.1016/j.sjbs.2022.103352

Isa, S.F.M., Hamid, U.M.A. & Yahya, M.F.Z.R. 2022. Treatment with the combined antimicrobials triggers proteomic changes in *P. aeruginosa-C. albicans* polyspecies biofilms. ScienceAsia, 48(2): 215-222. https://doi.org/10.2306/scienceasia1513-1874.2022.020

Johari, N.A., Aazmi, M.S. & Yahya, M.F.Z.R. 2023. FTIR spectroscopic study of inhibition of chloroxylenol-based disinfectant against *Salmonella enterica* serovar *Thyphimurium* biofilm. Malaysian Applied Biology, 52(2): 97-107. https://doi.org/10.55230/mabjournal.v52i2.2614

Kamaruzzaman, A.N.A., Mulok, T.E.T.Z., Nor, N.H.M. & Yahya, M.F.Z.R. 2022. FTIR spectral changes in *Candida albicans* biofilm following exposure to antifungals. Malaysian Applied Biology, 51(4): 57-66. https://doi.org/10.55230/mabjournal.v51i4.11

Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., Won, S. & Chun, J. 2012. Introducing EzTaxon-e: a prokaryotic 16s rRNA gene sequence database with phylotypes that represent uncultured species. International Journal of Systematic and Evolutionary Microbiology, 62(3): 716-721. https://doi.org/10.1099/ijs.0.038075-0

Kuete, V. 2010. Potential of Cameroonian plants and derived products against microbial infections: A review. Planta Medica, 76(14): 1479-1491. https://doi.org/10.1055/s-0030-1250027

Kumar, S. 2021. Antimicrobial resistance: A top ten global public health threat. EClinicalMedicine, 41: 101221. https://doi.org/10.1016/j.eclinm.2021.101221

Lam, K.S. 2006. Discovery of novel metabolites from marine actinomycetes. Current Opinion in Microbiology, 9(3): 245-251. https://doi.org/10.1016/j.mib.2006.03.004

Li, Q., Chen, X., Jiang, Y. & Jiang, C. 2016. Morphological identification of actinobacteria. In: Actinobacteria-Basics and Biotechnological Applications. D. Dhanasekaran & Y. Jiang (Eds.). InTech, Rijeka. pp. 59-86. https://doi.org/10.5772/61461

Makade, C.S., Shenoi, P.R., Bhongade, B.A., Shingane, S.A., Ambulkar, P.C. & Shewale, A.M. 2024. Estimation of MBC: MIC ratio of herbal extracts against common endodontic pathogens. Journal of Pharmacy and Bioallied Sciences, 16(Suppl 2): S1414-S1416. https://doi.org/10.4103/jpbs.jpbs_735_23

Man, C.A.I.C., Razak, W.R.W.A. & Yahya, M.F.Z.R. 2022. Antibacterial and antibiofilm activities of *Swietenia macrophylla* King ethanolic extract against foodborne pathogens. Malaysian Applied Biology, 51(4): 45-56. https://doi.org/10.55230/

mabjournal.v51i4.10
Manoharan, N. 2018. Antibacterial effect of endophytic actinomycetes from marine algae against multi drug resistant gram negative bacteria. Examines in Marine Biology & Oceanography, 1(5): 555572. https://doi.org/10.31031/EIMBO.2018.01.000522
Mazumdar, R. & Thakur, D. 2024. Antibacterial activity and biosynthetic potential of *Streptomyces* sp. PBR19, isolated from forest rhizosphere soil of Assam. Brazilian Journal of Microbiology, 55(4): 3335-3352. https://doi.org/10.1007/s42770-024-01454-3
Mogana, R., Adhikari, A., Tzar, M.N., Ramliza, R. & Wiart, C. 2020. Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates. BMC Complementary Medicine and Therapies, 20(1): 55. https://doi.org/10.1186/s12906-020-2837-5
Mustapha, Z., Mat, N., Othman, R. & Zakaria, A.J. 2017. Quantification of BRIS soil bacteria at Tembila, Besut Terengganu. Agrivita, 39(3): 252-256. https://doi.org/10.17503/agrivita.v39i3.1292
Nafis, A., Raklami, A., Bechtaoui, N., Khalloufi, F.El, Alaoui, A.El, Glick, B.R., Hafidi, M., Kouisni, L., Ouhdouch, Y. & Hassani, L. 2019. Actinobacteria from extreme niches in Morocco and their plant growth-promoting potentials. Diversity, 11(8): 139. https://doi.org/10.3390/d11080139
Ozturk, S. & Ercisli, S. 2006. Chemical composition and in vitro antibacterial activity of Seseli libanotis. World Journal of Microbiology and Biotechnology, 22(3): 261-265. https://doi.org/10.1007/s11274-005-9029-9
Paul, S., Saha, S., Panigrahi, S., Sengupta, D., Das, S., Chatterjee, A., Chattopadhyay, D., Manna, A., Roy, S., Khanra, R., Tripathy, S., Ghosh, C. & Khanra, K. 2024. Antimicrobial activity, phytochemical screening by HPTLC, FTIR and GC-MS of the extracts of *Cissus quadrangularis* (L). African Journal of Biomedical Research, 27(1S): 1203-1220. https://doi.org/10.53555/AJBR.v27i1S.1212
Ríos, J.L. & Recio, M.C. 2005. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, 100(1-2): 80-84. https://doi.org/10.1016/j.jep.2005.04.025
Rosandy, A.R., Ishak, S.S.O., Sabri, N.A., Ahmad, W.Y.W. & Al Muqarrabun, L.M.R. 2021. Antibacterial activity of lupeol from the bark of *Dehaasia cuneate* (Lauraceae). Current Research on Biosciences and Biotechnology, 2(2): 145-148. https://doi.org/10.5614/crbb.2021.2.2/BOFY6724
Safini, I.N.M., Zakaria, N.F.S., Saad, M.I.H., Yahya, M.F.Z. & Jamil, N.M. 2024. Understanding bacterial persistence under antibiotic pressure: a review. Science Letters, 18(2): 56-69.
Selim, M.S.M., Abdelhamid, S.A. & Mohamed, S.S. 2021. Secondary metabolites and biodiversity of actinomycetes. Journal of Genetic Engineering and Biotechnology, 19(1): 72. https://doi.org/10.1186/s43141-021-00156-9
Sivasankar, P., Rekadwad, B., Poongodi, S., Sivakumar, K., Venkateswaran Parli, B. & Kumar, N.A. 2018. Bioinformatics delimitation of the psychrophilic and psychrotolerant actinobacteria isolated from the Polar Frontal waters of the Southern Ocean. Data in Brief, 18: 576-584. https://doi.org/10.1016/j.dib.2018.03.014
Tamura, K., Stecher, G. & Kumar, S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution, 38(7): 3022-3027. https://doi.org/10.1093/molbev/msab120
Vijayakumar, R., Muthukumar, C., Thajuddin, N., Panneerselvam, A. & Saravanamuthu, R. 2007. Studies on the diversity of actinomycetes in the Palk Strait region of Bay of Bengal, India. Actinomycetologica, 21(2): 59-65. https://doi.org/10.3209/saj.SAJ210203
Wang, Y.C., Li, W.Y., Wu, D.C., Wang, J.J., Wu, C.H., Liao, J.J. & Lin, C.K. 2011. In vitro activity of 2-methoxy-1, 4-naphthoquinone and stigmasta-7, 22-diene-3β-ol from *Impatiens balsamina* L. against multiple antibiotic-resistant *Helicobacter pylori*. Evidence-Based Complementary and Alternative Medicine, 2011: 704721. https://doi.org/10.1093/ecam/nep147
Yahya, M.F.Z.R., Jalil, M.T.M., Jamil, N.M., Nor, N.H.M., Alhajj, N., Siburian, R. & Majid, N.A. 2025. Biofilms and multidrug resistance: an emerging crisis and the need for multidisciplinary interventions. Frontiers in Bioengineering and Biotechnology, 13: 1625356. https://doi.org/10.3389/fbioe.2025.1625356