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# Taxonomic characterization and isolation of antitrypanosomal compound from *Streptomyces* sp. FACC-A032 isolated from Malaysian forest soil

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## ABSTRACT

**Aims:** The present study is aimed at taxonomic characterization and isolation of active compound MS01 from *Streptomyces* sp. FACC-A032 which exhibited strong antitrypanosomal activity ($IC_{50}$ 0.02 µg/mL).
**Methodology and results:** Isolate FACC-A032 was characterized based on its cultural, morphological, physiological and genomic properties. Isolate FACC-A032 was tentatively identified as *Streptomyces* sp. Biochemical analysis of diaminopimelic acid (DAP) isomer of whole-cell hydrolysates further confirmed the isolate FACC-A032 that contained LL-DAP isomer as species belonging to the genus *Streptomyces*. The inoculum for submerged cultures of isolate FACC-A032 was prepared from cultures on ISP2 agar. After eight days of growth at 28 ± 2 °C and 200 rpm in fermentation medium M3, fermentation broth was extracted with butanol and the crude extracts (solvent layer) were separated and dried *in vacuo*. Further studies were carried out to isolate the active compound from the culture extracts of isolate FACC-A032. Using bioassay-guided isolation, crude extract was partitioned based on different polarity. After which, the resulting elutes were tested for antitrypanosomal activity. The active fraction was analyzed with HPLC-DAD analysis. Based on the analysis, major peak in the active fraction was collected using HPLC preparative. Active compound MS01 was isolated and structure elucidated using NMR spectroscopy.
**Conclusion, significance and impact of study:** Bioassay-guided isolation techniques used in this study had discovered an active antitrypanosomal compound, staurosporine, from *Streptomyces* sp. FACC-A032. This is the first discovery of staurosporine, a protein kinase inhibitor, from Malaysian soil actinobacteria *Streptomyces* sp. Therefore, the study demonstrated the potential of Malaysian soil actinobacteria as antitrypanosomal therapeutic agent.

*Keywords:* Antitrypanosomal, Streptomyces, Malaysian soil actinobacteria, bioassay-guided isolation, Staurosporine

## INTRODUCTION

African trypanosomiasis is caused by the protozoan parasites *Trypanosoma brucei* infection and this disease still remains as a serious health problem to humans and farm animals on the WHO list of neglected tropical diseases. Most pharmaceutical industries have declined their investment in drug development for trypanosomiasis because this disease affects populations who do not represent a profitable market. Thus, the option for new lead compound which are safer and effective is an urgent need since the currently registered drugs are limited due to age, toxicity, difficulty to administer, cost and all current treatments suffer from significant drawbacks (Abdel Sattar *et al.*, 2009).

Actinobacteria are well known as secondary metabolite producers and also a group of physiologically diverse bacteria. This diversity is seen both in the production of extracellular enzymes and secondary metabolic products that they synthesize and excrete. Many of these products are antibiotics and have a high pharmacological and commercial interest. There are previous published findings of several microbial compounds which have been shown to exhibit antitrypanosomal activity. For example, Isyiyama *et al.* (2008) discovered two compounds from soil microorganism KS-505a and alazopeptin, which showed potent antitrypanosomal activity with $IC_{50}$ values of 1.03 and 0.51 µg/mL respectively against *T. brucei* strain GUT at 3.1.

The genus *Streptomyces* is the most noteworthy producers of bioactive compound. It is a prolific producer of structurally diverse bioactive metabolites and has yielded some of the most important products in the drug industry including erythromycin, kanamycin, tetracyclines, gentamicin and other classes of antibiotics. They have provided over two-thirds of the naturally occurring antibiotics discovered and continue to be a major source of novel and useful compounds (Berdy, 2005). In a previous study to search for antitrypanosomal compounds from soil actinobacteria, we successfully identified a number of isolates showing strong activity (Lili-Sahira *et*

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*al.*, 2013). The most active isolate, *Streptomyces* sp. FACC-A032, produced metabolites with strong antitrypanosomal activity ($IC_{50}$ 0.23 ug/mL) in culture broth. In the present study, *Streptomyces* sp. FACC-A032 was characterized based on its taxonomic characteristics and the active compound from the crude extract was identified.

## MATERIALS AND METHODS

### Actinobacteria inoculum and test organism

Isolate FACC-A032 was isolated from soil samples collected at the Penang National Park (Getha *et al.*, 2008). Pure culture of the isolate which was maintained in cryovials containing 20% glycerol, at the FRIM Actinobacteria Culture Collection (FACC) laboratory was revived for this study. An aliquot of 10 µL of culture suspension was transferred from the cryovials and aseptically lawned on yeast extract-malt extract agar (ISP2) (Shirling and Gottlieb, 1966). The plate was then incubated at 28 ± 2 °C for seven days. The test organism for the antitrypanosomal assay, *T. brucei* strain BS221 was obtained from the Swiss Tropical Institute, Basel (Jean-Robert *et al.*, 2009). The trypanosome culture was grown in minimum essential medium (MEM) with Earle's salts (powder, GIBCO) supplemented with 25 mM HEPES, 1 g/L additional glucose, 2.2 g/L $NaHCO_3$, and 10 mL/L MEM non-essential amino acids (100×). The medium was further supplemented with Balz supplement (Raz *et al.*, 1997), 0.2 mM 2-mercaptoethanol and 15% heat inactivated fetal bovine serum. Cultures were maintained in 24-well tissue culture plates and incubated at 37 °C and 5% $CO_2$ according to the methods described by Lili-sahira *et al.* (2013).

### Phenotypic characterization

*Cultural and morphological characterization*

Isolate FACC-A032 was streaked on different media such as ISP2, ISP3 and ISP4 agar (Shirling and Gottlieb, 1966). Plates were incubated at 28 ± 2 °C for 7 to 14 days. After incubation, the colour of aerial mycelium or surface growth, substrate mycelium as viewed in the reverse side of agar plate and diffusible pigments were observed. An in-house colour chart was used for morphological characterization and this colour chart was based on NBS/ISCC Colour System (Mundie, 1995). The spore chain type was observed using slide culture techniques. Sterile cover slips were inserted at an angle of 45° in the isolates growth medium. After incubation for 14 days, mycelia adhering to cover slips were removed and transferred to a microscope slide with the upper surface of coverslips mounted in 5 µL of 1% NaCl. Slide was examined at 600× magnification using light microscope.

### Physiological characterization (pH, salt and temperature tolerance)

The effect of pH on the growth of actinobacteria isolate FACC-A032 was studied using MBA agar (Jones, 1949) with pH ranging from 4.0 to 10.0. For the salt tolerance, ISP4 agar was supplemented with 0 to 10% (w/v) of NaCl. Plate was inoculated with a loop-full of spore suspension and plate with pH value of seven and plate without NaCl were assigned as controls. All the plates were incubated at 28 ± 2 °C for 14 days. After incubation, growth was observed and compared with control plates. The ability of isolate to grow on MBA agar with different temperatures of 4°, 10°, 28° and 45 °C was also studied. Plates were incubated at 28° and 45 °C for 14 days while growth at 4 °C and 10 °C is read after six weeks of incubation.

### Biochemical characterization

The type of diaminopimelic acid (DAP) in actinobacteria cell wall was determined by TLC using a method described by Hasegawa *et al.* (1983). Isolate FACC-A032 was grown on ISP2 agar at 28 ± 2 °C for 7 days (*Streptomyces*) and 14 days (non-*Streptomyces*). The aerial and substrate mycelia of one or two colonies were scraped from the agar plates. These were then hydrolyzed in 0.1 mL of 6N HCl in sealed test tubes. The tubes were autoclaved 15 min and cooled at room temperature. Spotting of cellulose plates with whole cell hydrolysate was done using a capillary tube. About 10 µL of the hydrolysate was spotted on a thin cellulose sheet (Cat no. 105577, Merck). A spot of 10 µL mixture of 0.01M DL-DAP that contained isomers LL-DAP and meso-DAP was spotted at the same sheet as a standard. Hydrolysates and standard were then dried under a stream of warm air furnished by a hair dryer. The TLC sheet was developed in a solvent system containing methanol: distilled water: 6N HCl: pyridine (80:26:4:10, v/v). After three hours, the developed sheet was removed, dried at room temperature and sprayed with 0.2% (w/v) ninhydrin reagent, followed by heating at 100 °C until characteristic spots appeared. Based on the cell wall composition study, the isolate FACC-A032 was assigned to the genus *Streptomyces* or non-*Streptomyces*.

### Genomic characterization

Genomic DNA was extracted from the selected actinobacteria isolate using NucleoSpin Tissue Kit from Macherey-Nagel. Isolation protocols are according to the manufacturer's instruction manual. PCR reactions were carried out in 50 µL aliquots containing 5 U of *Taq* polymerase (Fermantas Corp.), 10 µL of 10X Fermantas buffer (Fermantas Corp.), 25 mM $MgCl_2$, 10 Mm dNTP, 1 µL of primers (10 µM), 1 µL of genomic DNA, and autoclaved distilled water. PCR amplification was performed with a Perkin-Elmer thermal cycler (Perkin-Elmer Corp., Norwalk, CT) programmed for the following cycles: one initial denaturation step at 95 °C for 2 min followed by 30 cycles of denaturation at 94 °C for 30 sec,

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annealing at 53 °C for 30 sec, and extension at 72 °C for 90 sec, with a final extension step at 72 °C for 10 min. After PCR, 1 µL of 5× loading buffer was mixed well with 5 µL of the PCR product and then the mixture was loaded into a 1% agarose gel and electrophoresed in 0.5× TBE buffer at room temperature for 40 min at 100 V/cm. The gel was stained with 0.5 µg of ethidium bromide for 10 to 15 min, de-stained with distilled water, and photographed. Successfully purified PCR products were sent for sequencing at the First Base Laboratories Sdn. Bhd., Shah Alam. The obtained 16S rRNA sequences were compared to sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST). Sequences of strains with maximum identity of ≥ 99% are cutoff points in species determination.

**Bioassay-guided isolation of antitrypanosomal compound**

*Fermentation and extraction of Streptomyces sp. FACC-A032 culture broth*

Isolate FACC-A032 inoculum was prepared as mentioned above. By using a sterile cork borer, two pieces of 5 mm diameter agar-plugs were cut aseptically from rich surface growth of seven days culture and inoculated into 50 mL medium M3 (Getha and Vikineswary, 2002) in 250 mL Erlenmeyer flasks. Flasks were incubated at 28 ± 2°C, and 200 rpm for three days to produce seed culture. The seed culture at a concentration of 5% (v/v) was then inoculated into 200 mL medium M3 in 1-L Erlenmeyer flasks. Six flasks with a total of 1.2 L culture broth were incubated for eight days. After incubation, the culture broth was centrifuged to separate the mycelium and culture filtrate. The filtrate was extracted with butanol BuOH (1:1, v/v) in separating funnel and the solvent layer was collected. Solvent layers were evaporated to dryness using a rotary evaporator. Crude extract was tested for antitrypanosomal activity using methods described by Lili-Sahira *et al.* (2013). Antitrypanosomal activity of the crude extract was estimated by a dose response curve using Alamar Blue® sensitivity assay according to the method of Raz *et al.* (1997).

**Isolation and elucidation of active compound**

A total of 1.15 g butanol crude extract was subjected to partitioning using solid phase extraction (SPE). Extract was adsorbed onto isolute HM-N before loading into 150 mL SPE cartridge packed with 56 g of C-18 silica gel. The column was eluted with eluent A (water with 0.1% of trifluoroacetic acid) and B (methanol with 0.1% of trifluoroacetic acid) in order of decreasing polarity. Eluates were collected in round bottom flask and dried. Each fraction was tested for antitrypanasomal activity and their profiles were recorded with HPLC-DAD analysis. Fractions which showed high antitrypanasomal activity were selected to be further purified using HPLC preparative.

In HPLC-DAD analysis, sample was prepared in methanol at a concentration of 2 mg/mL. The analysis was performed using Agilent 1200 Series HPLC system comprising a quaternary pump, dual loop auto sampler and an Agilent Diode Array Detector (DAD). The UV detector was set at 230 nm, 254 nm, and 320 nm and data was acquired using ChemStation Software. XDB-C18 column 3.5 µm (4.6 × 100 mm) was used with the temperature maintained at 25 °C. Gradient elution of the samples was performed using ammonium formate buffer (solvent A) and acetonitrile (solvent B) at flow rate of 1.2 mL/min. The gradient elution initial conditions were set at 15% of solvent B with linear gradient to 40% from the third to sixth minute. These conditions were maintained up to 12 min. After which solvent B was increase to 55% (in 7 min) and subsequently to 85% (in 3 min). At this point, the proportion of the solvent was kept for 7 min. Lastly the column was flushed by returning it to initial condition in 3 min. The sample injection volume was 50 µL.

Fractions, which were selected for HPLC preparative, were prepared in methanol at 50 mg/mL concentration. 500 µL was injected into XDB-C18 column (5 µm, 21.2 × 150 mm). Deionized water and acetonitrile were chosen as solvent A and solvent B, respectively. In order to optimize the gradient profile, solvent B was set to achieve 30% in 3 min from initial conditions of 15%. This portion was increased to 50% (in 7 min) and maintained for 10 minutes. In the next 12 min, the mobile phase must achieve 60% of solvent A and 40% of solvent B. After this condition was achieved, solvent B was decrease to 15% (in 4 min). Finally, the column was flushed out by reaching equilibrium condition in 2 min.

Active compound was prepared in deuterated methanol ($CD_3OD$) at 5 mg/mL concentration for proton nuclear magnetic resonance ($^1$H-NMR) analysis. $^1$H-NMR analysis was recorded on Bruker Avance 400 Spectrometer operating at 400 MHz. Mass spectra and fragmentation pattern of active compound was obtained using direct injection into LCQ DECA XP Plus with sample concentration prepared at 2 mg/mL.

**RESULTS AND DISCUSSION**

**Cultural and morphological characteristics of *Streptomyces* sp. FACC-A032**

Growth of actinobacteria isolate FACC-A032 on ISP2, ISP3 and ISP4 was observed after 14 days of incubation at 28 ± 2 °C. Soluble pigment was not produced by the isolate in all three agar tested. Morphological characteristic of the isolate was typical to the genus *Streptomyces* such as fast-growing colonies with thick aerial mycelium and powdery appearance. The initial white aerial mycelium of the isolate turned to brownish nearing 14 days of incubation in all agar media indicating mature sporulating aerial mycelium. The reverse colour was also brown on all agar media. The morphology of spore chains of isolate FACC-A032 was observed under light microscope as shown in Figure 1. Isolate was observed after two weeks of incubation. According to

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Ensign (1992) the morphology of aerial mycelium exhibits richness in variation when observed the length of hyphae, their type of branching, the arrangement of sporogenous hyphae and their morphology such as straight, flexous and spiral shape. Isolate FACC-A032 was fully matured with good spore formations after two weeks of incubation where its aerial hyphae were straight to irregularly curved, often with cork-screw shape (Figure 1).

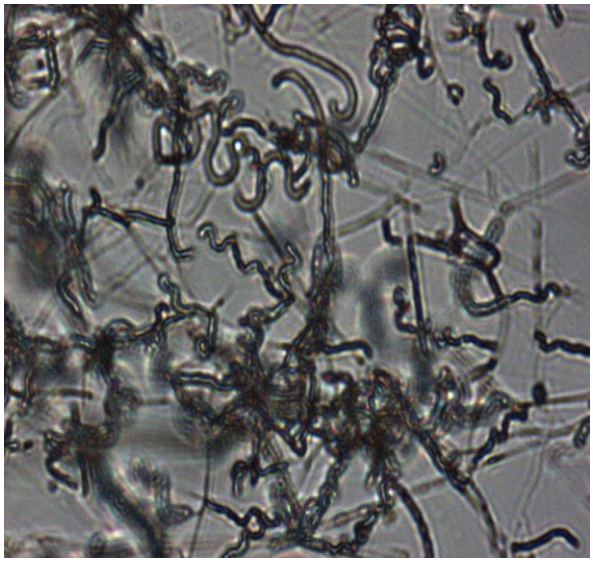

**Figure 1:** Light micrograph of spore chain structure of 14 days old FACC-A032 cultures observed under 600x magnifications.

**Physiological characterization**

The optimum growth of isolate FACC-A032 was observed at pH 6.0 to 8.0 (Table 1). The isolate failed to grow at pH 4.0 and weak growth was observed at pH 5.0, 9.0 and 10.0. Only some bacteria groups can grow at extreme pH values of less than two or greater than 10. Organisms that live at low pH are types of extremophiles called acidophiles, while those in a high pH are known as alkaliphiles. However, the majority of the microorganisms are referred to as neutrophiles, where the optimum pH for growth is between pH 6.0 to 8.0 (Madigan *et al.*, 2000). Goodfellow and Williams (1983) stated that most soil actinobacteria behave as neutrophiles in culture, growing between pH 5.0 and 9.0 with an optimum close to neutrality. This statement is in accordance with the pH range 6.0 to 8.0 that was observed in growth for isolate FACC-A032. Thus, the isolate obtained in this study could be classified as neutrophilic actinobacteria.

Isolate FACC-A032 exhibited higher tolerance towards NaCl, with growth up to 5% NaCl (Table 1). Good growth of isolate was observed at 0 to 2% NaCl and isolate fail to grow in NaCl ranging between 6 to 10%. Tresner *et al.* (1968) suggested the salt tolerance test as a criterion in the classification of *Streptomyces*. This test has become widely accepted as a method to recognize the capability of *Streptomyces* to grow in the presence of various concentration of sodium chloride (Kavitha and Vijayalaksmi 2007; Reddy *et al.*, 2011). Kavitha and Vijayalaksmi (2007) had reported that *Streptomyces rochei* was able to grow in media containing NaCl up to 7% and Reddy *et al.* (2011) reported the same strain exhibited salt tolerance up to 6%. Both studies suggested that the isolate can be placed in moderate salt tolerance group. It is known that microorganisms have the ability to tolerate high concentrations of NaCl in their substrate media (Tresner *et al.*, 1968). Based on the results obtained in this study, *Streptomyces* isolate FACC-A032 was able to grow in media containing NaCl up to 5%.

**Table 1:** Morphological, physiological and biochemical characteristics of isolate FACC-A032.

| Characteristics | Results |
|---|---|
| Aerial mycelium colour | Brown |
| Substrate mycelium colour | Brown |
| Diffusible pigment produced | No |
| Spore chain morphology | Hooks, Cork-screw |
| Growth at: | |
| pH 4 | - |
| pH 5 | + |
| pH 6-8 | ++ |
| pH 9-10 | + |
| NaCl (0-2) | ++ |
| NaCl (3-5) | + |
| NaCl (6-10) | - |
| 4 °C** | - |
| 10 °C** | - |
| 28 °C* | ++ |
| 45 °C* | + |
| DAP isomer in whole cell hydrolysate | LL-DAP |

*Observed after 14 days of incubation. **Observed after 6 weeks of incubation.
–, No growth; +, Growth weak then control; ++, Growth similar or dense than control.

The temperature tolerance of isolate FACC-A032 exhibited good growth at temperature 28 ± 2 °C but weak growth was observed at 45 ± 2 °C (Table 1). Isolate was unable to grow at low temperatures of between 4 °C and 10 °C. Temperature is one of the most important environmental factors affecting growth and survival of microorganisms (Madigan *et al.*, 2000). Most of the actinobacteria behave as mesophiles in the laboratory, with maximum growth at 25 °C to 30 °C (Goodfellow and Williams, 1983). However, some actinobacteria have the ability to grow at temperatures above the mesophilic range of 50 °C to 60 °C. Chun *et al.* (1999) reported that

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**Table 2:** Closest match from GenBank using BLAST to *Streptomyces* sp. FACC-A032.

| Strain | Closest matches | Accession number | % similarity |
|---|---|---|---|
| FACC-A032 | *Streptomyces filipinensis* strain NRRL 2437 | NR_115456.1 | 99 |
| | *Streptomyces durhamensis* strain CSSP538 | NR_043352.1 | 99 |
| | *Streptomyces achromogenes* strain NBRC 12735 | NR_112251.1 | 99 |

**Table 3:** Macromorphology comparison between different literature on a species closely related.

| Isolate | Agar medium | Growth | Surface colour | Reverse colour | Soluble pigment | Spore chain type |
|---|---|---|---|---|---|---|
| *Streptomyces* sp. FACC-A032 | ISP2 | Good | Brown | Brown | None | Hooks, Cork-screw |
| *Streptomyces filipensis* strain NRRL 2437 | ISP2 | Good[1] | Brownish grey[1] | Dark brown[1] | Dark brown[1] | Brunched spirals[1] |
| *Streptomyces durhamensis* strain CSSP538 | ISP2 | Good[2] | Telegrey[2] | Ochre brown[2] | None[2] | Hooks, open and closed loops[2] |
| *Streptomyces achromogenes* strain NBRC 12735 | ISP2 | Good[3] | Cream[3] | Maize yellow[3] | None[3] | Straight (smooth spores)[4] |

References: [1] Zimmerman *et al.*, 1978; [2] Gordon and Lapa, 1966; [3] Okami and Umezawa, 1953; [4] Tresner *et al.*, 1961.

the soil actinobacteria *Amycolatopsis thermaflava* can grow at a temperature of 55 °C which represents the thermophilic actinomycetes. In this study, isolate FACC-A032 could be classified as mesophiles actinobacteria. Although the isolate was identified based on morphological and physiological characteristics, cell wall analysis was carried out to confirm the classification of this isolate into the *Streptomyces* or non-*Streptomyces* group.

**Biochemical characterization**

One of the biochemical properties that have been found to be practical for the separation of *Streptomyces* from other genera of the actinobacteria is determination of diaminopimelic acid (DAP) isomers in bacterial cell wall where the occurrence of LL-DAP or meso-DAP can be established by thin-layer chromatography (TLC) of whole cell hydrolysates (Korn-Wendich and Kutzner, 1992). In this study, TLC of whole-cell hydrolysates of isolate FACC-A032 contained LL-DAP isomer (Table 1). Bacteria generally contain one of the isomers, the LL-form or meso-form, mostly located in the cell wall peptidoglycan (Sivakumar, 2001). The LL-DAP is characteristic of the genus *Streptomyces*, whereas meso-DAP is found in members of other genera. Coupled with results of morphological examinations, it is permitted a clear assignment that the *Streptomyces* isolate FACC-A032 with LL-DAP are members of the genus *Streptomyces*.

**Genomic characterization**

Based on BLAST maximum identity, strain FACC-A032 showed closest 16S rRNA sequence similarities with a total of three species belonging to the *Streptomyces* genus as listed in Table 2. However, it was only 99% of similarities. Thus, a comparison was done on the morphology characteristics between the test strain and the three different species based on reports from previous studies (Table 3). The test isolate showed closest similarity in terms of macromorphology to *Streptomyces durhamensis*, with similar spore chain structure, reverse colour and absence of soluble pigments on ISP2 agar (Table 3). Although the determination of bacterial species must be made by a hybridization test of genomic DNA and the homology value of the 16S rDNA sequence does not become the determination criterion for species, there is close correlation between these two species.

**Bioassay-guided isolation and identification of active compound**

Antitrypanosomal assay revealed that crude extract FACC-A032 have $IC_{50}$ 0.23 µg/mL. From fractionation of this crude, a total of ten eluates were collected and tested for activity. Fraction 1Fr3 showed high $IC_{50}$ activity 0.56 µg/mL. From HPLC analysis (Figure 2), one peak (peak MS01) at retention time 7.2 min was detected. This peak was further purified using HPLC preparative to give MS01

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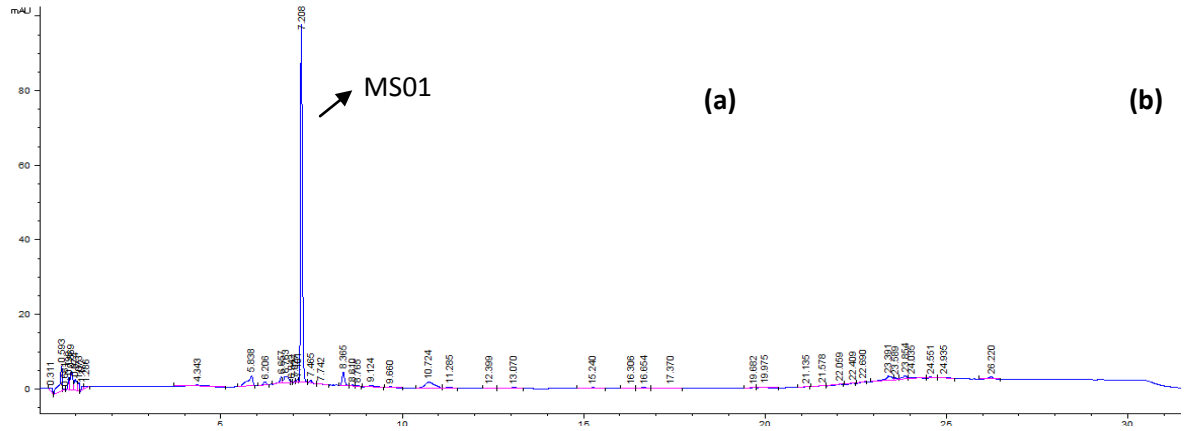


**Figure 2:** HPLC chromatogram of active fraction 1Fr3.

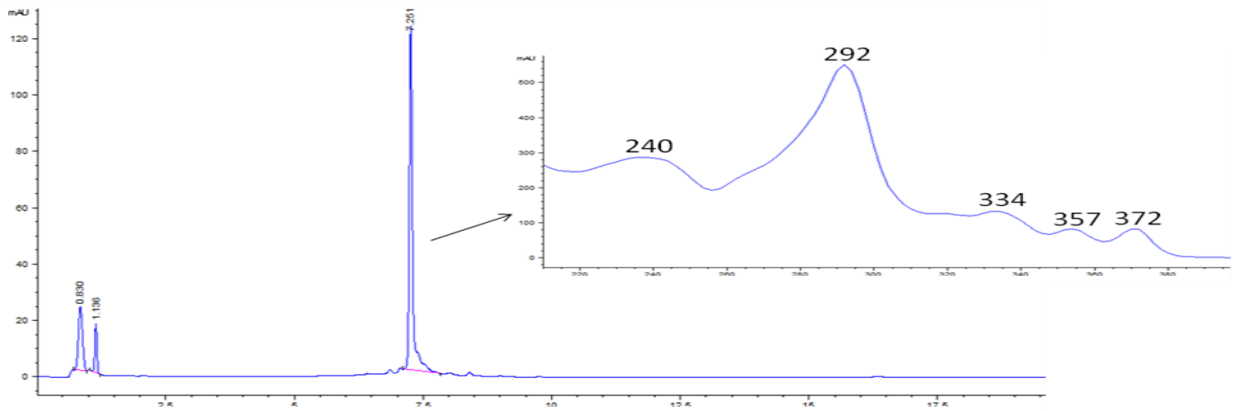


**Figure 3:** HPLC chromatogram of active compound MS01 (a) and UV visible spectra of peak at 7.2 min with detection at the λ max at 292 nm (b).

as a light yellow solid in 8.2 mg with $IC_{50}$ 0.02 µg/mL (Figure 3a). The isolation of MS01 was summarized in Figure 4.

UV analysis of MS01 (Figure 3b) showed absorption maxima at 240, 292, 334, 357, and 372 nm which showed a similarity with a choromophore of an indolo[2,3a]carbazole system (Figure 5a) (Koshino *et al*., 1991; Fu *et al.*, 2012). The existence of an indolo[2,3a]carbazole system explained the signals at 7.0 – 8.0 ppm, observed in 1H NMR of MS01 (Table 4) which was assigned for proton in the benzene ring. The NMR data also suggested the existence of amide functional group which was indicated by signal at 6.75 ppm. A singlet at 9.48 ppm indicated a proton from benzene ring that interacts with carbonyl from amide functional group. Three methyl singlets were also observed at 3.40, 2.38, and 1.50 ppm. The difference in chemical shifting value, point out that each methyl group have a different chemical environment.

The 1H NMR data of MS01 was compared with staurosporine (Figure 5b), the first reported indolocarbazole compound isolated from cultures of *Streptomyces staurosporeus* AM-2282 (Omura *et al*., 1977; Sanchez *et al*., 2006) (Table 4). From this table, it can be seen that the chemical shift of MS01 and staurosporine is very similar. UV characteristic of staurosporine standard compound was detected at UV maximum of 292 nm (Freel *et al.*, 2011). This, together with shoulder peak pattern, is comparable to UV spectra of MS01. Analysis on the ESI-MS fragmentation pattern of MS01 ($[M+H]^+$ *m/z* 465) (Figure 6) corroborated the fact that compound MS01 is indeed staurosporine (Yang and Cordell, 1997).

Studied showed that staurosporine, as protein kinase inhibitor, can induce apoptosis in uni- and multicelullar organisms including trypanosoma parasites (Arnoult *et al*., 2002; Johansson *et al*., 2003). Due to this, staurosporine is also known for its anticancer properties (Sun *et al.*, 2011; Burgos-Díaz *et al.*, 2013; Fares *et al.*, 2014; Moela *et al.*, 2014). The compound acts as an inhibitor by binding to the adenosine triphosphate (ATP) binding site (Leclercq *et al*., 2013). Another factor that contributes to

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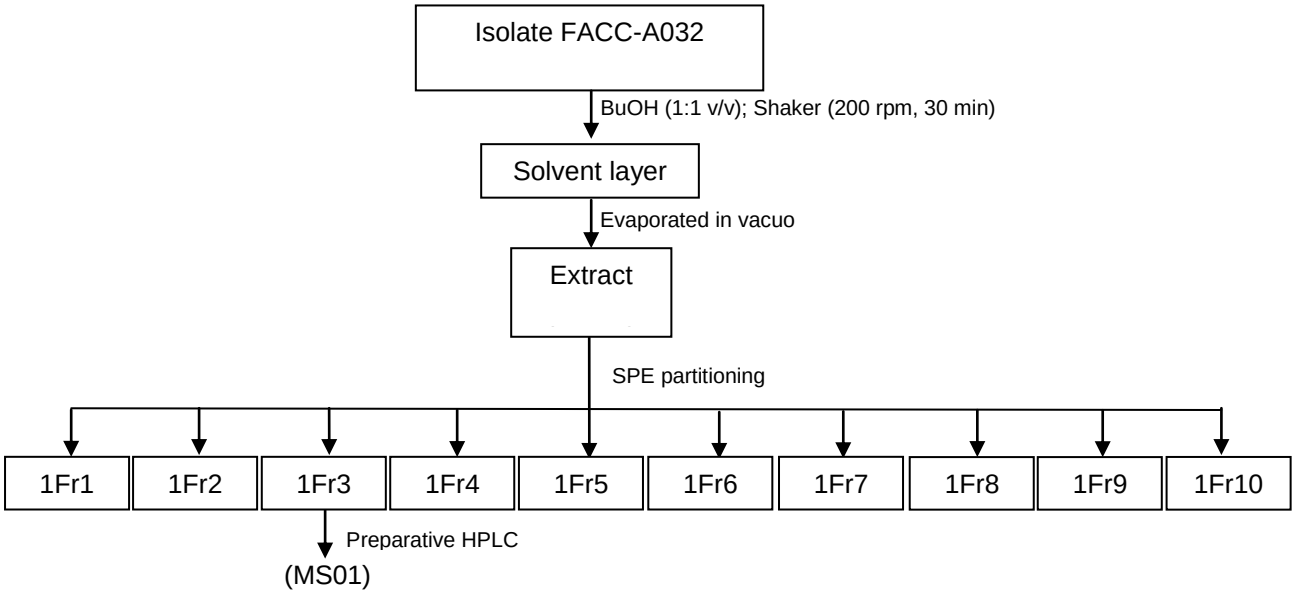


**Figure 4:** Bioassay-guided separation of active compound from culture filtrate extract of *Streptomyces* sp. FACC-A032.

its strong anticancer activity is inhibition of eukaryotic DNA topoisomerase (Lassota *et al*., 1996; Hasinoff *et al*., 2012). Topoisomerase catalyses are processes such as DNA replication, transcription, recombination and repair of strands (Pommier, 1998; Meng *et al*., 2003). These processes are interrupted by staurosporine through inhibition of the active tyrosine site (Lassota *et al*. 1996). Using these protein as a potential targets for staurosporine, further study to look at new derivatives of the compound as antitrypanosomal drug is an interesting subject to be carried out.

## CONCLUSION

Actinobacteria isolate FACC-A032 from Malaysia soil which produces strong antitrypanosomal activity was identified as *Streptomyces* sp. This isolate was identified based on taxonomic characterization. Bioassay-guided isolation of crude extract successfully determined one active compound MS01 ($IC_{50}$ 0.02 µg/mL). MS01 was identified as staurosporine based on its spectrometry and spectroscopy data. Based on previous literature search, this is the first discovery of staurosporine, a protein kinase inhibitor, from Malaysian soil actinobacteria *Streptomyces* sp. This study stresses the potential of *Streptomyces* sp. FACC-A032 as an antitrypanosomal therapeutic agent.

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