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# Antimicrobial, antidermatophytic, and cytotoxic activities from *Streptomyces* sp. MER4 isolated from Egyptian local environment

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## Abstract

**Background:** Recently, actinomycetes have attracted the attention of researchers worldwide. They can produce secondary metabolites with antibacterial, antifungal, antiviral, and antitumoral properties.

**Results:** *Streptomyces* sp. MER4 (accession no. KM099241) was isolated from the pyramid region, Giza, Egypt. This strain was previously mentioned for its ability to produce antidermatophytic bioactive metabolites. Scale-up fermentation and fractionation of the extract has been established using different solvents. The ethanol fraction exhibited a considerable antidermatophytic effect (19.8, 21.2, and 20.3 mm for *Trichophyton mentagrophytes*, *Microsporum canis*, and *Microsporum gypseum*, respectively), antimicrobial (10, 8, 7, 9, and 9 mm for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Bacillus subtilis*, and *Aspergillus niger*, respectively), and cytotoxic activity (inhibition of 78.48%). Further purification of the ethanol fraction was done, and one promising compound was produced. This compound was intensely characterized and elucidated by studying its spectral data including nuclear magnetic resonance (NMR) and liquid chromatography mass spectrometry (LC/MS). The produced compound was identified as JBIR-58 (salicylamide) derivative compound.

**Conclusions:** *Streptomyces* sp. MER4 has been identified genetically and screened for its ability to produce bioactive compound with antibacterial antitumor and antidermatophytic agent. The scale-up fermentation, purification, and structure elucidation led to pure compound named salicylamide derivatives JBIR-58 from the fermentation broth of *Streptomyces* sp. MER4.

**Keywords:** *Streptomyces* sp., Antimicrobial, Antidermatophytic, Cytotoxicity, Salicylamide derivative (JBIR-58)

## Introduction

Actinomycetes have been known as a group of the major soil population. They are widespread in nature and found in dry than wet soil (Wellington et al. 1992). Actinomycetes are good producers of wide diversity of structurally unique bioactive secondary metabolites with various biological activities including therapeutically and agriculturally important compounds (Tanaka and Omura 1993). Furthermore, they can produce a group of secondary

metabolites, many of which have antibacterial or antifungal properties. *Streptomyces* is the major genus of Actinobacteria and in the family Streptomycetaceae (Hong et al., 2009). More than 500 species of *Streptomyces* have been known and identified (Lee et al. 2005). *Streptomyces* species are chemoorganotrophic in their mode of nutrition, filamentous Gram-positive bacteria but not acid-alcohol fast. Although they occur in the same habitat of fungi and are superficially similar, streptomycetes are not fungi (Ikeda et al. 2003). They have genomes with high-GC content 69–78% (Kavitha et al. 2010). The filaments and spores are tiny usually 1 μm or less in diameter (Willemse et al. 2011). The colonies are slow-growing and often have a soil-like smell because of production of geosmin, a

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volatile metabolite (Jüttner and Watson 2007). The most important characteristic of streptomycetes is their potency to produce secondary metabolites with antibacterial, anti-fungal, antiviral, and antitumoral properties. *Streptomyces griseus* and *Streptomyces coelicolor* are used for industrial production of Streptomycin and novel antibiotics such as dihydrogranticin respectively. Streptomycetes produced Doxorubicin as anticancer agents (Mukhtar et al. 2012) and the immunomodulatory agents Rapamycin. Another metabolite of *Streptomyces* known as the “Geosmin” and sidrophore are responsible for the earthy odor (Sangler et al. 1993). However, volatile product secreted by *Streptomyces* may also be responsible for the specific smell (Bais et al. 2012). The most antibiotics developed for human pharmaceutical use represents about 50% of all known antibiotics and are mainly come from actinomycete metabolites especially from *Streptomyces* spp. (Goodfellow & Williams, 1983, Hopwood 2007). The genus *Streptomyces* was first illustrated by Waksman and Henrici (1943) and is considered as a rich source of bioactive natural products which are extensively used as pharmaceuticals (Eguira et al. 2005). Genus *Streptomyces* alone produces a large number of bioactive molecules. It has an enormous biosynthetic potential that remains unchallenged without a potential competitor among other microbial groups. A large number of *Streptomyces* spp. have been isolated and screened from soil in the past several decades (Watve et al. 2001).

Dermatophytes are a group of fungi that exist and live on keratin substrate and thus called “Keratinophilic fungi.” These fungi were considered as the etiological agents responsible for causing skin infection (Deepika and Kannabiran 2010). Dermatophytes invade skin, hair, and nails caused as a result of fungal colonization of the keratin-containing layers of the body. Three fungal genera are mainly responsible for keratin colonization namely *Trichophyton*, *Microsporum*, and *Epidermatophyton* (Emmons et al. 1977; Luilma et al. 2005).

The work is undertaken with the aim of isolating different *Streptomyces* strains from the Egyptian habitats and screening them for their ability to produce bioactive compounds with antidermatophytic effects. Also, isolation, purification, and structure elucidation of promising compound (s) could take place. The promising *Streptomyces* strain could also be molecularly identified.

## Materials and methods

### Chemicals

All reagents used were of analytical grade, and solvents were of spectroscopic grade; methanol, ethanol, dichloromethane, and ethyl acetate HPLC grade (Fischer Chemical, UK). All other media components, analytical grade chemicals, and reagents were obtained from Sigma

Chemical Co., USA, and Qualikemes Fine Chem Pvt. Ltd., India.

### Sampling

Samples were collected during 2011, between June and December 2011 from soil surroundings of pyramid area. Samples were collected, kept in sterile tubes in refrigerator until processed in laboratory.

### Isolation of streptomycetes

Isolation of streptomycetes from soil samples has been done using the serial dilution method of Hayakawa and Nonomura (1987). Suspensions were plated for isolation of streptomycetes. Petri dishes were prepared 1 day before plating and incubated at room temperature overnight to eliminate films of moisture on the agar surface; 0.1 ml inocula of the proper dilution was placed on each plate and spread with a sterile glass rod using starch nitrate agar medium for isolation of streptomycetes. This medium contains (g/l) starch, 20.0; KNO<sub>3</sub>, 2.0; K<sub>2</sub>HPO<sub>4</sub>, 1.0; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5; NaCl, 0.5; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.01; CaCO<sub>3</sub>, 3.0; agar, 20.0; and distilled water 1000 ml. The pH was adjusted to 7.2. The inoculated plates were incubated at 28 °C and examined for streptomycete growth after 7–10 days. The isolation of streptomycetes based on their special morphological characteristics (deep sitting colonies, sporulation, characteristic color, etc.) The plates that showed countable single colonies were selected and purified by streak plate technique.

### Molecular identification of *Streptomyces* isolate P4

*Streptomyces* isolate (P4) was identified according to a molecular biological protocol by DNA isolation, amplification (PCR), and sequencing of the ITS region. The primers 27F (AGAGTTTGA TCM TGG CTC Ag) and 1492R (TAC GGY TAC CTT GTTACG ACT T) were used at PCR. The purification of the PCR products was carried to remove unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore). Sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA).

### Extraction of crude secondary metabolite from actinomycetes

Spore suspensions of the candidate actinomycete strains were inoculated in 1 L Erlenmeyer flasks each containing 250 ml ISP2 broth medium of the following constituents (g/l): yeast extract, 2.0; malt extract, 1.0; and dextrose, 0.5. The flasks were incubated on shaker at 150 rpm rotation at 30 °C for 10 days. The cultures were centrifuged at 4 °C, 5000 rpm for 30 min. The culture

supernatant was extracted with 500 ml ethyl acetate and concentrated in rotary vacuum (Augustine et al. 2005).

#### Determination of antidermatophytic activity of crude extract from actinomycetes

The test fungal cultures *Trichophyton mentagrophytes* (RCMB 09285), *Microsporium canis* (RCMB 07321), and *Microsporium gypseum* (RCMB 07336) obtained from Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University. The antidermatophytic activity of ethyl acetate extract was tested using the agar well diffusion method (Tepe et al., 2004), 100  $\mu$ l of the actinomycetes broth culture was placed in the wells made with a sterile cork borer on Sabouraud dextrose agar plates (pH 5.6) seeded with the test fungal cultures *Trichophyton mentagrophytes* (RCMB 09285), *Microsporium canis* (RCMB 07321), and *Microsporium gypseum* (RCMB 07336). The plates were incubated at 28 °C and observed for antibiosis after 3–4 days (Augustine et al. 2005). Amphotericin B was used as controls. The Sabouraud dextrose agar (Fluka) medium contains (g/l) peptone extract, 1.0; dextrose, 0.5; and agar, 20.0. The pH was adjusted to 5.6.

#### Determination of antimicrobial activity

The obtained extract was dissolved in 10% dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) in methanol (MeOH) at a concentration of 1 mg/ml. Aliquots of 50  $\mu$ l were soaked on filter paper discs (5 mm, Whatman no.1 filter paper) (Bauer et al. 1966) and dried at room temperature under sterilized conditions. The paper discs were placed on agar plates seeded with test microbes and incubated for 24 h at the appropriate temperature of each test organism. Both bacterial and yeast microbes were grown on nutrient agar medium. The fungal strain was on other hand grown on Czapek-Dox agar medium (DSMZ130). The culture of each microorganism was diluted by sterile distilled water to 10<sup>7</sup> to 10<sup>8</sup> CFU/ml. The inoculated agar plates were first put in the refrigerator for 2 h and then incubated for 24 h (yeast and bacteria) at 37 °C (bacteria) and 48 h (fungi) at 30 °C. After incubation, the diameter of inhibition zones was measured against a wide range of test microorganisms comprising Gram-positive bacteria (*Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC6538-P), Gram-negative bacteria (*Escherichia coli* ATCC14169 and *Pseudomonas aeruginosa* ATCC27853), yeasts (*Candida albicans* ATCC10231 and *Saccharomyces cerevisiae* ATCC9080), and the fungus (*Aspergillus niger* NRRLA-326). All test microbes were obtained from the culture collection center, Microbial Chemistry Department, National Research Center, Egypt.

#### Evaluation of the antitumor activity against Ehrlich cell

This test was performed using in vitro assay. Viability of tumor percentages of tumor cells which was measured

by modified cytotoxic Trypan blue exclusion technique of Bennett et al. (1976). Female Swiss albino mice were kept under healthy environmental and nutritional conditions for 2 weeks then injected by Ehrlich Ascites carcinoma cells (EACC).

Ehrlich ascites carcinoma cells were isolated from previously transplanted mice after 7 days of transplantation using a sterilized syringe and obtained from Dr. Mohamed El-awady, Microbial Biotechnology Department, National Research Center. The cells were used immediately for viability study and could be diluted by phosphate buffer solution (PBS) if needed. The number of tumor cells/milliliter was calculated by using the appropriate microscope technique used culture medium which prepared using Roswell Park Mark Institute 1640 (RPMI 1640) media supplemented with 10% fetal bovine serum and 10% L-glutamine.

The viability percentages of tumor cells were measured after incubation with the examined sample as well as PBS as control. Two milliliters of Ehrlich cells (1.6  $\times$  10<sup>6</sup> cells/ml) were added to 1 ml sample. Then, the tubes were incubated under an atmosphere of 5% carbon dioxide at 37 °C for 72 h. The living Ehrlich cells at the end of the 72-h incubation period were determined by a colorimetric assay based on Trypan blue solution prepared by Trypan blue (0.4%) which was dissolved in 100-ml distilled water and then kept in brown closed glass bottle. Ten microliters of trypan blue solution added to 10  $\mu$ l of sample as well as control with 80  $\mu$ l PBS solution and were mixed for count living cells. The number of living cells was calculated using hemocytometer slide, survival cells appeared as unstained bodies while non-viable cells were stained blue color. The in vitro results were expressed as the inhibition ratio of tumor cell proliferation calculated as:

$$\begin{aligned} \text{The inhibition ratio of tumor cell proliferation (\%)} \\ = [(A-B)/A] \times 100 \end{aligned}$$

where *A* and *B* are the average numbers of viable tumor cells of the control and the samples, respectively.

#### Scale-up fermentation and extraction

*Streptomyces* sp. MER4, which provide higher antidermatophyte activity, was used for scale-up fermentation on ISP2 broth medium. One-liter-volume Erlenmeyer flasks each containing 250 ml of ISP2 medium (Pridham et al. 1956-1957) were inoculated with 5 disks of agar from well-grown subculture of this strain. The flasks were incubated at 28 °C on rotary shaker (150 rpm) and harvested after 7 days. After centrifugation, the supernatant of each flask containing extracellular metabolites was extracted by ethyl acetate as mentioned above. In this study, ethyl acetate was selected for further

secondary metabolite purification and isolation. Ethyl acetate extracts were concentrated in vacuo till dryness, yielding 1.9 g of a reddish brown crude extract.

#### Purification and structure elucidation of bioactive compounds

Sephadex LH-20 column was used for the separation of different extract components. The components were separated and distributed on the stationary phase according to their molecular size. Solvent extraction and partitioning is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. Characterization and structure elucidate for the purified compound have been established using modern spectroscopic techniques including NMR,  $^{13}\text{C}$ , and LCMS.

## Results

#### Molecular identification of the most potent *Streptomyces* sp. (P4)

Sequencing of the soil isolate *Streptomyces* (P4), which displayed high-antidermatophytic activity in preliminary evaluation (Hamed et al. 2016), revealed high similarity (99%) with *Streptomyces* spp. (Fig. 1). The (P4) presented new *Streptomyces*-specific sequence which was deposited in Gene Bank as *Streptomyces* sp. MER4 (accession number KM099241). The phylogenetic tree of the *Streptomyces* sp.

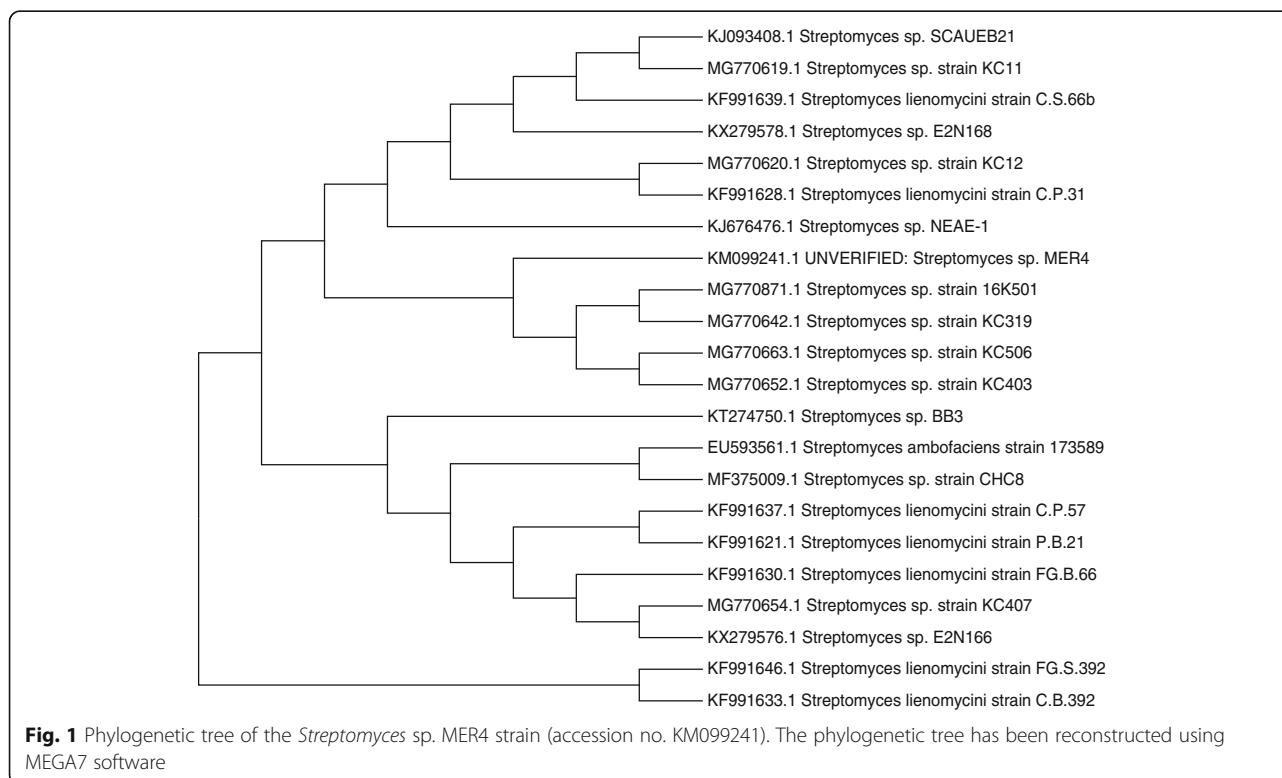
MER4 by applying the neighbor joining method is illustrated in Fig. 1.

#### Fractionation of the ethyl acetate extract from liquid culture of *Streptomyces* sp. MER4

*Streptomyces* sp. MER4 ethyl acetate extract (1.9 g) was fractionated by Sephadex LH20 column with a gradient mobile phase DCM: Methanol to yield four fractions (F1, F2, F3, and F4). F3 fractionated by liquid-liquid partition using ( $\text{H}_2\text{O}$ : n-hexane: ethanol: n-butanol) to yield another four subfractions (P4- $\text{H}_2\text{O}$ , P4-Hex, P4-but, and P4-eth). Kim et al. (2008) used Sephadex LH-20 Column for purification of protocatechualdehyde (PA) isolated from *Streptomyces lincolnensis* M-20. Figure 2 illustrated the overall fractionation, biological activities, and purification steps of the ethyl acetate extract from *Streptomyces* sp. MER4.

#### Antidermatophytic activity of subfractions

Ethyl acetate subfractions were assessed for their antidermatophytic activity (Figs. 2 and 3). Fraction (P4-eth) revealed the highest inhibition ratio against all dermatophytic test microbes with an inhibition zones of 19.8, 21.2, and 20.3 mm for *Trichophyton mentagrophytes* (RCMB 09285), *Microsporium canis* (RCMB 07321), and *Microsporium gypseum* (RCMB 07336), respectively. Fraction P4-water followed P4-eth with inhibition zones of 13.6, 17.4, and 15.2 for *T. mentagrophytes*, *M. canis*,



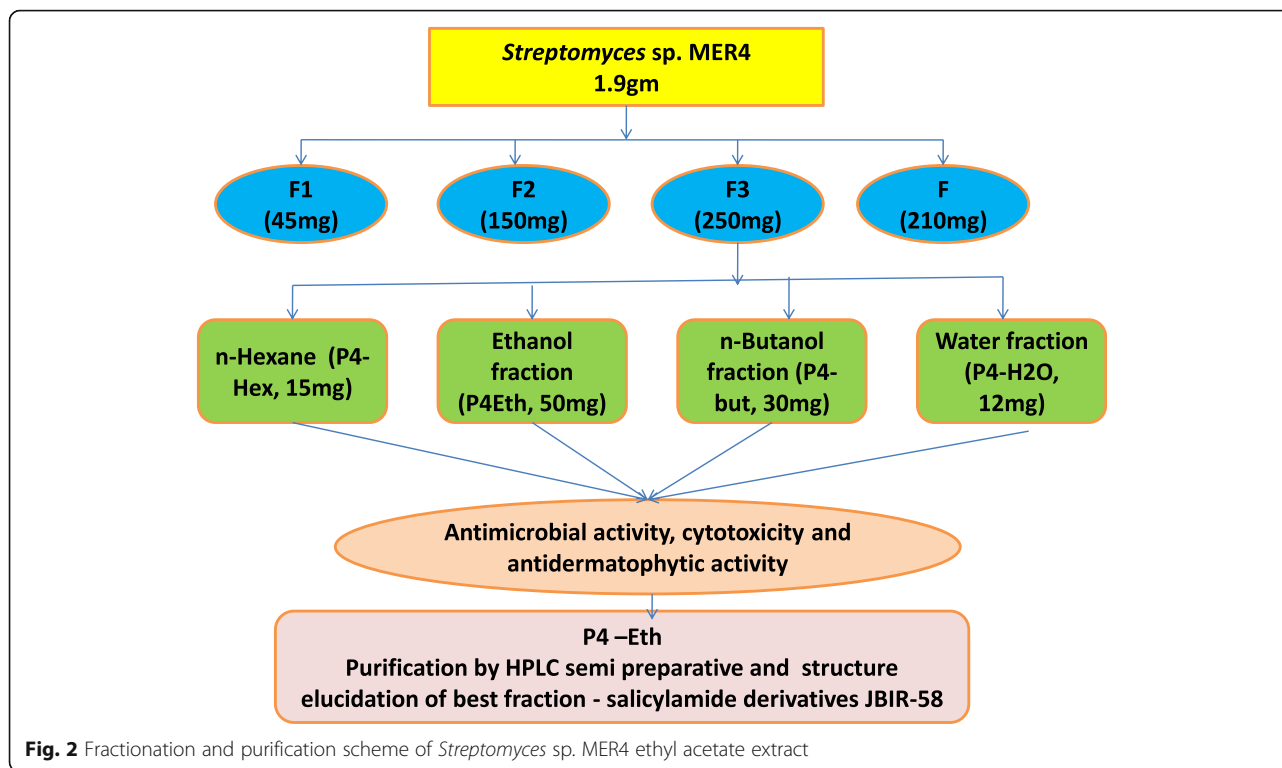


Fig. 2 Fractionation and purification scheme of *Streptomyces* sp. MER4 ethyl acetate extract

and *M. gypseum*, respectively. Moderately lower antidermatophytic effect has been reported for fraction P4-Hex with inhibition zones of 9.2, 15.2, and 12.3 mm for *T. mentagrophytes*, *M. canis*, and *M. gypseum*, respectively. Fraction P4-water did not show any activity against all tested dermatophytes. Amphotericin B was used as antidermatophytic standard, and it exhibited clear zones of 22.8, 20.3, and 26.7 mm for *T. mentagrophytes*, *M. canis*, and *M. gypseum*, respectively.

#### Antimicrobial activity of subfractions

The antimicrobial activities of the four subfractions were tested against (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *E. coli*, and *Bacillus subtilis*). It has been revealed that three fractions exhibited obvious antimicrobial activities (Figs. 4 and 5). The subfraction (P4-eth) showed antimicrobial activities against almost all test organisms *Staphylococcus aureus* (8 mm), *Pseudomonas aeruginosa* (10 mm), *Candida*

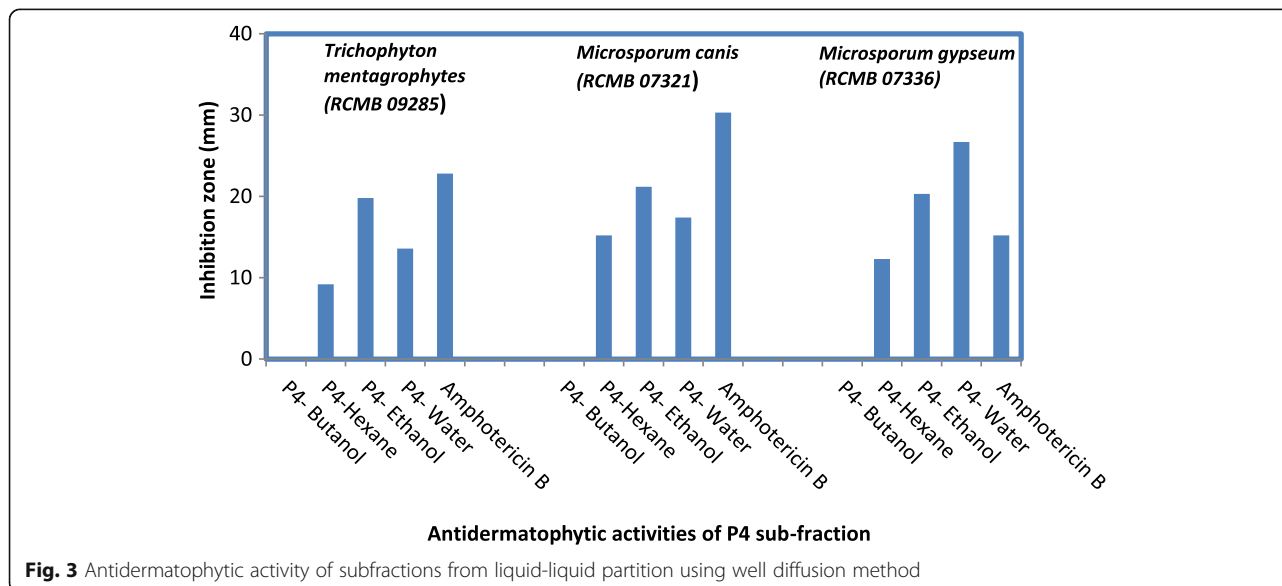
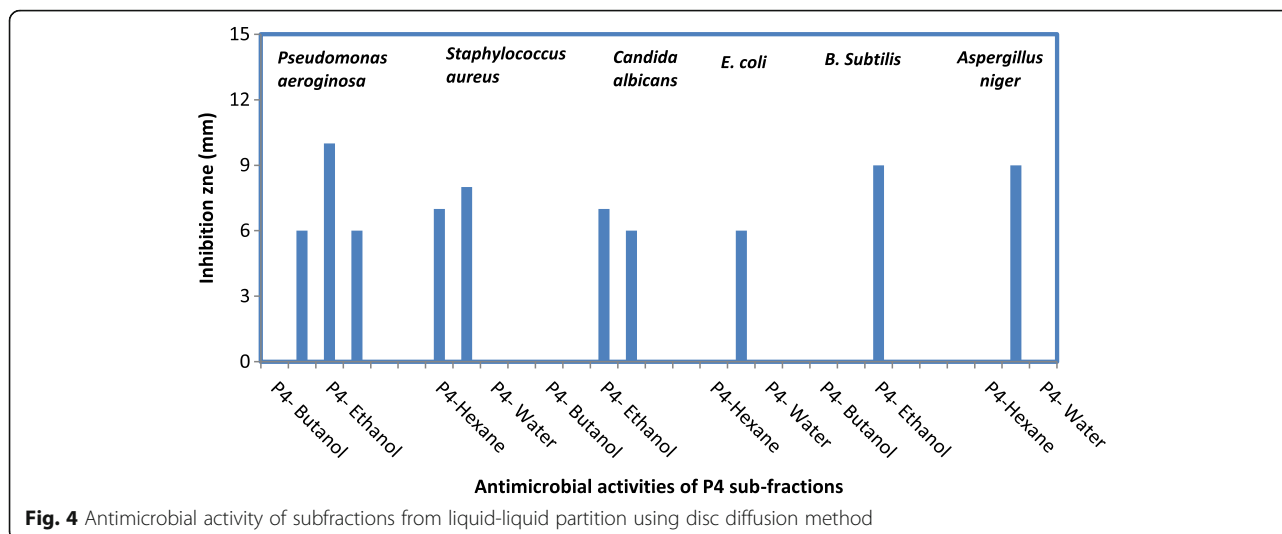


Fig. 3 Antidermatophytic activity of subfractions from liquid-liquid partition using well diffusion method



*albicans* (7 mm), *Aspergillus niger* (9), and *Bacillus subtilis* (9), and no activity against *E.coli*. Meanwhile, the subfraction (P4-hex) showed antimicrobial activities against the test organisms: *Staphylococcus aureus* (7 mm), *Pseudomonas aeruginosa* (6 mm), and *E.coli* (6 mm), and no activity against *Candida albicans*, *Aspergillus iger*, and *Bacillus subtilis*. Moreover, the subfraction (P4-water) showed antimicrobial activities against the test organisms *Pseudomonas aeruginosa* (6 mm) and *Candida albicans* (6 mm), and no activity against *E. coli*, *Staphylococcus aureus*, *Aspergillus niger*, and *Bacillus subtilis*. No antimicrobial activities have been recorded for fraction P4-but. Two antimicrobial standards: neomycin (antibacterial) at concentration of 100 µg/disc has been used and exhibited a clear zone of 20 mm (*Staphylococcus aureus*), 23 (*Bacillus subtilis*), 22 mm (*Pseudomonas aeruginosa*), 17 mm (*Candida albicans*), and showed negative effect with the fungus (*A. niger*). Moreover, the antifungal cyclohexamide was used and exhibited a clear zone of 32 mm against *A. niger*.

#### Antitumor activity of subfractions

The antitumor activity by determined by Ehrlich Ascites carcinoma method. Hidaka et al. (1979) used Ehrlich ascites carcinoma in the examination of antitumor activity for macromycin extracted from the culture broth of *Streptomyces macromyceticus*. The biological activity of the *Streptomyces* sp. MER4 ethyl acetate subfractions was also evaluated, and the subfraction (P4-eth) showed the highest inhibition ratio (78.46%) to the Ehrlich cells followed by P4-hex with an inhibition percent of 63.07 and P4-but with 32.3% cell inhibition while the P4-water fraction showed the lowest inhibition percent of 23.07% (Fig. 6).

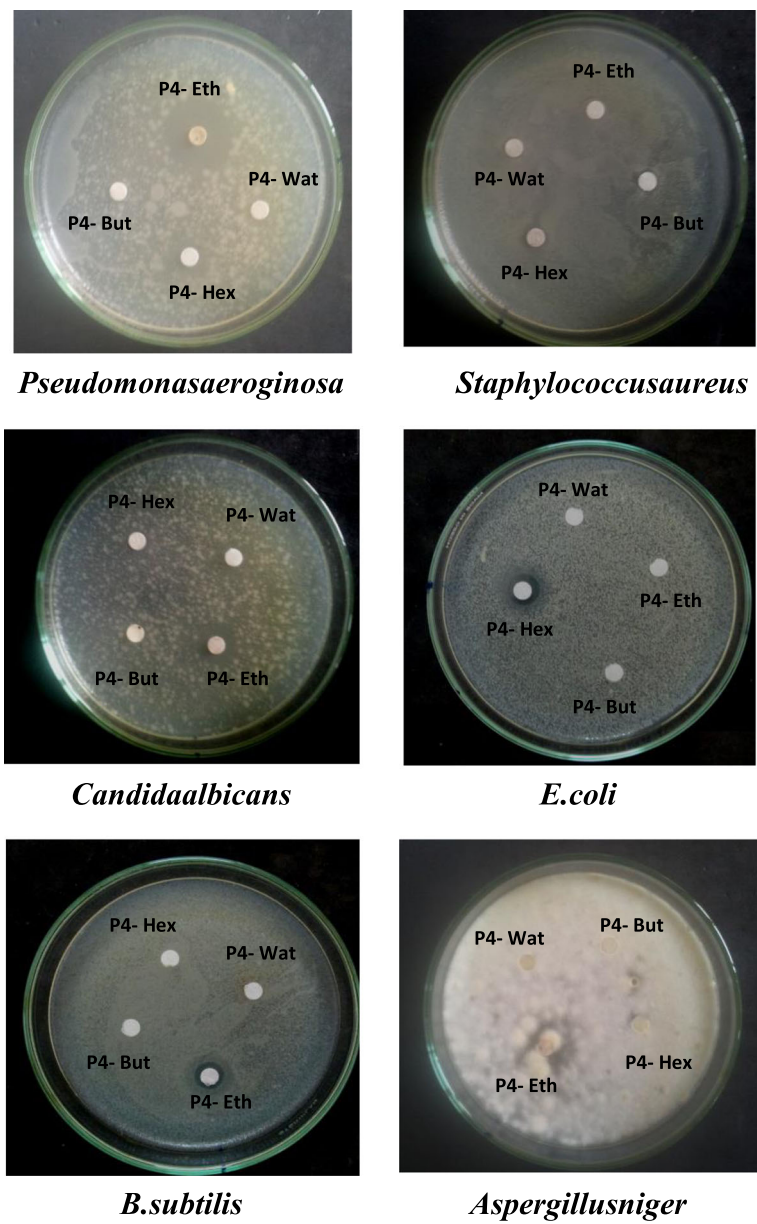
#### Purification and structure elucidation of sub fraction P4-eth

Pre-purified (P4-eth) fraction was further purified using high-performance liquid chromatography (HPLC)

semi-preparative technique. Three milligram of the (P4-eth) fraction dissolved in 100 µl of the solvent system. Then, the solvent system was pumped through the column and collected separately in Erlenmeyer flasks. The obtained fractions were dried subjected to further spectral analyses to elucidate their structures. The chemical structure of active sub-fraction (P4-Eth), isolated from *Streptomyces* sp. MER4 was investigated by electron ionization mass spectroscopy (EI-MS) and electron spray ionization mass spectroscopy (ESI-MS) (Additional file 1: Figure S1, Additional file 2: Figure S2 and Additional file 3: Figure S3), nuclear magnetic resonance (NMR) (Additional file 1: Figure S3), and ultraviolet (UV) spectroscopy. It was obtained as a yellow powder. The HPLC chromatogram shows that the compound separated at retention time of about 17 min as detected by UV (Fig. 7).

#### Discussion

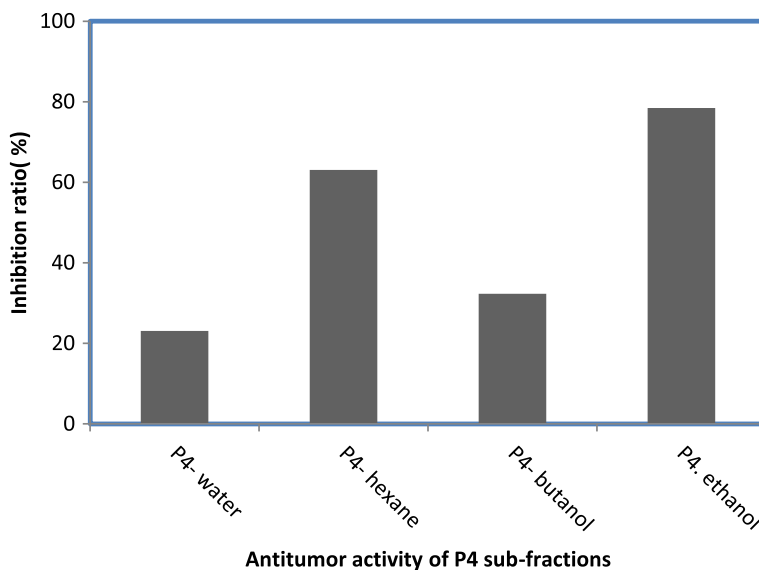
The molecular identification of the isolated strain revealed high similarity with *Streptomyces* sp. and has been deposited at GenBank under the accession no. KM099241 as *Streptomyces* sp. MER4 (accession number KM099241). The molecular technique is now a common and accurate technique for identifying microorganisms (Chen et al. 2001), and it is now used as an alternative to the traditional inaccurate and time-consuming methods (Zhao et al. 2007). These traditional methods depend on the study of morphological, physiological, and biochemical characters as well as the parietal components, in particular the amino acids and the glucides, and these provides very distinctive specific information. However, these characters in certain cases fail to classify an isolate in only one genus (Provost et al. 1997). Therefore, molecular studies are one of the strongest and easiest methods to identification (Ramazani et al., 2010).



**Fig. 5** Clear zone of *Streptomyces* sp. MER4(P4) subfractions using disc diffusion method with different test microbes

The biological activity of the *Streptomyces* sp. MER4 ethyl acetate subfractions were also evaluated; one subfraction (P4-ethanol) showed the highest inhibition ratio to the Ehrlich cells. So, all the four subfractions were also assessed for antidermatophytic activity, and the highest fraction revealed the highest inhibition ratio against all dermatophytic test microbes was P4-ethanol fraction. The first report about the antidermatophytic secondary metabolites from streptomycetes was established from *Streptomyces rochei* AK39 (Vijyakumar et al., 2007). Three *Streptomyces* spp. isolated from Saltpan region exhibited potential antidermatophytic activity, and

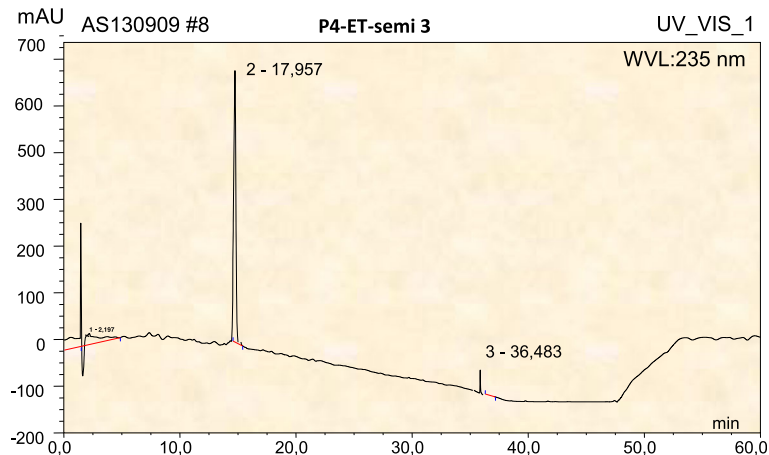
these strain were identified using 16S rRNA technique as *Streptomyces* sp. VITDDK1, *Streptomyces* sp. VITDDK2, and *Streptomyces* sp. VITDDK3 (Deepika and Kannabiran 2009a & b). The subfraction (P4-eth) showed broad spectra of antimicrobial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, and *Bacillus subtilis*. Another study on the crude extract from *Streptomyces fradiae* BW2-7 showed broad spectra of antimicrobial activities against the bacterial skin pathogen *Staphylococcus aureus* and *Staphylococcus epidermis* as well as the fungal pathogen (dermatophyte) *Trichophyton rubrum*



**Fig. 6** Antitumor activity of *Streptomyces* sp. MER4 (P4) secondary metabolites fractionated from liquid-liquid partitioning

(Kumar et al. 2014). The pre-purified subfraction (P4-eth) was further purified using HPLC semi-preparative technique. The purified compound was identified using different techniques to measure the molecular weight including LC-MS, EI-MS, and ESI-MS and the spectral analysis using NMR and Proton Nuclear Magnetic Resonance (1HNMR). A search in AntiBase using the obtained data as well as the molecular weight determined by ESI-MS and EI-MS suggested that the isolated compound is very closely similar to the structure of salicylamide derivative (JBIR-58) (C<sub>14</sub> H<sub>15</sub> NO<sub>7</sub>), which was further confirmed comparing with literatures. JBIR-58 is salicylic acid derivatives which are organic chemical compounds related to

class of structurally very diverse secondary metabolites. Ueda et al. (2010) isolated salicylamide derivatives JBIR-58 from the fermentation broth of *Streptomyces* sp. SpD081030ME-02. Salicylic acid is a monohydroxybenzoic acid, a type of phenolic acid and a beta hydroxy acid. It is derived from the metabolism of salicin. Salicylic acid derivatives are widely used for the treatment of various diseases. For example, methyl salicylate is a natural product of many species of plants which are used in flavoring for foods, candies, beverages, and pharmaceuticals. In addition, it is used as a perfumery like magnolia, meadowsweet, and root beer. It is also used in medicine mainly for dephlogistication and analgesia (Ekinici et al. 2011).



**Fig. 7** HPLC chromatogram of salicylamide derivative JBIR-58



## Conclusion

*Streptomyces* sp. MER4 (accession number KM099241) is isolated from terrestrial environment and screened for its ability to produce bioactive compound with antibacterial antitumor and antidermatophytic agents. Moreover, the work extended to scale-up fermentation and fractionation of the produced extract and purification and structure elucidation of salicylamide derivatives JBIR-58 from the fermentation broth of *Streptomyces* sp. MER4.

## Additional files

**Additional file 1: Figure S1.** EI-MS spectrum of salicylamide derivative JBIR-58. (DOCX 60 kb)

**Additional file 2: Figure S2.** ESI-MS spectrum of salicylamide derivative JBIR-58. (DOCX 35 kb)

**Additional file 3: Figure S3.** <sup>1</sup>HNMR spectrum of salicylamide derivative JBIR-58. (DOCX 1555 kb)

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## Availability of data and materials

Data are available upon request from the authors.

## Authors' contributions

MA-A, MF, MG, and AH contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript. AH performed the experiments. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable

## Consent for publication

Not applicable

## Competing interests

The authors declare that they have no competing interests.

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## References

- Augustine SK, Bhvsar SP, Kapadnis BP (2005) Production of a growth dependent metabolite active against dermatophytes by *Streptomyces rochei* AK 39. *Indian J Med Res* 121:164–170
- Bais Y, Nimbekar T, Wanjari B, Timande S (2012) Isolation of antibacterial compound from marine soil actinomycetes. *International journal of biomedical and advance. Research* 3(3):193–196
- Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am J ClinPathol* 45(4):493–496
- Bennett JM, Catovsky D, Danniel MT, Galton DAG, Graanlink HR, Sultan C (1976) Proposal for the classification of the acute leukemias. *Br J Haem* 33:451–458
- Chen YCJD, Eisner MM, Kattar SL, Lafe RK, Bui U (2001) Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. *J Clin Microbiol* 39:4042–4051
- Deepika TL, Kannabiran K (2009a) A morphological, biochemical and biological studies of halophilic *Streptomyces* sp. isolated from saltpan environment. *Am J Infect Dis* 5(3):207–213
- Deepika TL, Kannabiran K (2009b) A report on antidermatophytic activity of actinomycetes isolated from Ennore coast of Chennai, Tamil Nadu, India. *Int J Integr Biol* 6(3):132–136
- Deepika TL, Kannabiran K (2010) Review on dermatomycosis: pathogenesis and treatment. *Nat Sci* 2:726–773
- Ekinci D, Senturk M, Kufrevioglu OI (2011) Salicylic acid derivatives: synthesis, features and usage as therapeutic tools. *Expert Opin Therapeut Patents* 21:1831–1841
- Emmons CW, Bindford CH, Utz JP, KwonChung KL (1977) *Dermatophytes medical mycology*, 3rd edn. Lea and Febiger, Philadelphia, pp 117–167
- Fguira LF, Serge F, Raoudha BA, Lotifi M, Harmut L (2005) Purification and structure elucidation of antifungal and antibacterial activities of newly isolated *Streptomyces* sp. Strain US80. *Res Microbiology* 156:341–347
- Goodfellow M, Williams ST (1983) Ecology of actinomycetes. *Annu Rev Microbiol* 37:189–216
- Hamed AA, Abdel-Aziz MS, Fadel M, Ghali MF (2016) Antidermatophytes from bioactive secondary metabolites of local *Streptomyces* spp. *JIPBS* (2):155–165
- Hayakawa M, Nonomura H (1987) Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J of Ferm Bioengin* 65:501–509
- Hidaka T, Yano Y, Yamashita T, Watanabe K (1979) Biological activity of macromomycin. *The j of antibio*:340–346
- Hong, K., Gao, A., Xie, Q., Gao, H., Zhuang, L., Yu, H., Mand Ruan, J. (2009). Actinomycetes for marine drug discovery isolation from Mangrove soils and plants in china microbiological research. *Mar Drugs*, 7(1), 24–44.
- Hopwood DA (2007) *Streptomyces* in nature and medicine: the antibiotic markers. Oxford University Press, NY,USA
- Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Ikuchi H, Shiba T, Omura S (2003) Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. *Nat Biotechnol* 21:526–531
- Jüttner F, Watson S (2007) Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. *Appl Environ Microbiol* 73(14):4395–4406
- Kavitha A, Vijayalakshmi M, Sudhakar P, Narasimha G (2010) Screening of actinomycete strains for the production of antifungal metabolite. *Afr J Microbiol Res* 4(1):27–32
- Kim KJ, Kim MA, Jung JH (2008) Antitumor and antioxidant activity of protocatechualdehyde produced from *Streptomyces lincolnensis* M-20. *Arch Pharm Res* 31:1572–1577
- Kumar RM, Priyadarisini BV, Sriganpalram S, Kumar S, Arasan T, Nou S (2014) Studies on a marine *Streptomyces fradiae* BW2-7 producing glycopeptide antibiotic vancomycin effective against skin pathogens. *Scholars Academic Journal of Biosciences* 2(11):746–761
- Lee J, Jung H, Hwang B (2005) *Streptomyces koyangensis* sp nov., a novel actinomycete that produces 4-phenyl-3-butenic acid. *Int J SystEvolMicrobiol* 55:257–262
- Luilma AG, Sidrim JJC, Domingos TM, Cechinel VF, Vietla SR (2005) *In vitro* antifungal activity of dragon's blood from *Croton urucurana* against dermatophytes. *J Ethnopharmacol* 97(2):409–412
- Mukhtar H, Ijaz S, Ul-Haq I (2012) Production of antitumor antibiotic by *Streptomyces capoamus*. *Pak J Bot* 44(1):445–452
- Pridham TG, Anderson P, Foley C, Lindenfelser HA, Hesseltn CW, Benedict RG (1956-1957) A selection of media for maintenance and taxonomic study of *Streptomyces*. *Antib Annu* pp. 947–953

- Provost F, Laurent F, Uzcategui LR, Boiron P (1997) Molecular study of persistence of *Nocardia asteroides* and *Nocardia otitidiscaviarum* strains in patients with long-term nocardiosis. *J Clin Microbiol* 35(5):1157–1160
- Ramazani A, Moradi S, Sorourim R, Javani S, Garshasbi M (2010) Screening for antibacterial activity of *Streptomyces* species isolated from Zanjan province Iran. *IJPCBS* 3(2):342–349
- Sanglier J, Haag H, Huck T, Fehr T (1993) Novel bioactive compounds from actinomycetes. *Res Microbiol* 144(8):661–663
- Tanaka YT, Omura S (1993) Agroactive compounds of microbial origin. *Annu Rev Microbiol* 47:57–87
- Tepe B, Donmez E, Unlub M, Candanc F, Dafererad D, Vardar-Unlub G, Polissiod M, Sokmen A (2004) Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* and *Salvia multicaulis*. *Food Chem* 84(4):519–525
- Ueda J, Khan ST, Takagi M, Shinya K (2010) JBIR-58, a new salicylamide derivative, isolated from a marine sponge derived *Streptomyces* sp. D081030ME02. *J Antibiot* 63:267–269
- Vijayakumar R, et al. (2007) Studies on the diversity of actinomycetes in the Palk Strait region of Bay of Bengal, India. *Actinomycetologica*, 21: 59–65.
- Waksman SA, Hinrici AT (1943). The nomenclature and classification of the actinomycetes. *J. Bacteriol.* 46, 337.
- Watve MG, Tickoo R, Jog MM, Bhole BD (2001) How many antibiotics are produced by the genus *Streptomyces*? *Arch Microbiol* 176(5):386–390
- Wellington EMH, Stackbrandt E, Sarder D, Wolstrup J, Torgensen NOG (1992) Taxonomic status of *Kitasatosporia* and proposed unification with *Streptomyces* on the basis of phenotypic and 16SrRNA analysis and emendation of *Streptomyces*
- Willemsse J, Borst J, Waal E, Bisseling T, Wezel G (2011) Positive control of cell division: FtsZ is recruited by SsgB during sporulation of *Streptomyces*. *Genes Dev* 25:89–99
- Zhao GZ, Liu XZ, Wu WP (2007) *Helicosporus hyphomycetes* from China. *Fungal Diversity*. 26:313–524

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