British Microbiology Research Journal
4(10): 1057-1068, 2014

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Isolation and Identification of *Streptomyces rochei* Strain Active against Phytopathogenic Fungi

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAEH designed the study, performed the statistical analysis, wrote the protocol and prepared the final draft of the manuscript. Author REMA conducted the major parts of the practical work. Author SAA did the identification of the fungal species. Author MAES managed the literature searches, wrote the first draft of the manuscript and performed DNA analysis. All authors read and approved the final manuscript.

Original Research Article

Received 25th April 2014
Accepted 18th May 2014
Published 7th June 2014

ABSTRACT

A total of 104 actinomycete isolates were recovered from farming soil samples collected from 11 states in Sudan. Upon screening for potential antifungal activity, an actinomycete isolate (R92) was found to be highly antagonistic against all of the tested phytopathogenic fungi. It was identified as *Streptomyces rochei* on the basis of its morphology, chemotaxonomy and 16S rDNA sequence analysis. *In vivo* antagonistic activities of the *n*-butanol extract of R92 culture were significant since the progress of *Drechslera halodes* leaf spot on sorghum and *Alternaria alternata* early blight on tomato was highly restricted and incidence of both diseases was greatly suppressed. Trials to evaluate the *In vivo* control efficacy of this extract under field conditions is recommended.

Keywords: Streptomyces rochei; In vivo activity; 16S rDNA; phytopathogens; Sudan.

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1. INTRODUCTION

Streptomyces is the largest antibiotic producing genus in the microbial world discovered so far. Approximately 60% of the antibiotics were isolated from species of this genus [1]. Watve et al. [2] presented a mathematical model which estimated the total number of bioactive compounds that this genus is capable of producing to be in the order of a 100.000, a tiny fraction of this has been discovered so far.

About 80% of plant diseases are traced to fungi [3] which cause very serious crop diseases. Examples of important fungal plant diseases in Sudan include: *Alternaria* early blight on tomato, *Alternaria* leaf spot on sesame, *Macrophomina* charcoal rot on sesame, *Drechslera* leaf spot on sorghum, *Colletotrichum* tissue necrosis in beans*, Drechslera maydis* leaf blight on corn and *Drechslera halodes* leaf spot on sorghum [4] and fusarium wilt caused by *Fusarium* spp. [5].

Although antifungal agents have an important role in the control of mycotic plant and animal diseases, they are very few [6]. This is because fungi, like plant cells, are eukaryotes and therefore agents that inhibit fungal growth have greater potential for toxicity on plant cells as well [7]. Nevertheless, reports have shown that *Streptomyces* continue to remain an important source of antifungals, examples included: 24-Demethyl-bafilomycin C_1 [8], Levorin [9], Phenyl-1-napthyl-phenyl acetamide and DPTB16 [6], and (6S,8aS,9S,11S,12aR)-6-hydroxy-9,10-dimethyldecahydrobenzo[*d*]azecine-2,4,12(3*H*)-trione [10].

Streptomyces species can therefore contribute significantly to agricultural fungicides. This means that if the screening efforts are maintained, new antifungal compounds are expected to be discovered regularly. It should be emphasized that, the search for such compounds requires a large number of isolates [11]. The aim of this study was to screen locally isolated *Streptomyces* for production of potent antifungal agents that can be used to control some selected important fungal plant pathogens.

2. MATERIALS AND METHODS

2.1 Source and Isolation of Actinomycetes Isolates

Streptomyces isolates (*n*=104) used throughout this study were isolated from different soil samples collected from different locations in 11 States in Sudan. Soil samples were taken from a depth of 15-20cm after removing approximately 3cm of the earth surface, and were then air-dried at room temperature for two days. The soil suspension technique described by Oskay et al. [12] was used. Isolation was performed in Glycerol Arginine Agar (GAA) medium supplemented with Nystatin (50µg/ml) and Chloramphenicol (1µg/ml). Colonies characteristic of Streptomycetaceae that appeared on the incubated plates were selected, repeatedly subcultured for purification and preserved in the maintenance medium (Glycerol Asparagine Agar) at 4ºC.

2.2 Screening of Presumptive *Streptomyces* Isolates for Antifungal Activity

In vitro antifungal activities of *Streptomyces* were assessed against four phytopathogenic fungi according to the Agar diffusion method described by Taechowisan et al. [13].

Phytopathogens tested were: *Drechslera halodes, Alternaria alternata, Alternaria sesami* and *Macrophomina phaseolina*.

2.3 Characterization and Identification of R92 *Streptomyces* Isolate

R92 was characterized following the directions given by the International *Streptomyces* Project ISP [14,15]. Cell wall composition was determined according to the method of Becker et al. [16].

R92 was then identified through alighment of its 16S rDNA sequence with sequences in the NCBI. Two universal primers: pA (5'-AGAGTTTGATCCTGGCTCAG-3') and R1492 (5'-TACGGTTACCTTGTTACGACTT-3') were used for this purpose. Amplification reactions were performed in volumes of 25 μL containing 20 ng template DNA, 0.4μM of each primer, 1X buffer with Mg^{2+}, 1U of Taq DNA Polymerase (Promega, USA) and 0.2mM dNTPs. Nucleases free water was used to bring the reaction volume to 25μL. After initial denaturation at 95ºC for 2min, samples were cycled for 35 PCR cycles using the following cycle profile: 95ºC denaturation for 1 min, primer annealing at 55ºC for 1 min, and extension at 72°C for 2 min, followed by a final 5 min elongation step at 72ºC. The amplified PCR product (>1300bp) was separated on 1.2% (w/v) Agarose gel, purified and was fully sequenced. The obtained 16S rDNA gene sequence data were analyzed using BLASTnt search program at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences were deposited in the gene bank and were aligned via MEGA version 6.0 [17] and the phylogenetic tree was constructed using the neighbor-joining method [18]. The statistical significance of the tree was obtained by 2000 bootstrap analysis.

2.4 Production of Antifungal Compound by R92 in Submerged Culture

Isolate R92 was grown in submerged culture using Bennet Broth medium. A pure colony from 14-days old culture was transferred to Bennet Agar medium, pH 7.2, and incubated at 28ºC. After ten days, a pure colony was used to inoculate 150ml of Bennet Broth in 250ml Erlenmeyer flasks. Flasks were incubated at room temperature (28-30ºC) for 30 days at 180 rpm. At the end of the incubation period, the fermentation broth was mixed with *n*-butanol (1:1 v/v) and centrifuged for 10 min. at 3000rpm. Supernatant was concentrated under reduced pressure in a freeze-drier, and 250µg of the extract were dissolved in 5ml of dimethyl sulfoxide (DMSO) to give a final concentration of 50µg/ml. To this, 500ml of water: methanol (19:1 v/v) and 125ml of Tween 80 were added. The *In vitro* and *In vivo* antifungal activities of this extract preparation were tested.

2.4 *In vitro* Antifungal Activity of the Broth Culture Extracts

A piece of a well-grown Corn Meal Agar (CMA) cultures of *D. halodes, A. alternata*, *A. sesami* and *M. phaseolina* were cut with a cork borer, placed each in the centre of a freshly prepared CMA plate and incubated for 2 days. Filter paper discs (6mm diameter) were loaded, each with ten µl of each of three selected commercial antifungal agents (Nystatin, Mycostatin and Itracon) and the broth culture extract of isolate R92. The discs were left to dry and placed onto the pre-seeded CMA plates at 30mm distance from the fungus culture. The plates were incubated for 3 days at 24ºC and the diameters of growth inhibition zones were measured and compared with those of the commercial antibiotics [19].

2.5 Minimum Inhibitory Concentration (MIC) Of R92 Broth Culture Extract against *D. halodes*

The *n*-butanol extract of R92 culture was dissolved in DMSO and then serially diluted to final concentrations (µg/ml) of 480, 240, 120, 60, 30 and 15. Filter paper discs (6.0mm in diameter) were loaded each with one of the prepared culture extract concentrations and were left to dry. A piece of a well grown CMA culture of *D. halodes* was cut with a cork borer, seeded in the center of a freshly prepared CMA plate and incubated for two days. Loaded filter paper discs were then placed in the CMA plate at 30mm distance from the seeded *D. halodes* plug culture, incubated for three days and growth inhibition zones were measured.

2.5 In vivo Anti-Fungal Activity of R92 Broth Culture Extract

Seedlings of sorghum and tomato were raised in vinyl pots in the greenhouse at 25±5°C for 5 weeks. The raised seedlings were sprayed with R92 broth culture extract and left to grow for 24hrs before they were inoculated with *D. halodes* and *A. alternata* spores' suspension (10^3-10^7 spores/ml), respectively. Seedlings were then incubated in the dark for one day at 25 ±2ºC at 100% Relative Humidity (RH), transferred to the green house and left to grow under the conditions of 70-80% RH, 25 ±2ºC with 12h of light per day. A control set, in which seedlings were sprayed with Tween 80 and water-methanol alone instead of R92 broth culture extract was included. Inoculated seedlings were examined daily for the appearance of disease symptoms. The percentage of seedlings showing disease symptoms were calculated according to Lee and Hwang [20].

In another *In vivo* experiment, a set of seedlings of each plant were first inoculated each with its respective spores' suspension (10^3-10^7 spores/ml) of the test fungi and incubated in the dark for one day at 25±2ºC and 100% RH. The seedlings were then transferred to the greenhouse, left to grow under the conditions of 70-80% RH, 25±2ºC with 12h of light per day for 72h. The diameters of necrotic spots were measured and recorded. Seedlings of each test plant (sorghum and tomato) were then divided into two lots, the first was sprayed with R92 broth culture extract preparation while the second was not (control). Both lots were left to grow for 24 hours, their spot diameters were then re-measured and the % increments in spot diameter in both lots were calculated.

3. RESULTS

3.1 Isolation and *In vitro* Screening of Presumptive *Streptomyces* for Antifungal Activity

One hundred and four presumptive *Streptomyces* isolates were recovered and tested *In vitro* for antifungal activity. Only actinomycetes showing inhibition zone diameters of 5mm and above, against any of the tested fungi, were considered active. Approximately, 60% were found to be inhibitory (inhibition zone ≥ 5mm) to the growth of one or more of the phytopathogenic fungi tested. While 53% of the isolates were active against all of the tested fungi, 21% have failed to exhibit any activity against any of these fungi (Table 1; Plate 1).

Representative data for the most active actinomycetes isolates with inhibition zone diameters of 16mm and above is shown in Table 2. The presumptive *Streptomyces* isolate encoded R92 was selected for characterization, identification and for *In vivo* experiments due to the strong suppression it caused on all of the tested phytopathogenic fungi; results of

characterization are shown in Table 3. The isolate produces a brown vegetative mycelium with powdery surface and a grey aerial mycelium with spiral-shaped conidia. It utilized rhaminose, L-arabinose, glucose, fructose, sucrose, maltose, lactose and mannitol as sole carbon sources. It was also positive for some biochemical activities such as production of melanin, H_2S, organic acids and can liquefy gelatin, hydrolyze starch and express catalase, oxidase, nitrate reductase and urease. The isolate possesses LL-diaminopimelic acid but not mycolic acid in its cell wall. These later criteria place R92 in the genus *Streptomyces* [21].

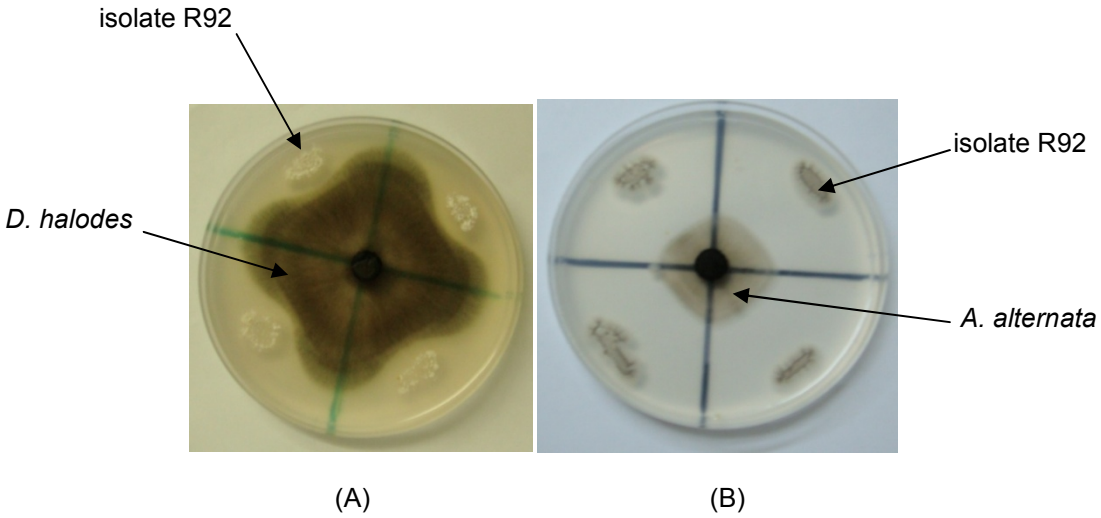

Plate 1. Effect of *Streptomyces* R92 on: (a) *D. halodes* and (b) *A. alternata*

Table 1. Inhibition zones diameters (mm) shown by *Streptomyces* isolates

Range of inhibition zone diameters in mm	Number of presumptive *Streptomyces* isolates active against				
	Drechslera halodes	***Alternaria alternata***	***Alternaria sesami***	***Macrophomina phaseolina***	**All tested fungi**
26-34 (highly active)	1	1	1	1	1
16-25 (active)	10	10	10	8	7
5-15 (moderately active)	50	51	51	52	48
0-4 (inactive)	43	42	42	43	48
Total	**104**	**104**	**104**	**104**	**104**

3.2 16S rDNA-Based Identification

The 1306 bp sequence of *Streptomyces* R92 showed 100% similarity (Fig.1) with the nucleotide sequences of *Streptomyces rochei* strain SCSIOZ-SH13 (accession number KC747481) and *S. rochei* strain S41 (accession number JX007969) from China, and 99% similarity with each of *S. rochei* HBUM 174697 (accession number FJ532458) and *S. rochei* KMB-1 from Pakistan (accession number KJ020689). The strain was designated as *Streptomyces rochei* R92 (accession number KJ689444).

3.3 Production of *S. Rochei* R92 Active Metabolite in Submerged Culture

The crude extract of the cell free culture broth of *S. rochei* R92 showed higher inhibition zone diameters (17-19mm) against all tested phytopathogens (Table 4) when compared to

the three commercial antibiotics tested *viz* Nystatin (6-9mm), Mycostatin (4-6mm) and Atracin (3-5mm). The MIC values recorded for the extract against *D. halodes* was 30µg/ml.

Fig. 1. Phylogenetic tree of the 16S rDNA based on the neighbour-joining method

Table 2. Inhibition zones diameters (mm) shown by selected *Streptomyces* isolates against plant pathogenic fungi

Isolate code	*Drechslera halodes*	*Alternaria alternata*	*Alternaria sesami*	*Macrophomina phaseolina*
RI	14	15	14	16
R2	15	14	16	15
R6	17	17	16	15
R8	17	20	19	16
R9	17	18	17	20
R10	15	16	16	14
R13	16	16	15	14
R15	16	13	13	14
R19	17	16	18	20
R28	18	16	18	17
R29	19	20	17	19
R39	21	20	22	23
R43	20	23	22	23
R92	**33**	**34**	**32**	**33**

3.4 *In vivo* Activity of *S. rochei* R92 Culture Extract

Sorghum and tomato seedlings inoculated, each with its respective fungal pathogen, and treated with *S. rochei* R92 broth culture extract were found to be free of disease symptoms (zero infection percentages). Seedlings of the positive control, have recorded 100% infection (Table 5). Table 6 shows the development of disease symptoms expressed as percentage increment in the diameter of leaf spot on sorghum and tomato seedlings sprayed with R92 culture extract 72 hours after their infection by *D. halodes* and *A. alternata*, respectively. Sorghum and tomato seedlings sprayed with *S. rochei* R92 culture extract have shown less percentage increments in spot diameters. The increment was 37.9% in the case of sprayed sorghum seedlings compared to 251.7% for non-sprayed seedlings. Similarly, sprayed and non-sprayed tomato seedlings have recorded 110% and 230% increments, respectively.

Table 3. Characteristics of potential *Streptomyces* R92 isolate

Colony characteristics		**Biochemical and physiological characteristics:**	
Shape	Concentric	Oxidase	+ ve
Choromogenesis	Grey	Catalase	+ ve
Edge	Smooth	Melanin production	+ ve
Opacity	Opaque	Nitrate reduction	+ ve
Elevation	Umbonate	H_2S production	- ve
Surface	Powdery	Casein hydrolysis	- ve
Consistency	Dry	Organic acid fermentation	+ ve
Microscopical characteristics:		Gelatin liquefaction	+ ve
Gram stain	+ ve	Urease	+ ve
Acid fast	- ve	Starch hydrolysis	+ ve
Aerial mycelium	Present	Sugar utilization	Rhaminose, Arabinose, Glucose, Fructose, Sucrose, Maltose, Lactose, Mannitol are utilized
Conidia shape	spiral		
Cell wall composition:			
LL-diaminopimelic acid		Present	
Meso-diaminopimelic acid		Absent	
Mycolic acid		Absent	

Table 4. Inhibition zones diameters (mm) shown by R92 culture extract and some commercial antifungal agents

Bioactive agent	***Drechslera halodes***	***Alternaria alternata***	***Alternaria sesami***	***Macrophomina phaseolina***
R92 extract	19	18	18	17
Nystatin	10	9	9	12
Mycostatin	7	8	7	9
Itracon	5	3	4	5

Table 5. Effect of spraying by R92 culture extract on percentage infection due to inoculation of sorghum and tomato by pathogens under test

Treatment	Percentage infection on	
	Sorghum (infected by *D. halodes*)	Tomato (infected by *A. alternata)*
Seedlings not treated with R92 extract and uninoculated with pathogenic spores (-ve control)	Zero	Zero
Seedlings not treated with R92 extract but inoculated with pathogenic spores (+ve control)	100	100
Seedlings treated with R92 extract and inoculated with pathogenic spores	Zero	Zero

Table 6. Effect of sparying with R92 extract on percentage increments in spot diameters caused by phytopathogens on sorghum and tomato

Treatment	Diameters of spot area in mm					
	Sorghum			Tomato		
	After 72 hrs	After 96 hrs	% increment in spot diameter	After 72 hrs	After 96 hrs	% increment in spot diameter
Seedlings infected, and then sprayed with R92 culture extract after 96 hrs.	2.9*±0.76	4.3±1.4	37.9	1.0±0.21	2.1±0.29	110
Control seedlings infected but not sprayed	2.9±0.76	10.2±1.38	251.7	1.0±0.21	3.3±0.41	230

**mean of 30 spots ±SD*

4. DISCUSSION

All recovered actinomycetes (*n*=104) were considered as presumptive *Streptomyces* depending on their mycelium configuration and their abilities to grow on GAA medium supplemented with Nystatin (50µg/ml) and Chloramphenicol (1µg/ml). This medium was reported to be specific and sensitive for *Streptomyces*, since it contains glycerol that most actinomycetes use as sole carbon source [12,22,23]. Screening of the 104 presumptive *Streptomyces* for antifungal production revealed 60% antagonistic activity against one or more of the tested fungi. In similar screening studies, researchers have reported varying percentages of actinomycetes active against fungi. For example, out of 110 *Streptomyces* isolates only 14 (12.7%) were reported to exhibit antifungal activities against eight phytopathogenic fungi [24]. Similarly, 14% of 218 actinomycetes isolates obtained from 26 soil and water samples were found to be active against dermatophytic fungi [25]. The total percentage of antagonistic activities against different fungi in recent studies were reported as 6.8% [26,27], 31% [28,29] and 7% against *Fusarium oxysporium* [30].

In this study, 21% of the isolated *Streptomyces* failed to exhibit inhibitory effect against any of the tested fungi. As *Streptomycetes* are known to possess 10-20 gene clusters that code for the production of active metabolites, it is possible that these isolates be active against some members of other groups of microorganisms or they require a different set of cultivation conditions for active secondary metabolites production. Previous reports have shown that the expression of genes responsible for active metabolite production is dependent on growth conditions [31,25].

16S rDNA-based identification indicated that R92 is 100% *S. rochei* (accession number KJ689444). The antibacterial activity of *S. rochei* is well known against both Gram negative and Gram positive bacteria [32- 34]. Ugur and Sahin [35] reported that *S. rochei* MU119 was inactive against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The activity of *S. rochei* S785-16 against *Aspergillus fumigatus* was reported by Kotake et al. [35]. Augustine et al. [25] reported that *Streptomyces rochei* AK39 was active only against dermatophytes whereas yeast and molds like *Aspergillus niger* and *Fusarium oxysporium* were resistant. Contradictory to this, Kavitha and Vijayalakshmi [34] reported a strong activity of *S. rochei* MTCC8376 metabolite against *Apergillus niger* and *Fusarium oxysporium*. The inconsistent results reported by different investigators may be due to different *S. rochei* strains or to different sensitivity pattern exhibited by different strains of the tested bacteria and/or fungi.

The *n*-butanol extract of the 6-days old cell free *S. rochei* R92 culture was more potent against the phytopathogenic fungi when compared with the tested commercial antifungal agents. Results are also comparable to the results recorded by Ouhdouch et al. [36] in Morocco, El-Naggar et al. [37] in Egypt, Hoon et al. [38] in Korea and Khamna et al. [27] in Japan for their actinomycetes isolates against some phytopathogenic fungi. The MIC value of R92 culture extract against *D. halodes* was 30µg/ml; this again indicates the strong potency of this extract. Taechowison et al. [39] reported higher MIC values of 480 and 30µg/ml for a *Streptomyces* sp. culture extract against *Fusarium oxysporium* and *Colletotrichum gloeosporioides*, respectively.

Results of the *In vivo* experiments indicated a strong curing value of *S. rochei* R92 cell free culture extract. The *In vivo* control efficacies of the extract was substantial since the progress of *D. halodes* leaf spot on sorghum and *A. alternata* early blight on tomato was significantly (p=0.01) restricted and incidence of both diseases was completely suppressed.

Results of *In vitro* and *In vivo* experiments provided strong evidence that R92 culture extract could protect sorghum and tomato crops from *D. halodes* leaf spot and *A. alternata* early blight, respectively. This is in agreement with previous reports which highlighted the potentiality of *Streptomyces* as biocontrol agents. For example, *Streptomyces ambofaciens* was suggested for the control of damping-off in tomato and *Fusarium* wilt in cotton [40]. Lee and Hwang [20] recommended the use of *S. lipmanii* as a control agent against rice blast caused by *Magnaporthe grisea*. Kanini et al. [30] reported the ability of *Streptomyces rochei* ACTAI55I to protect tomato seeds from the pathogenic effect of *Fusarium oxysporiu*m. The culture filtrate of *S. spectabilis* CMU-PA101 has also been reported to control shallot blotch caused by *Alternaria porri* [27].

5. CONCLUSION

S. rochei R92 was found to be very effective not only *in vitro* inhibition of growth of *D. halodes*, *A. alternata*, *A. sesami* and *M. phaseolina* but also in preventing both incidence and development of leaf spot diseases caused by *A. alternata* and *D. halodes* on tomato and sorghum, respectively. Trials are required to evaluate the efficiency of this isolate as a biofungicide under field conditions. Results also demonstrated that isolation of *Streptomyces* species from diverse geographical locations may present a significant capacity for the discovery of potent natural antifungal agents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abouwarda A, Abu El-Wafa WM. Production of anti-mycobacterial agents by Egyptian *Streptomyces* isolates. Int. J. Microbiol. Res. 2011;2(1):69-73.
2. Watve M, Tickoo R, Jog M, Bhole B. How many antibiotics are produced by the genus *Streptomyces*? Arch. Microbiol. 2001;176:386-390.
3. Someya N. Biological of fungal plant disease using antagonistic bacteria. J. Gen. Plant. Bath. 2008;74:459-460.
4. Kendrick B. Fungal Plant Pathology. In: The Fifth Kingdom all about Fungi, Canada Mycologue publications; 2001.
5. Kim GH, Hur J, Choi W, Kohand YJ. *Fusarium* wilt of winter daphne (*Daphne odora Thunb.*) caused by *Fusarium oxysporeum*. J. Plant Pathol. 2005;21:102-105.
6. Dhanasekaran DH, Thajudin N, Panneerselvam A. An antifungal compound: 4' phenyl-1-napthyl-phenyl acetamide from *Streptomyces* sp. DPTB16. Facta Universitals Series: Medi. Biol. 2008;15:7-12.
7. Georgopapadakou NH, Tkacz JS. Human Mycoses: Drugs and targets for emerging pathogens. Science 1994;264:371-373.
8. Lu CH, Shen YM. Two new macrolides produced by *Streptomyces* sp. J. Antibiotics. 2004;56:597-600.
9. Kozhuharova L, Gochev V, Koleva L. Isolation, purification and characterization of Levorin produced by *Streptomyces levoris* 99/23. World J. Microbiol. Biotechnol. 2008;24:1-5.
10. Wu X, Huang H, Chen G, Sun Q, Peng J, Zhu J, Bao Sh. A novel antibiotic produced by *Streptomyces noursei* Da 07210. Antonie Van Leeuwenhoek. 2009;1:109-112.

11. Sahin N, Ugur A. Investigation of the antimicrobial activity of some *Streptomyces* isolates. Turk.J. Biol. 2003;27:79-84.
12. Oskay M, Tamer AÜ, Azeri C. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. African J. Biotechnol. 2004;3(9):441-446.
13. Taechowisan T, Lu C, Shen Y, Lumyong S. Secondary metabolites from endophytic *Streptomyces aureofaciens* CMUAc130 and their antifungal activity. J. Microbio. 2005;151:1691-1695.
14. Shirling EB, Gottlib D. Methods of characterization of *Streptomyces S*pecies. Int .J. Sys. Bacteriol. 1966;16:313-340.
15. Locci R. *Streptomyces* and related genera In: Bergey's Manual of systematic Bacteriology, Williams and Wilkins Company, Baltimore. 1990;4:2451-2508.
16. Becker B, Lechevalier MP, Gordon RE, Lechevalier HA. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. Microbiol. 1964; 12: 421-423.
17. Kumar S, Tamura K, Jakobsen IB, Nei M. MEGA2: Molecular evolutionary genetics analysis software. Bioinformatics 2001;17(12):1244-1245.
18. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 1987;4(4):406–425.
19. Jain PK, Jain PC. Production of hetero antifungal antibiotic by *Streptomyces purpeofuscus* CM 1261. Ind. J. Exp. Biol. 2005;43:342.
20. Lee JY, Hwang BK. Diversity of antifungal actinomycetes in various vegetative soils of Korea. Canidian J. Antibiot. 2002;48:407-417.
21. Kutzner HJ. The family Streptomycetaceae. In: The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria. Starr MP, Stolp H, Tru¨per HG, Balows A, Schlegel H. (eds). Berlin; Springer-Verlag. 1981;2028–2090.
22. Smith AJ, Hall V, Thakker B, Gemmell CG. Antimicrobial susceptibility testing of Actinomyces species with 12 antimicrobial agents. J. Antimicrob. Chem. 2005;56:407–409.
23. Hingston CD, Hingston EJ, Wise MP. Impact of nystatin on *Candida* and the oral microbiome. Crit. Care. 2012;16:440.
24. Aghighi S, Bonjar GHS, Rawashdeh R, Batayneh S, Saadoun I. First report of antifungal spectra of activity of Iranian actinomycetes strains against *Alternaria solani*, *Alternaria alternata*, *Fusarium solani*, *Phytophthora megasperma*, *Verticillium dahliae* and *Saccharomyces cerevisiae*. Asian J. Plant Sci. 2004;3:463-471.
25. Augustine SK, Bhavsar SP, Kapadnis BP. Production of a growth dependent metabolite active against dermatophytes by *Streptomyces rochei* AK 39. Indian J. Med. Res. 2005;121(3):164-170.
26. Prapagdee B, Kuekulvong C, Mongkolsuk S. Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against Phytopathogenic Fungi. Int. J. Boil. Sci. 2008;4(5):330-337.
27. Khamna S, Yokaota A, Peberdy FJ, Aiumyong S. Antifungal activity of *Streptomyces* spp. isolated from rhizosphere of Thai medicinal plants. Int. J. Integ. Biol. 2009;6:143-147.
28. Bharti A, Kumar V, Gusain O, Bisht GS. Antifungal activity of actinomycetes isolated from Garhwal region. J. Sci. Eng. Technol. Manag. 2010;2(2):3-9.
29. Al-Askar AA, Abdul Khair WM, Rashad YM. *In vitro* antifungal activity of *Streptomyces spororaveus* RDS28 against some phytopathogenic fungi. African J. Agric. Res. 2011;6(12):2835-2842.
30. Kanini GS, Katsifas EA, Savvides AL, Karagouni AD. *Streptomyces rochei* ACTA1551, an indigenous Greek isolate studied as a potential biocontrol agent against *Fusarium oxysporum* f. sp. *lycopersici*. BioMed Res. Int. 2013;11.

31. Porter JN. Prevalence and distribution of antibiotic- producing Actinomycetes. Adv. Appl. Microbiol. 1971;14:73-92.
32. Ellaiah P, Raju KV, Adinarayana K, Adinarayana G, Saisha V, Madhavi S, et al. Bioactive actinomycetes from Krishna river sediments of Andhra Pradesh. Hind. Antibiot. Bull. 2002;44:8-16.
33. Sahin NS. Antimicrobial activity of *Streptomyces* species against mushroom blotch disease pathogen. J. Basic Microbiol. 2005;45:64-71.
34. Kavitha M, Vijayalakshmi M. Studies on cultural, physiological and antimicrobial activities of *Streptomyces rochei.* J. Appl. Sci. Res. 2007;3(12):2026-2029.
35. Kotake C, Yamasahi T, Moriyama T, Shinoda M, Komiyama N, Furumai T, Konishi M, Oki T. Butyrolactols A and B, new antifungal antibiotics. J. Antibiot. 1992;45:1442-1450.
36. Ouhdouch Y, Barakate M, Finanse C. Actinomycetes of Moroccan habitats: Isolation and screening for antifungal activities. Eur. J. Soil Biol. 2001;37:69-74.
37. El-Naggar MY, Hassan MA, Said WA. Isolation and characterization of an antimicrobial substance production by *Streptomyces violatus.* Egyptian. J. Biol. 2001;3:11-21.
38. Hoon L, Kim B, Choi JG, Cho YK, Yang H, Shin CH, Min S, Lim Y. *Streptomyces* with Anti-fungal activity against rice blast causing fungus, *Magnaporthe grisea*. J. Microbiol.Biotechnol. 2002;12:1026-1028.
39. Taechowisan T, Chanaphat S, Ruensamran W, Phutdhawong WS. Antifungal activity of 3-methylcarbazoles from *Streptomyces* sp. LJK109; an endophyte in *Alpinia galangal*. J. Appl. Pharm. Sci. 2012;2(3):124-128.
40. Reddi GS, Rao AS. Antagonism of soil actinomycetes to some soil-borne plant pathogenic fungi. Indian Phytopathol. 1971;24:649–657.

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