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GC-MS Analysis and Bioactivity of *Streptomyces* sp. nkm1 Volatile Metabolites against some Phytopathogenic Fungi

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HIGHLIGHTS

- *Streptomyces* sp. nkm1 was isolated from a marine soil sample and identified using morphological and molecular techniques.
- *Streptomyces* sp. nkm1 had the highest antifungal activity against some plant pathogenic fungi on M6 medium.
- On *Aspergillus flavus*, the minimum inhibitor concentration of ethyl acetate Crude extract of *Streptomyces* sp. nkm1 was more effective.
- The GC-MS analysis of the ethyl acetate extract revealed 26 components, with nonadecane being the most important bioactive compound.

Abstract: The main concern of today's agricultural production around the world is a cleaner and greener approach to crop production. *Streptomyces* species with distinct characteristics can be found in soil. The current research focuses on *Streptomyces* species isolated from marine sediment samples. To evaluate the morphology and biochemical properties of the isolated *Streptomyces* strain ukm1, it was grown in Starch Casein Nitrate (SCN) agar medium. Morphological, biochemical, and 16s rRNA analysis were performed on the strain. *Streptomyces* sp. was identified based on these findings. The production of bioactive metabolites by strain nkm1 was carried out in five different fermentative media. After 10 days of incubation, M6 medium was found to be efficient and was extracted with ethyl acetate. The MIC values revealed that the ethyl acetate extract inhibited the growth of plant pathogenic fungi such as *Phytophthora palmivora* (31.25 g/ml), *Aspergillus flavus* (15.62 g/ml), *Rhizoctonia solani* (31.25 g/ml), and *Cladosporium herbarum* (31.25 g/ml). The GC-MS analysis of the ethyl acetate extract revealed 26 components, the major volatile bioactive compound being nonadecane.

Keywords: *Streptomyces* sp.; ethyl acetate; plant pathogen; nonadecane.

INTRODUCTION

Fungal pathogens have caused millions of dollars' worth damage each year to economically important crops all over the world, despite the extensive use of synthetic pesticides. Recently, synthetic fungicides are widely used in modern farming practices. In addition, some of them are toxic and cause environmental pollution [1-3]. The concern over toxicity and the development of resistance to some fungicides make it necessary to find a safer and effective fungicide. Microbial metabolites have attracted the attention as potential alternatives to synthetic antifungal agents [4-6]. Several antifungal compounds were isolated from various microbial sources. Actinomycetes are the most appealing sources because they are powerful producers of a wide range of secondary metabolites with diverse biological activities, including therapeutically and agriculturally important bioactive compounds [7-9]. Rare actinomycetes have been important natural resource sources of novel and efficient antibiotics [10]. A variety of actions are associated with secondary metabolites generated by *Streptomyces* sp., which includes antimicrobial, antifeedant and enzyme inhibitors [11, 12]. Marine sediments and invertebrates are relatively untapped sources for prospective secondary metabolites [13]. The genus *Streptomyces* is the largest bioactive metabolites producing actinomycete [14-16]. Moreover, majority of antibiotics that has been reported so far, is obtained from *Streptomyces* species [17]. These antibiotics are used in evaluating bioactivity against various plant pathogens causing plant diseases [18, 19]. Currently, the wide-spread occurrence of antibiotic resistance by microorganisms is a threat to both human health and agricultural production. As a result, the current study was conducted to investigate the potential of Streptomyces species isolated from marine soil sediment as antifungal agents against fungal plant pathogens, with volatile bioactive compounds identified using GC-MS.

MATERIAL AND METHODS

Isolation of *Streptomyces* sp. from marine soil samples

The isolation of *Streptomyces* sp. was performed by serial dilution plate technique [20] with starch casein nitrate (SCN) agar medium (g/l: starch 10.00, casein 0.3, KNO_3 2.00, NaCl 2.00, K_2HPO_4 2.00, $MgSO_4$-$7H_2O$ 0.05, $CaCO_3$ 0.02, $FeSO_4$-$7H_2O$ 0.01, agar 20.00). After incubation, the plates containing isolated *Streptomyces* sp. were purified, sub cultured and stored at 4^oC. For long storage, the isolates were grown in International *Streptomyces* Project-2 (ISP-2) broth for 5 days and stored at -20^o C in 15 % glycerol stock.

Characteristics of *Streptomyces* sp. isolate

Standard methods of Shirling and Gottlieb (1961) [21] and Waksman (1961) [22], were used to determine the biochemical, morphological, and physiological characteristics of the prospective strain nkm1. Subsequently, growth was observed after incubation at 28^oC for 7 days and colors were determined according to the methods described by Prauser (1964) [23]. In addition, pigmentation of aerial mycelium and structure, as well as the arrangement of spores were observed through the cultivation of the strains in ISP4. The utilization of carbon and nitrogen sources by the strain was carried out according to the method of Gottlieb (1961) [21].

Fungal plant pathogens

The following plant pathogenic fungi were used; *Fusarium oxysporum, Phytophthora palmivora, Aspergillus flavus, Botrytis conerea, Alternaria alternata, Cerscospora capsici, Rhizoctonia solani, Cladosporium herbarum* and *Helminthosporium papulosum*.

Preparation of fungal spores

The filamentous fungi were grown on Sabouraud Dextrose Agar (SDA) slants at 30ºC for 5 days and the spores were collected using sterile doubled distilled water and homogenized.

Preliminary screening

The strain nkm1 was inoculated on Yeast Peptone Glucose (YPG) agar plates by single streak in the center. The plates were incubated at 28ºC for 5 days. The plant pathogenic fungi (inoculum size was 1×10^5 PFU/ml) were spotted and incubated at 30ºC for 3 days. The fungal inhibitions were observed by determining the inhibition zone.

Antifungal activity of strain nkm1

The antifungal activity of strain nkm1 against plant pathogenic fungi was determined using the standard cup plate method. Plant pathogenic fungi were inoculated into 20 ml of Mueller Hinton agar medium to create assay plates. Agar-cups (6mm diameter) were filled in triplicate with 50 l of mycelia-free culture filtrate and incubated at 30oC for 3 days. The diameter of the inhibition zone was measured in millimeters.

Antifungal metabolites' production with different media

In a shake flask with five different designated media, antifungal secondary metabolites were produced. The following were the media compositions: (g/L): Sabouraud Medium Dextrose Broth (SDB) 1: 20.00 Dextrose, 10.00 Peptone; Yeast Peptone Glucose (YPG) - Medium 2.00 Peptone, 3.00 Yeast extract, and 10.00 Glucose NaCl2 3.00 M6 - Moderate 3: Starch 10.00, K2HPO4.7H2O 1.00, MgSO4.7H2O 1.00, (NH4)2SO42.00; Medium Tryptic Soy Broth (TSB) 4: Casein peptone 17.00, K2HPO4.7H2O3.00, Glucose 3.00, NaCl 25.00, Soy peptone 3.00; Medium Modified Nutrient Glucose Broth (MNGB) 5: Peptone (5.00), Beef extract (3.00), Glucose (10.00), Yeast extract (3.00), NaCl (23.00). The pH of each medium was adjusted to 7.0 earlier using the autoclaving. In a 500 ml Erlenmeyer flask containing 150 ml respective fermentation medium, a loop full of culture nkm 1 was inoculated and incubated at 28°C on shaker (200 rpm) for 5 days. Secondary metabolite production was observed in all media after seed culture was inoculated into a 500ml flask (100 ml medium) and grown for 10 days under the same conditions.
The supernatant of fermented broth cultures was tested against plant pathogenic fungi. The antifungal activity of the culture broth was tested using a well diffusion assay for 7 days [24]. Briefly, the fungal spore suspension (inoculum size was 1x 10^5PFU/ml) was seeded over the Mueller Hinton Agar (MHA). Six mm diameter well was filled with 50µl of culture supernatant. After three days, the diameters of the inhibition zones were measured and the results were recorded and tabulated. The experiment was carried out three times.

Extraction of volatile antifungal metabolites

The culture's spore suspensions were inoculated on M6 production medium and incubated for 10 days at 28°C on a shaker at 200 rpm. The supernatant was collected after centrifuging the fermented cultures. Later, the pH of the supernatant was reduced with HCl to 4.00. The supernatant was then extracted three times with ethyl acetate in a 1:1 (v/v) ratio. This supernatant was kept at 4°C for three days, until two layers formed. The organic phase was then separated using a separating funnel, and the aliquot was decanted and concentrated at 40 °C using a vacuum rotary evaporator. The crude extract was stored at 4°C before being dissolved in dimethyl sulfoxide (DMSO) for further use. The crude extract obtained was used to test antifungal activity.

Minimum inhibitory concentrations (MIC)

The crude ethyl extract (2 mg) was dissolved in 1 ml of DMSO: water (1:9) which was utilized for antifungal investigation using standard broth microdilution method [25] and the MIC was calculated. Mueller Hinton broth (MHB) was prepared and sterilized by autoclaving at 121ºC, 15 lbs for 20 minutes. In addition, the required concentration of the extract (µg/ml) (500.00, 250.00, 125.00, 62.50, 31.25, 15.65 and 7.80) was

added to the 96 well micro titer plate containing 0.1 ml MHB. The 10 µl of fungal spore suspension was introduced into the respective wells and the final inoculum size was at $1x10^5$PFU/ml. The titer plates were incubated at 28 ˚C for 3days; Amphotericin B and solvent DMSO were also included as positive and negative control respectively. MIC was determined as the lowest concentration of the crude extract which inhibited complete growth of tested fungi.

Gas chromatography-mass spectrometry (GC-MS) analysis

The active ethyl acetate extract was analyzed using a gas chromatograph (GC-MS-Shimadzu) equipped with a CPB-capillary column (mm inner diameter X 50 m length) mass spectrometer (ion source 200°C, RI 70 eV) set to 40-280°C at a rate of 4°C/min. The injector temperature was 280°C, and the carrier gas was He (20 psi). Using a hot-needle, sample volumes of 1 µl were injected with a split ratio of 25:1. Sargam Laboratory Service, Private Ltd, Chennai-600 089, India, performed the GC-MS analysis.

RESULTS AND DISCUSSION

Isolation and Characterization of strain nkm1

This isolate was identified as *Streptomyces* sp. strain nkm1 using morphological, biochemical, and molecular methods (Figure 1, 2 and Table 1). The sequence was submitted to the NCBI under the accession number HM125709. The Neighbor-Joining method was used to infer the evolutionary history. The optimal tree is shown, with a total branch length of 40.13326794. The evolutionary distances were measured in base substitutions per site and calculated using the Maximum Composite Likelihood method. Eleven nucleotide sequences were examined. First+second+third+noncoding codon positions were included. All positions with gaps and missing data were removed. The final dataset contained 260 positions. MEGA 5 was used to perform evolutionary analyses. Gram staining identified the strain nkm1 as a Gram-positive filamentous bacterium. The colonies were brown, opaque, rough, leathery, and difficult to remove due to filaments branching that had grown into the M6 medium, according to morphological studies. Streptomyces sp. was identified as the antifungal antibiotic-producing strain through biochemical and morphological analysis. The strain grew on a variety of agar media and displayed typical *Streptomyces* sp. morphology [26]. The color of the aerial mycelium was white and it did not produce diffusible pigments on several agar media. The strain exhibited salt tolerance (up to 0.8%) that could be placed in the intermediate salt tolerance group. The strain also demonstrated various biochemical activities and capability to produce different enzymes such as amylase, protease and lipase. The utilization of carbohydrates, nitrogen sources, and growth characteristics on different temperatures, pH and other characteristics are depicted in Table 1. Similar *Streptomyces* sp., has been isolated from different sources by several researchers [27-29].

Antifungal activities of volatile metabolites against plant pathogenic fungi

The preliminary screening revealed that strain nkm1 showed activity against tested plant pathogenic fungi (Figure 3 and Table 2). In M6 production medium, strain nkm1 demonstrated a broad spectrum of antifungal activity. As a result, it was chosen as the most efficient medium for mass production of secondary metabolites. The antifungal activity significantly started at exponential phase of growth; the maximum activity was observed on seventh day of incubation. The production medium's optimum pH and temperature were 7.0 ± 0.5 and 28^{o}C respectively. The other production media used for secondary metabolites production were not effective. The fermented culture broth from M6 production medium inhibited the growth of plant pathogenic fungi such as *F. oxysporum* (18.00±0.05), *P. palmivora* (6.00±0.05), *A. flavus* (21.00±0.07), *B. cinerea* (20.00±0.30), *A. alternata* (21.00±0.60), *C. capsici* (23.00±0.85), *R. solani* (20.00±0.40), *E. unguis* (18.00±0.30), *C. herbarum* (17.00±0.70) and *H. papulosum* (9.00±0.10) (Table 3 and Figure 4). Based on the inhibition zones, the M6 production medium was determined to be the best for the production of volatile antifungal secondary metabolites. The carbon and nitrogen sources were also preferred for antibiotic secondary metabolites synthesis but they did not favor high specific growth rate of *Streptomyces* sp. strain nkm1. The limitation of nutrients was responsible for the onset of antifungal metabolites biosynthesis [30-32]. The study's findings revealed that M6 production medium was the most efficient nutritional source for the production of antifungal metabolites.

Minimum inhibitory concentration (MIC)

200 mg crude extract was obtained from the concentrated organic phase. All of the tested plant pathogenic fungi were inhibited by the ethyl acetate extract, with MIC values as follows: (µg/ml); *F. oxysporum*

at 62.50, *P. palmivora* at 31.25, *A. flavus* at 31.2565, *B. cinerea* at 125.00, *A. alternata* at 125.00, *C. capsici* at 62.50, *R. solani* at 31.25, *E. unguis* at 15.62, *C. herbarum* at 31.25 and *H. papulosum* at 250.00 (Table 4). The above results indicated that the ethyl acetate extract of nkm1 significantly inhibited the growth of all the tested plant pathogenic fungi specifically *A. flavus, R. solani, E. unguis, C. herbarum, F. oxysporum* and *A. alternata*. Lyu and coauthors [33] reported the antifungal activity of actinomycetes against plant pathogenic fungi. Marimuthu and coauthors [34] also reported antifungal metabolites from *Streptomyces* sp. against the tea fungal plant pathogen *Pestalotiopsis theae* [35]. Previous research revealed that Streptomyces species culture extracts inhibited the growth of *A. niger*, *A. flavus*, and *F. oxysporum* [36, 37]. However, current findings showed that *Streptomyces* sp. strain nkm1 inhibited the growth of *F. oxysporum* and *A. flavus* at low concentrations of 62.50 and 15.65 µg/ml respectively. Various researchers have reported the antifungal activities of marine derived *Streptomyces* [38-41]. Our results showed that MIC value for *A. flavus* was 15.65 µg/ml. This value was low compared to previous reports [38, 41]. Many actinomycete genera, particularly those of the genus *Streptomyces*, are well known for producing antifungal agents that inhibit a variety of plant pathogenic fungi [42, 43]. *Streptomyces* species' antagonistic activity against plant fungal pathogens is usually associated with the production of antifungal compounds [44-46]. Many researchers have reported *Streptomyces* sp. antifungal activity in their studies [47-50].

GC-MS analysis of the active fraction

The active ethyl acetate extract of strain nkm1 was analyzed with GC-MS chromatograph as shown in Table 5, and the GC-MS spectrum showed the presence of various compounds. The major compounds from the GC-MS spectrum were: 3-Trifluoroacetoxypentadecane (11.9%), Hexadecane(10.5%), Phthalic acid, butyl undecyl ester(10.5%), 1-Iodo-2-methylundecane(10.2), Tetradcane (8.42%), Tetradecane, 2,6,10-trimethyl- (7.6%), Pterin-6-carboxylic acid (7.34%), 1-Octadecanesulphonyl chloride(6.77%), Decane, 2,4,6-trimethyl-(5.78%), Nonadecane (5.10%), 4-Trifluoroacetoxypentadecane(4.57%), Phthalic acid, isobutyl octadecyl ester(4.15%), 3-Trifluoroacetoxytetradecane(3.22%), Decane, 2,3,5,8-tetramethyl(3.09%), Tridecane (2.97%), Didodecyl phthalate(2.93%), 2-Trifluoroacetoxytetradecane(2.85%), 10-Heneicosene(2.84%), 5-Eicosene, (E) (2.52%), 1-Hexadecanol (2.41%) and Dodecane, 2,6,11-trimethyl-(2.24%) (Table 5). According to the literature, Nonadecane ($C_{19}H_{40}$) was the bioactive compound which exhibited significant activity towards tested plant fungal pathogens. However, several compounds were previously reported as antifungal agents from *Streptomyces* species [51, 52].

The production of microbial metabolites can be substantially increased by optimizing the nutritional conditions, physical parameters, and genetic makeup of the respective organisms. The nature and concentration of some components of the fermentation medium also have a marked effect on secondary metabolite production [53, 54]. The present study looked into the production of antifungal metabolites by *Streptomyces* sp. strain nkm1 in various media. The antifungal activity of crude ethyl acetate extract was significant. The majority of antifungal compounds were extracted using ethyl acetate [49, 55, 56]. The current results demonstrated that the antifungal compounds were produced extra cellularly in fermented medium. Most of the secondary metabolites and antifungal antibiotics are extracellular in nature. Extra cellular products of marine actinomycetes showed potent antifungal activities [57, 58].

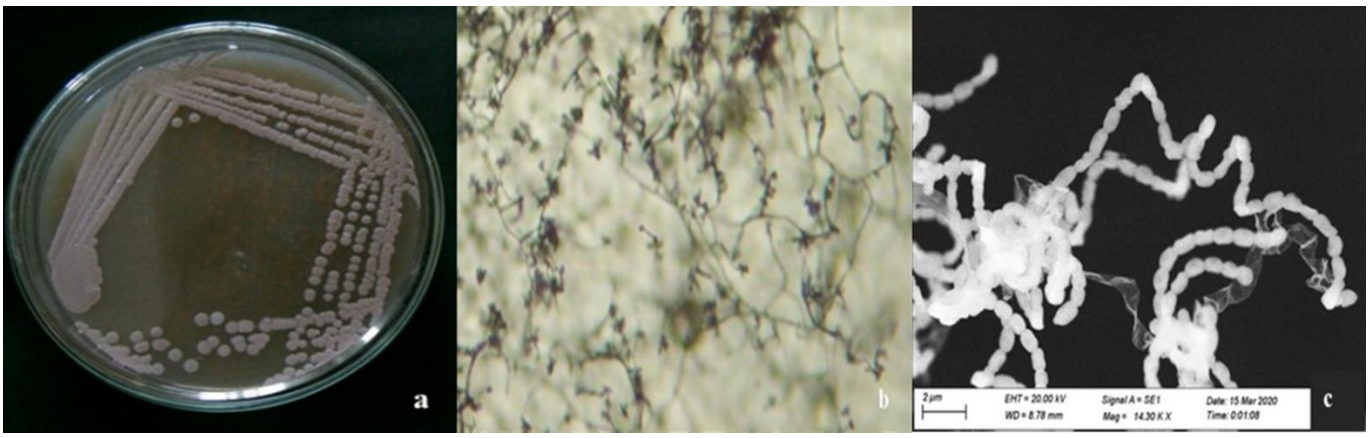

Figure 1. Morphological characterizations of *Streptomyces* sp. nkm1, a) on M6 medium, b) Microscopic image of spores' chain at 1500 X, c) Scanning electron microscope image of spores.

Table 1. Morphological and biochemical tests for identification of strain nkm1 on International *Streptomyces* project (ISP) medium 2.

Characteristics features	**Strain nkm1**
Gram reaction	Positive
Mycelium	Aerial mycelium
Color of the mycelium	White
Production of diffusible pigment	-
Range of temperature for growth	28° C- 38° C
Optimum temperature for growth	30° C
Range of pH for growth	5.0- 7.5
Optimum pH for growth	7.0
Hydrolysis of	
Protease	+
Catalase	+
Amylase	+
Lipase	+
Gelatinase	-
H_2S production	-
Utilization of carbon source	
L-Arabinose	-
Fructose	+
Galactose	+
Inositol	-
D-Mannitol	+
Rhamnose	-
Soluble starch	+
Sucrose	+
Xylose	-
Cellulose	-
Glucose	+

[1](+) means presence; [2](-) means absence

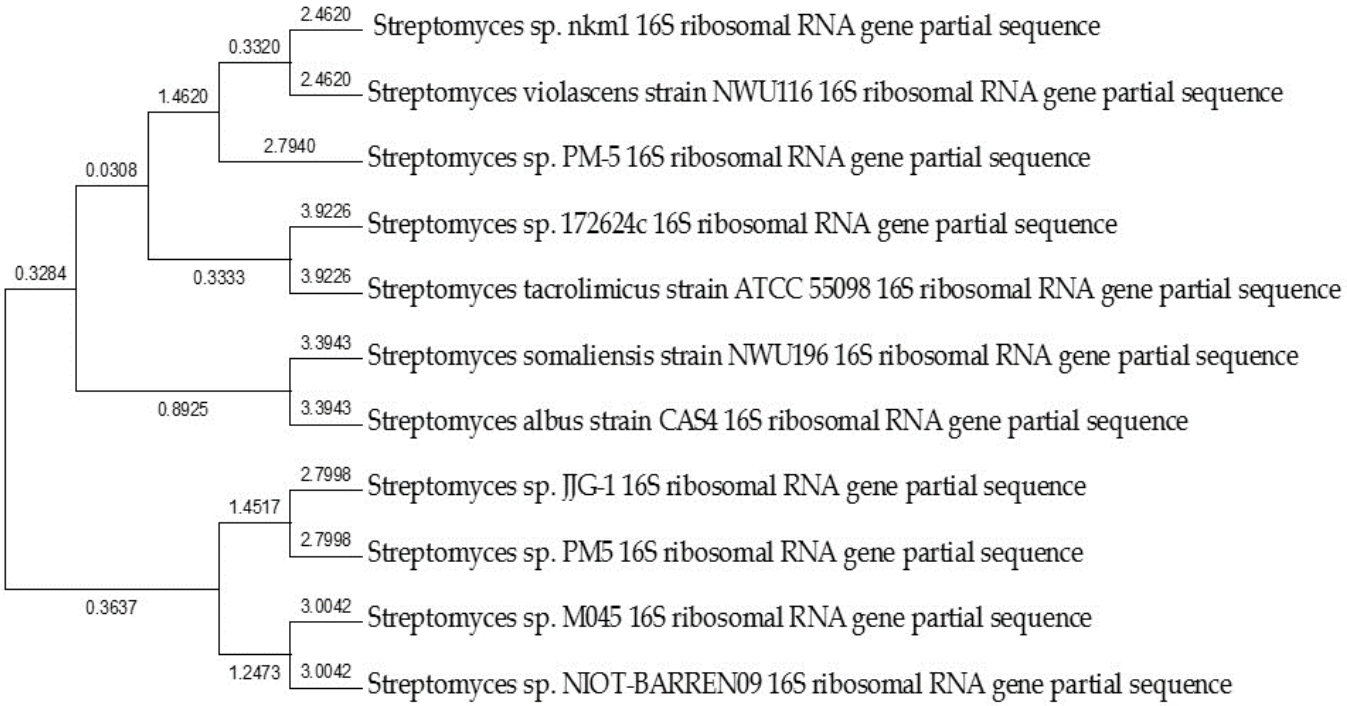

Figure 2. Evolutionary relationships of taxa of *Streptomyces* sp. nkm1.

Table 2. Preliminary screening of antifungal activity of *Streptomyces* sp. nkm1 fungi by cross streak method on Modified Nutrient Glucose Medium (MNGA)

Plant pathogenic Fungi	**Inhibition***
Fusarium oxysporum	+
Phytophthora palmivora	-
Aspergillus flavus	+
Botrytis conerea	+
Alternaria alternata	+
Cerscospora capsici	+
Rhizoctonia solani	+
Emericella unguis	+
Cladosporium herbarum	+
Helminthosporium papulosum	-

[1] (+) means Activity; [2](-) means no activity

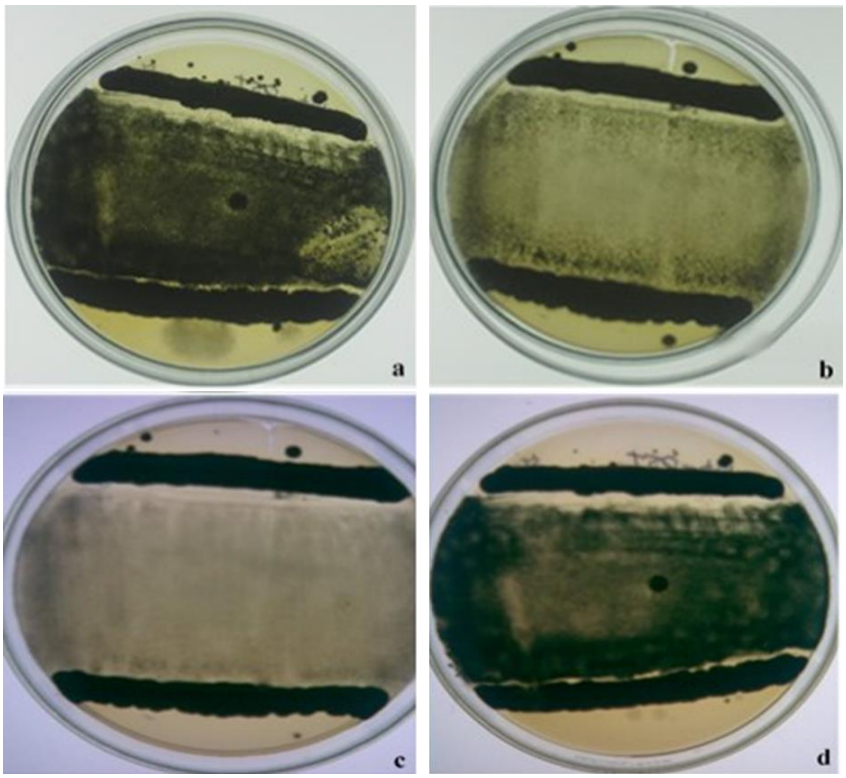

Figure 3. Preliminary screening of antifungal activity of *Streptomyces* sp. nkm1 against plant pathogenic fungi; a) *F. oxysporum*; b) *A. flavus*; c) *A. alternate*; d) *C. herbarum.*

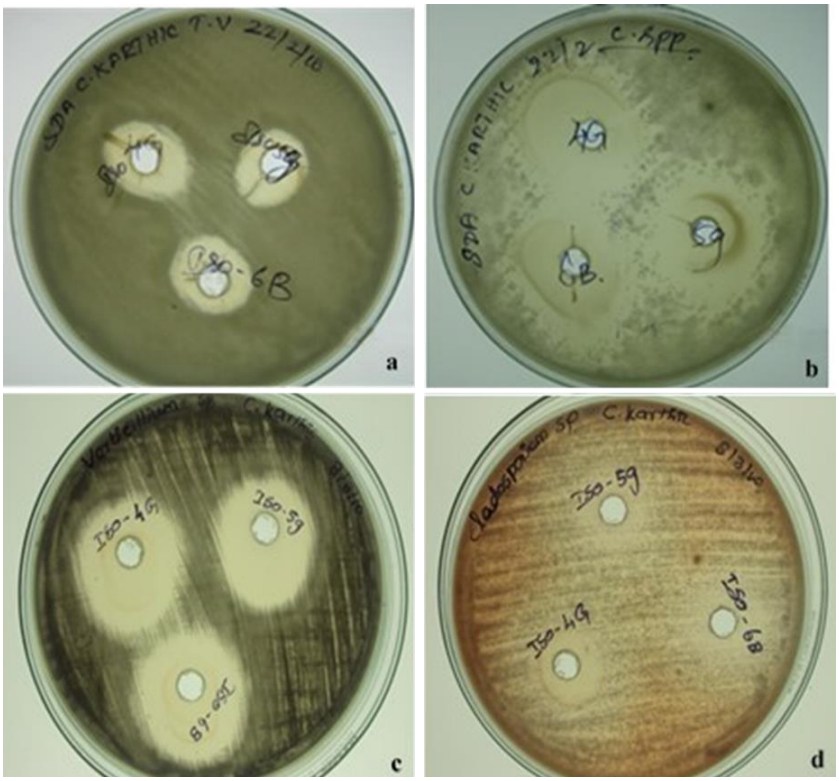

Figure 4. Antifungal activity of *Streptomyces* sp. nkm1 against plant pathogenic fungi by well diffusion method; a) *F. oxysporum*; b*) A. flavus*; c) *A. alternate*; d) *C. herbarum*.

Table 3. Antifungal activity of *Streptomyces* sp. nkm1 against plant pathogenic fungi with different production media by well diffusion method.

Plant Pathogenic Fungi	Zone of inhibition (mm)/production media*				
	SB	**YPGM**	**M6**	**TSB**	**MNGM**
F. oxysporum	13.00±0.50	17.00±0.15	18.00±0.05	11.00±0.10	15.00±0.25
P. palmivora	NZ[$]	NZ	6.00±0.05	NZ	NZ
A. flavus	15.00±0.10	18.00±0.55	21.00±0.07	21.00±0.12	18.00±0.18
B. conerea	14.00±0.21	19.00±0.25	20.00±0.30	10.00±0.05	16.00±0.15
A. alternata	12.00±0.20	20.00±0.75	21.00±0.60	19.00±0.40	19.00±0.25
C. capsici	11.00±0.25	21.00±1.05	23.00±0.85	20.00±0.55	24.00±0.75
R. solani	12.00±0.16	19.00±1.15	20.00±0.40	17.00±0.30	18.00±0.60
E. unguis	14.00±0.20	20.00±0.75	18.00±0.30	15.00±0.25	21.00±0.40
C. herbarum	13.00±0.50	22.00±1.00	17.00±0.70	21.00±0.90	25.00±1.50
H. papulosum	NZ	NZ	9.00±0.10	NZ	NZ

*SDA means (Sabourad Dextrose Broth), YPGM means (Yeast Peptone Glucose Medium), TSB means(Tryptic Soy Broth), M6 means (Fermentation Media 6), MNGM means (Modified Nutrient Glucose Medium), *NZ means No Zone

Table 4. MIC value of *Streptomyces* sp. strain nkm1 ethyl acetate extract.

Plant Pathogenic Fungi	MIC µg/mL	
	nkm1	**Amphotericin B**
F. oxysporum	62.50	<12.50
P. palmivora	31.25	<12.50
A. flavus	31.25	<12.50
B. conerea	125.00	<12.50
A. alternata	125.00	<12.50
C. capsici	62.50	<12.50
R. solani	31.25	25.00
E. unguis	15.62	25.00
C. herbarum	31.25	25.00
H. papulosum	250.00	25.00

MIC= minimal inhibition concentration, nkm1 = strain of Streptomyces

Table 5. GC-MS analysis of active ethyl acetate extract of *Streptomyces* sp. nkm1.

Name of the volatile compound	RT	Molecular Formula	Peak %	MW
3-Trifluoroacetoxypentadecane	4.626	$C_{17}H_{31}F_3O_2$	11.9	324.4
Hexadecane	13.122	$C_{16}H_{34}$	10.5	226.44
Phthalic acid, butyl undecyl ester	14.279	$C_{23}H_{36}O_4$	10.5	376.5
1-Iodo-2-methylundecane	20.70	$C_{12}H_{25}I$	10.2	296.23
Tetradecane	10.637	$C_{14}H_{30}$	8.42	198.39
Tetradecane, 2,6,10-trimethyl-	23.043	$C_{17}H_{36}$	7.60	240.50
Pterin-6-carboxylic acid	4.712	$C_7H_5N_5O_3$	7.34	207.15
1-Octadecanesulphonyl chloride	15.222	$C_{18}H_{37}ClO_2S$	6.77	352
Decane, 2,4,6-trimethyl-	10.18	$C_{13}H_{28}$	5.78	184.36
Nonadecane	15.435	$C_{19}H_{40}$	5.10	268.52
4-Trifluoroacetoxypentadecane	8.44	$C_{17}H_{31}F_3O_2$	4.57	324
Phthalic acid, isobutyl octadecyl ester	14.68	$C_{30}H_{50}O_4$	4.15	474.726
3-Trifluoroacetoxytetradecane	11.907	$C_{16}H_{29}F_3O$	3.22	310.39
Decane, 2,3,5,8-tetramethyl	12.1	$C_{14}H_{30}$	3.09	198.39
Tridecane	3.48	$C_{13}H_{28}$	2.9	184.36
Didodecyl phthalate	13.2	$C_{32}H_{54}O_4$	2.93	502.8
2-Trifluoroacetoxytetradecane	12.1	$C_{16}H_{29}F_3O_2$	2.85	310.39
10-Heneicosene	18.15	$C_{21}H_{42}$	2.84	294.6
Ethanol, 2-(hexadecyloxy)-	8.2	$C_{18}H_{38}O_2$	2.73	289.5
5-Eicosene, (E)-	20.255	$C_{20}H_{40}$	2.52	280.53
Trichloroacetic acid, tetradecyl ester	16.61	$C_{16}H_{29}Cl_3O_2$	2.51	359.8
3-Eicosene, (E)-	13.324	$C_{20}H_{40}$	2.50	280.54
1-Hexadecanol	9.770	$C_{16}H_{34}O$	2.41	242.26
Dodecane, 2,6,10-trimethyl	22.06	$C_{15}H_{32}$	2.33	212
Heptadecane, 2,6-dimethyl	21.2	$C_{19}H_{40}$	2.24	268.5
Dodecane, 2,6,11-trimethyl-	20.1	$C_{15}H_{32}$	2.24	212.41

CONCLUSION

The present study showed the influence of different nutritional media and culture conditions on antifungal compound production by *Streptomyces* sp. strain nkm 1. It also demonstrated that the maximum biological activities were found in the extract of M6 production medium. In addition, extracellular volatile metabolites in the culture extract of strain nkm1 inhibited the growth of plant pathogenic fungi more particularly *F. oxysporum* and *A. flavus* with minimum MIC values. Hence, the *Streptomyces* sp. strain nkm1 can be exploited as efficient biocontrol candidate for fungal diseases of economically important crops.

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REFERENCES

1. Barnard C, Padgitt M, Uri N. Pesticide use and its measurement. Int Pest Control. 1997;39(5):161-4.
2. Nguyen P-A, Strub C, Durand N, Alter P, Fontana A, Schorr-Galindo S. Biocontrol of *Fusarium verticillioides* using organic amendments and their actinomycete isolates. Biol Control. 2018;118:55-66.
3. Charousová I, Javoreková S, Medo J, Schade R. Characteristic of selected soil Streptomycetes with antimicrobial potential against phytopathogenic microorganisms. J Microbiol Biotechnol. Food Sci. 2020; 2020:64-8.
4. Zhao PJ, Wang HX, Li GH, Li HD, Liu J, Shen YM. Secondary metabolites from endophytic *Streptomyces* sp. Lz531. Chem Biodivers. 2007; 4(5):899-904.
5. Zhao Z, Wang Q, Wang K, Brian K, Liu C, Gu Y. Study of the antifungal activity of *Bacillus* vallismortis ZZ185 in vitro and identification of its antifungal components. Bioresour Technol. 2010; 101(1):292-7.
6. Qi D, Zou L, Zhou D, Chen Y, Gao Z, Feng R, et al. Taxonomy and broad-spectrum antifungal activity of *Streptomyces* sp. SCA3-4 isolated from rhizosphere soil of opuntia stricta. Front Microbiol. 2019;10.
7. Lange L, Sanchez Lopez C. Micro-organisms as a source of biologically active secondary metabolites. Crit Rep Appl Chem. 1996; 35:1-26.
8. Singh R, Manchanda G, Maurya I, Wei Y. Microbial versatility in varied environments. Springer Singapore; 2020.
9. Nandhini SU, Sangareshwari S, Lata K. Gas chromatography-mass spectrometry analysis of bioactive constituents from the marine Streptomyces. Asian J Pharm Clin Res. 2015; 8(2):244-6.
10. Lazzarini A, Cavaletti L, Toppo G, Marinelli F. Rare genera of actinomycetes as potential producers of new antibiotics. A Van Leeuw. 2001;79:219-405.
11. Wu X-C, Chen W-F, Qian C-D, Li O, Li P, Wen Y-P. Isolation and identification of newly isolated antagonistic *Streptomyces* sp. strain AP19-2 producing chromomycins. J Microbiol. 2007; 45(6):499-504.
12. Duraipandiyan V, Sasi A, Islam V, Valanarasu M, Ignacimuthu S. Antimicrobial properties of actinomycetes from the soil of Himalaya. J Mycol Med. 2010; 20(1):15-20.
13. Baltz RH. Antimicrobials from actinomycetes: back to the future. Microbe. 2007;2:6-7.
14. Imada C. Enzyme inhibitors and other bioactive compounds from marine actinomycetes. A Van Leeuw. 2005; 87(1):59-63.
15. Imada C, Koseki N, Kamata M, Kobayashi T, Hamada-Sato N. Isolation and characterization of antibacterial substances produced by marine actinomycetes in the presence of seawater. Actinomycetologica. 2007 Jun 25;21(1):27-31.
16. Raja S, Ganesan S, Sivakumar K, Thangaradjou T. Screening of marine actinobacteria for amylase enzymes inhibitors. Indian J Microbiol. 2010; 50(2):233-7.
17. Lin Q, Liu Y. A new marine microorganism strain L0804: taxonomy and characterization of active compounds from its metabolite. World J Microbiol Biotechnol. 2010; 26(9):1549-56.
18. Harikrishnan H, Shanmugaiah V, Balasubramanian N. Optimization for production of Indole acetic acid (IAA) by plant growth promoting *Streptomyces* sp VSMGT1014 isolated from rice rhizosphere. Int J Curr Microbiol Appl Sci. 2014; 3(8):158-71.
19. 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; 2013.
20. Ellaiah P, Ramana T, Raju K, Sujatha P, Sankar AU. Investigations on marine actinomycetes from bay of Bengal near Kakinada coast of Andhra Pradesh. Asian J Microbiol Biotechnol Environ Sci. 2004; 6:53-6.
21. Shirling ET, Gottlieb D. Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol. 1966; 16(3):313-40.
22. Waksman SA. The Actinomycetes. Vol. II. Classification, identification and descriptions of genera and species. The Actinomycetes Vol II Classification, identification and descriptions of genera and species. 1961.
23. Prauser H. Aptness and application of colour codes for exact description of colours of Streptomycetes. Z Allg Mikrobiol. 1964;4(1):95-8.
24. Egorov N. Microorganisms-antagonists and biological methods for evaluation of antibiotic activity. Moscowka High school. 1965;200.
25. Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activity of Flindersine isolated from the traditional medicinal plant, Toddalia asiatica (L.) Lam. J Ethnopharmacol. 2009; 123(3):494-8.
26. Anderson AS, Wellington E. The taxonomy of Streptomyces and related genera. Int J Syst Evol Microbiol. 2001; 51(3):797-814.
27. Oskay AM, Üsame T, Cem A. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. Afr J Biotechnol. 2004;3(9):441-6.

28. Srivibool R, Sukchotiratana M. Bioperspective of actinomycetes isolates from coastal soils: A new source of antimicrobial producers. Songklanakarin J Sci Technol. 2006; 28:493-9.
29. Dharmaraj S, Sumantha A. Bioactive potential of *Streptomyces* associated with marine sponges. World J Microbiol Biotechnol. 2009; 25(11):1971-9.
30. Doull JL, Vining LC. Nutritional control of actinorhodin production by *Streptomyces coelicolor* A3 (2): suppressive effects of nitrogen and phosphate. Appl Microbiol Biotechnol. 1990;32(4):449-54.
31. Sanchez S, Demain AL. Metabolic regulation of fermentation processes. Enzyme Microb Technol. 2002; 31(7):895-906.
32. Chauhan A, Zubair S, Tufail S, Sherwani A, Sajid M, Raman SC, et al. Fungus-mediated biological synthesis of gold nanoparticles: potential in detection of liver cancer. Int J Nanomed. 2011; 6:2305.
33. Lyu A, Liu H, Che H, Yang L, Zhang J, Wu M, et al. Reveromycins A and B from Streptomyces sp.3-10: antifungal activity against plant pathogenic fungi in vitro and in a strawberry food model system. Front Microbiol. 2017; 8:550.
34. Marimuthu S, Karthic C, Mostafa AA, Al-Enazi NM, Abdel-Raouf N, Sholkamy EN. Antifungal activity of *Streptomyces* sp. SLR03 against tea fungal plant pathogen *Pestalotiopsis theae*. J King Saud University Sci. 2020; 32(8):3258-64.
35. Kumar PS, Yuvaraj P, Paulraj MG, Ignacimuthu S, Al-Dhabi NA. Bio-prospecting of soil *Streptomyces* and its bioassay-guided isolation of microbial derived auxin with antifungal properties. J Mycol Med. 2018; 28(3):462-8.
36. Ara I, Bukhari N, Perveen K, Bakir M. Antifungal activity of some actinomycetes isolated from Riyadh soil, Saudi Arabia: An evaluation for their ability to control Alternaria caused tomato blight in green house pot trial. Afr J Agric Res. 2012;7(13):2042-50.
37. Mohseni M, Norouzi H. Antifungal activity of actinomycetes isolated from sediments of the Caspian Sea. J Maz Univ Med Sci. 2013; 23(104):80-7.
38. Wang Z, Yu Z, Zhao J, Zhuang X, Cao P, Guo X, et al. Community composition, antifungal activity and chemical analyses of ant-derived actinobacteria. Front Microbiol. 2020; 11:201.
39. Yi W, Qin L, Lian X-Y, Zhang Z. New Antifungal Metabolites from the Mariana Trench Sediment-Associated Actinomycete *Streptomyces* sp. SY1965. Mar Drugs. 2020;18(8):385.
40. Lu D, Ma Z, Xu X, Yu X. Isolation and identification of biocontrol agent *Streptomyces rimosus* M527 against Fusarium oxysporum f. sp. cucumerinum. J Basic Microbiol. 2016; 56(8):929-33.
41. Nafis A, Elhidar N, Oubaha B, Samri SE, Niedermeyer T, Ouhdouch Y, et al. Screening for Non-polyenic Antifungal Produced by Actinobacteria from Moroccan Habitats: Assessment of Antimycin A19 Production by *Streptomyces albidoflavus* AS25. Int J Mol Cell Med. 2018; 7(2):133.
42. Oskay M. Antifungal and antibacterial compounds from *Streptomyces* strains. Afr J Biotechnol. 2009; 8(13).
43. Bubici G. *Streptomyces* spp. as biocontrol agents against *Fusarium* species. CAB Rev. 2018;13:050.
44. Evangelista-Martínez Z. Isolation and characterization of soil *Streptomyces* species as potential biological control agents against fungal plant pathogens. World J Microbiol Biotechnol. 2014; 30(5):1639-47.
45. Colombo EM, Kunova A, Pizzatti C, Saracchi M, Cortesi P, Pasquali M. Selection of an endophytic Streptomyces sp. strain DEF09 from wheat roots as a biocontrol agent against Fusarium graminearum. Front Microbiol. 2019; 10:2356.
46. Kaur T, Kaur A, Sharma V, Manhas RK. Purification and Characterization of a New Antifungal Compound 10-(2, 2-dimethyl-cyclohexyl)-6, 9-dihydroxy-4, 9-dimethyl-dec-2-enoic Acid Methyl Ester from *Streptomyces hydrogenans* Strain DH16. Front Microbiol. 2016; 7:1004.
47. Kumar S, Kannabiran K. Antifungal activity of *Streptomyces* VITSVK5 spp. against drug resistant *Aspergillus* clinical isolates from pulmonary tuberculosis patients. J Mycol Med. 2010; 20(2):101-7.
48. Goudjal Y, Zamoum M, Sabaou N, Mathieu F, Zitouni A. Potential of endophytic *Streptomyces* spp. for biocontrol of *Fusarium* root rot disease and growth promotion of tomato seedlings. Biocontrol Sci. Technol 2016; 26(12):1691-705.
49. Ahsan T, Chen J, Zhao X, Irfan M, Wu Y. Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Express. 2017; 7(1):1-9.
50. Shaik M, Sankar GG, Iswarya M, Rajitha P. Isolation and characterization of bioactive metabolites producing marine *Streptomyces parvulus* strain sankarensis-A10. J Genet Eng Biotechnol. 2017; 15(1):87-94.
51. Xu F, Nazari B, Moon K, Bushin LB, Seyedsayamdost MR. Discovery of a cryptic antifungal compound from *Streptomyces albus* J1074 using high-throughput elicitor screens. J Am Chem Soc. 2017; 139(27):9203-12.
52. Elnahas MO, Amin MA, Hussein M, Shanbhag VC, Ali AE, Wall JD. Isolation, characterization and bioactivities of an extracellular polysaccharide produced from *Streptomyces* sp. MOE6. Molecules. 2017; 22(9):1396.
53. Wang D, Wang C, Gui P, Liu H, Khalaf SM, Elsayed EA, et al. Identification, bioactivity, and productivity of actinomycins from the marine-derived *Streptomyces heliomycini*. Front Microbiol. 2017;8:1147.
54. Gesheva V, Ivanova V, Gesheva R. Effects of nutrients on the production of AK-111-81 macrolide antibiotic by *Streptomyces hygroscopicus*. Microbiol Res. 2005; 160(3):243-8.
55. Lacret R, Oves-Costales D, Gómez C, Díaz C, De la Cruz M, Pérez-Victoria I, et al. New ikarugamycin derivatives with antifungal and antibacterial properties from Streptomyces zhaozhouensis. Mar Drugs. 2015; 13(1):128-40.

56. Lee L-H, Goh B-H, Chan K-G. Actinobacteria: Prolific producers of bioactive metabolites. Front Microbiol. 2020; 11:1612.
57. Wei Y, Zhao Y, Zhou D, Qi D, Li K, Tang W, et al. A newly isolated *Streptomyces* sp. YYS-7 with a broad-spectrum antifungal activity improves the banana plant resistance to Fusarium oxysporum f. sp. cubense tropical race 4. Front Microbiol. 2020; 11:1712.
58. He H, Hao X, Zhou W, Shi N, Feng J, Han L. Identification of antimicrobial metabolites produced by a potential biocontrol Actinomycete strain A217. J Appl Microbiol. 2020; 128(4):1143-52.