ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antifungal Compound against Phytopathogenic Fungi Produced by *Streptomyces griseofuscus* NV-38 Screening, Isolation and Identification.

Kamal YI El-shahed[1], Bakery M Haroun[2], Islam A. Elsehemy[1], and Hassan M Awad [1,3]*.

[1]Chemistry of Natural and Microbial Products Dept., Pharmaceutical Industries Div., National Research Centre,33 EL Bohouth St. (Former EL Tahrir St.) .,Dokki, Giza, Egypt. P.O.Box:12622.
[2]Botany and Microbiology Dept., Faculty of Science, Al-Azhar University, Yosief Abbas Street P.O 11787, Cairo, Egypt
[3]Deanship of Preparatory Year, Al jouf University, Al Jouf, Kingdom of Saudi Arabia. Sakaka P.O. Box 2014

ABSTRACT

As part of a screen for antifungal activity, a total of 110 isolates were recovered from Egyptian soil samples. The objective of this study was to determine the antifungal activity of compounds produced by a new *Streptomyces* isolate. The NV-38 isolate was found to be active against different microorganisms, especially fungi. The identification of this isolate based on culture, morphological, physiological and biochemical characteristics strongly indicated that the NV-38 strain belongs to the genus *Streptomyces*. Analysis of the nucleotide sequence of the 16S rRNA gene of the *Streptomyces* sp. NV-38 showed high similarity (99%) with the 16S rRNA of *Streptomyces griseofuscus* NRRL B-1989. Phenotypic, phylogenetic and chemotaxonomic analyses support the conclusion that NV-38 represents a novel strain within the genus *Streptomyces*; the name *Streptomyces griseofuscus* NV-38 was assigned to this strain. The nucleotide sequence of the 16S rRNA gene was submitted to GenBank under ID HQ839862. Partial characterization of the active antifungal compound using thin layer chromatography and chemical reactions was performed. The present results indicate that the NV-38 isolate is a potential antifungal producer that could be used for the biocontrol of plant pathogens.
Keywords: Antifungal compound, *Streptomyces griseofuscus* NV-38, isolation, phenotypic and phylogenetic identification

Corresponding author

INTRODUCTION

The incidence of fungal infection is increasing. Fungi infect humans, animals, and plants and cause many health and economic problems [1]. Fungal phytopathogens cause serious problems in the world's agriculture and food industry by destroying crops and economically important plants in the field and during storage, especially in the subtropical and tropical regions [2]. *Fusarium,* which is found worldwide in cereals and other food types for human and animal consumption, are the most important toxigenic fungi in northern temperate regions [3].

In Egypt, ***F. moniliforme*** **and** several other common species contaminate the maize and attack the mango, causing malformation (mangofirea) and prodcing fumonisin B1, a suspected human carcinogen (IARC) that is also toxic to pigs and poultry and fatal to horses [4]. Egyptian soils have been exposed from time to time to pollution from chemical fertilizers and watering with polluted water. These new conditions may cause new isolates of microflora to be obtained. However, *Streptomyces* are well known as biocontrol agents or lyses several soil borne and airborne plant pathogenic fungi [5]. Bressan, [6] found that two *Streptomyces sp.* isolated from maize rhizosphere, which inhibited *Aspergillus sp*. and *F. subglutinans,* seed pathogenic fungi.

Antifungal agents are used in areas such as human and animal therapies, agriculture to protect plants and crops, the food industry and in the treatment of wood. Plant diseases need to be controlled to maintain the quality and abundance of food and feed [7]. The toxicity of present antifungal therapies due to the biochemical similarity between fungal pathogens and the infected host (all eukaryotes) as well as the increase of mycosis caused by opportunistic fungi encourages research for novel antifungal antibiotics [1].

The antifungal potential of extracellular metabolites of *Streptomyces* strains against fungi has been reported around the world. However, there is no research on the antifungal activities of *Streptomyces* sp. isolated from the New Valley habitats as a novel source for the discovery of new bioactive compounds. Therefore, this study reported herein was undertaken to determine and elucidate the antifungal potential compound of a new isolated *Streptomyces* sp. against pathogenic fungi, especially *F. moniliforme,* and will use different methods for the taxonomy of the antibiotic producing strain.

MATERIALS AND METHODS

Isolation of pathogenic (target organism)

To obtain a pure culture from *Fusarium* species, the single-spore technique was used [8]. *F. moniliforme* var *subglatinus* (*F. moniliforme*) was isolated from (mango malformed panicles) an infected field at Giza Governorate. This fungal strain was identified at Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Cultural features in addition to microscopic examination were carried out for the fungal isolate and identification was based on current universal keys for fungi [9].

Soil samples collection and Streptomyces isolation

Soil samples were collected at a depth of 15-20 cm from different localities in Egypt (Alexandria, Hurghada, North Sinai, EL-Mansoura, New Valley, EL-Sharkia, Qualiubia and Giza). The isolation and enumeration of *Actinomycetes* were performed by a soil dilution plate technique using either a starch-nitrate medium or an *Actinomycetes* isolation agar medium (Difco, Michigan, U.S.). The starch-nitrate medium (g/l) was made up of soluble starch, 20; $NaNO_3$, 2; K_2HPO_4, 1; KCl, 0.5; $MgSO_4\ 7H_2O$, 0.5; $CaCO_3\ 2H_2O$, 2; agar, 20; at pH 7 [10].

Screening for antimicrobial activity of an actinomycete isolates

The starch-nitrate medium was used to culture *actinomycetes* isolates at 28ºC for seven days. The MO used are: *B. subtitles* ATCC-6633, *S. aurous* ATCC 6538, *P. aeruginosa* ATCC 9027, *E. coli* ATCC-7839, *C. albicans* ATCC-10231, *A. niger* NRRL 348, *A. flavus* ATCC-16883 and *F. moniliforme*. The nutrient agar (Merck, Darmstadt, Germany) was used for bacteria at 37ºC for 24 hours, Sabaroud's agar (Lab M., Bury, Lancashire, U.K.) for yeast at 30ºC for 24 hours and PDA (Merck, Darmstadt, Germany) for fungi at 25ºC for 48 hours. The bioassay method was carried out using the agar well diffusion method [11].

Conventional taxonomy of Streptomyces isolate

The morphological characteristics of the potential strain NV-38 were examined under a light and scanning electron microscope using starch nitrate agar medium at 30 ºC for 14-21 days, according to Locci [12]. Physiological and biochemical characteristics such as lipase [13], protease [14], α-amylase [15] and catalase [16] were tested. Also, hydrogen sulfide production and oxidase tests were investigated [15]. Melanin pigment, nitrate reduction, the utilization of C and N sources and cultural characteristics were studied in accordance with the guidelines established by the ISP [17]. The cell wall was performed by Lechevalier and Lechevalier's method [18]. Color characteristics were assessed on the scale developed by Tresner and Backus [19].

Molecular biology identification of the strain NV-38

Extraction of genomic DNA Streptomyces

The NV-38 isolate was inoculated with 50 ml of the ISP2 broth medium at 28 °C on 200 rpm for 24 hours. The total DNA was extracted according to Pospiech and Neumann [20].

PCR amplification

The 16S rDNA gene was amplified by PCR on Gene AMP, PCR System 9700, PE Applied Biosystems, (Perkin Elmer, Ohio, U.S.). *Streptomyces*-specific PCR primers were Strep B, 5'- ACA AGC CCT GGA AAC GGG T-3' (forward) and Strep F 5'-ACG TGT GCA GCC CAA GACA -3'(reverse) using Biolego BV software (Biolegio, Nijmegen, the Netherlands), [21]. The PCR reaction mixture (25µL) conditions using PCR beads (Amirsham Bioscience Europe GmbH, Freiburg, Germany) were approved according to Awad et al. [22]. PCR products were purified using Qia quick PCR purification kit (Qiagen, Hilden, Germany) and detected by the gel documentation system, (Alpha-Imager 2200, CA, U.S.).

Sequencing of PCR products and phylogenetic analysis

PCR products were sequenced by the Gene Analysis Unit in the genetic laboratories of an Egyptian company that produces vaccines, sera and drugs (VACSERA), El-Dokki, Cairo, Egypt. The DNA sequences were detected using an ABI PRISM 377 DNA sequencer and ABI PRISM BigDye Terminator Cycle Sequencing (Perkin Elmer, Ohio, U.S.) at a sequencing facility at Cornell University in the U.S. BLAST (www.ncbi.nlm.gov) was used to assess the DNA similarities. A multiple sequence alignment and molecular phylogeny were performed using BioEdit software [23]. The phylogenetic tree was constructed using the Tree-view program [24].

Fermentative production

Streptomyces isolated was cultivated at 28 °C and 200 rpm on a rotary incubator shaker for 6 days at 30 ºC. The cultivation was performed in a 250 ml Erlenmeyer flask containing 50 ml starch-nitrate medium at pH 7.0. The inoculation was carried out using a spore suspension of strain NV-38 slanted on ISP2. Then, the filtrate broth was used to determine antifungal activity and related analysis.

Extraction and purification of active antifungal compound

The extraction process was conducted using immiscible organic solvents ethyl acetate, chloroform, hexane, n-butanol and petroleum ether. The active filtrate was adjusted at pHS 2, 5, 7 and 9 and the organic solvent was used with ratios at 1:1 v/v for solvent and active filtrate. Then, the active substance was applied to bioassay against *F. moniliforme* by using a paper disc technique (0.5 mm).

The active compound was detected by TLC using silica gel aluminum sheet G 60 F 245 (Merck, Darmstadt, Germany). The active spot was tested by bioassay and the florescence characters using a short UV lamp. Then they were scratched, collected and eluted by methanol. The active metabolite was then prepared for further analyses by using spectroscopic characters.

Physico-chemical Properties

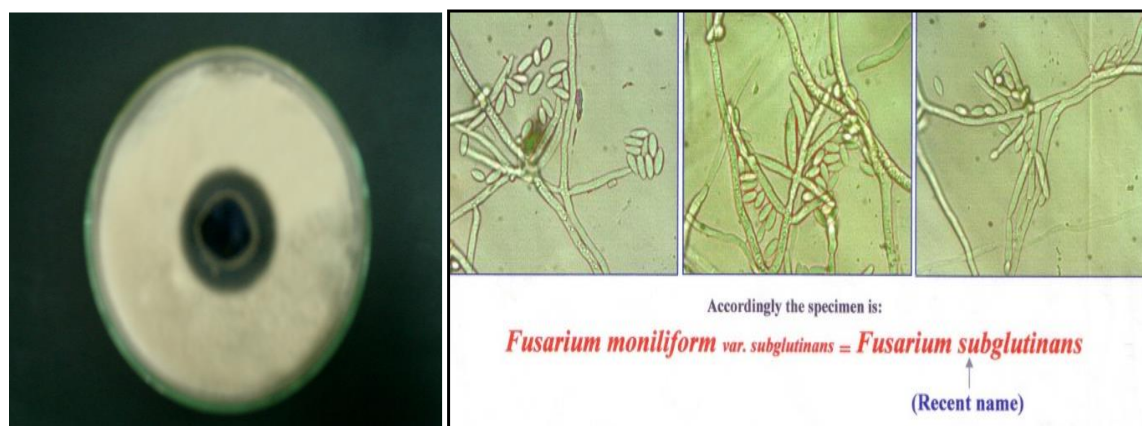
Reactions such as Molish's, Fehling, Sakaguchi, Ninhydrin, Ehrlish, Nitroprusside, ferric chloride and Mayer were carried out.

RESULTS AND DISCUSSION

Isolation and identification of F. moniliforme

The cultural examination of the isolated fungus indicated that the colonies attaining a diameter of 4.5 mm in 96 hours have pale pinkish mycelium and reverse peach salmon pigmentation (Fig. 1a). At the same time, the microscopic examination (Fig. 1b) shows that the conidiophores were unbranched at first, and then were later branched with mono and polyhialides. This indicates that the microcondia produced from polyhialides is uniform. Diagnostic character separates this variety from the parent strain and accumulates in globashead. It also observed that a chain is not produced; instead they are oval to obclavate (10 x 2.7 µm**)**. The macro-conidia is absent while meso-conidia appeared and chlamydospores were not produced. Therefore, according to these characteristics [9], the pathogenic fungus was identified as *F. moniliforme*, which is used as a target organism.

Fig 1: Bioassay of the isolate NV-38 against *Fusarium moniliforme* var *subglutinans* (a), conidiophores and micro-conidia of *F. moniliforme*.var *subglutinans* (b).



Screening of *Streptomyces* isolates producing antifungal agent

A total of 110 *Streptomyces* isolates were collected from eight localities in Egypt using the selective medium and cultivation conditions. The results showed a variation of the *Streptomyces* (87 isolate) where 53 were against G^{+}ve and 34 were against G^{-}vie with a variation of inhibition zone diameter (Table 1). The results obtained indicated that 23, 31, 19 and 38 isolates of *Streptomyces* produced bioactive substances against the pathogenic fungus *C. albicans, A. niger, A. flavas and F. moniliforme* respectively.

Seventy-five isolates (68.18%) showed antimicrobial activity against the tested microorganisms; 72% showed antibacterial activity, 55% showed antifungal activity and 30.9% showed antibacterial activity against either G^{+}ve or G^{-}ve bacteria while 28% exhibited a broad-spectrum activity against test bacteria, yeast and fungi. Soil is the most common habitat of *Streptomycetes* in most soils, *Streptomycetes* comprise 1-20% of the total viable count [25], and 64-97% of the cultivable actinomycetes [26]. The isolation of rare and uncommon actinomycetes has become an increasingly important part of natural product discovery [27].

Table 1: Initial screening and origin of samples for *Streptomyces* isolate on starch-nitrate medium by cup plate method.

Origin of soil samples	Soil samples properties	Total isolates No.	Total number of active isolates against microorganisms							
			Bacteria				Fungi			
			G+ve		G-ve		unicellular	filamentous		
			B. subtilits	*S .aureus*	*P.aerogenos*	*E. coli*	*C. albicans*	*A. niger*	*A. flavus*	*F. monilifor*
1. GZ	cultivated & sandy	14	4	2	2	0	0	3	3	6
2. Hu	uncultivated & sandy	23	3	5	2	5	3	7	4	7
3. NV	sandy	4	2	2	1	2	1	0	1	2
4. Alex	cultivated & sandy	12	0	3	2	0	4	5	3	8
5. QuB	cultivated& uncultivated	34	8	10	4	5	8	8	6	11
6. SH	cultivated	2	1	1	1	1	1	2	0	0
7. Ma	cultivated	1	0	0	0	0	1	1	0	0
8. NS	cultivated & sandy	20	7	5	5	4	5	5	2	4
Total isolates No		110	25	28	17	17	23	31	19	38

Where: localities are; Giza (GZ), Hurghada (Hu), New valley (NV), Alexandria (Alex), North Sinia (NS), Qualiubia (QuB).

Emphasized screening of isolates for antimicrobial activity

The subsequent screening of 17 promising isolates showed broad-spectrum activity against a test MO in an agar medium. The results in Table 2 show that NV-38 is the isolate that produces the most antifungal substance against *F. moniliforme*, and it exhibited a broad-spectrum activity against both test bacteria and fungi, except for *A. niger*.

Table 2: Effect of different incubation period of the promising *Streptomyces* isolates against *F. moniliforme*

Isolate	Mean diameter of inhibition zone (mm)/incubation time day									
	Solid medium	Submerged culture								
No	6^{th}day	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
GZ-6	20	0.0	0.0	0.0	14	17	18	20	19	15
Hu-22	18	0.0	0.0	0.0	0.0	18	17	0.0	0.0	0.0
Hu-24	19	0.0	0.0	0.0	0.0	0.0	17	20	18	22
Hu-28	18	0.0	0.0	0.0	0.0	15	20	19	13	13
Hu-33	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20
NV-38	**21**	**0.0**	**13**	**15**	**18**	**20**	**23**	**22**	**21**	**20**
ALX-42	18	0.0	0.0	0.0	0.0	14	12	20	20	22
ALX- 44	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18	18
ALX-45	18	0.0	0.0	13	20	22	19	18	21	15
ALX-49	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18	19
ALX- 50	20	0.0	0.0	0.0	0.0	13	13	21	18	18
QuB-59	17	0.0	0.0	0.0	0.0	16	16	16	0.0	0.0
QuB-60	21	0.0	0.0	0.0	13	21	19	0.0	0.0	0.0
QuB-74	19	0.0	0.0	0.0	0.0	15	18	17	17	17
QuB-82	21	0.0	12	15	15	18	21	20	22	21
QuB-84	1.8	0.0	0.0	0.0	0.0	1.8	1.8	2.1	0.0	0.0
NS–92	20	0.0	0.0	18	20	20	21	20	19	19

Where: localities are; Giza (GZ), Hurghada (Hu), New valley (NV), Alexandria (Alex), North Sinia (NS), Qualiubia (QuB).

A preliminary screening for antifungal activity production was done by a conventional agar plug method of isolates using the diffusion technique [28]. The formation of insoluble compounds may eliminate the availability of a nutrient, while inhibiting compounds that result from the interaction of medium components may result in a medium that is not conducive to growth or product formation [29]. Therefore, strain NV-38 was chosen for further study.

Conventional identification of strain NV-38

Cultural and morphological characteristics

Table 3 shows that the growth of NV-38 strain varies from abundant to fair depending on medium composition. The growth was abundant on media ISP-2, ISP-3, ISP-4, starch nitrate and Czapek's and weak on the other media. The color of aerial mycelium ranged from white to light gray. Consequently, the aerial mycelium is assigned to the gray series. The soluble pigment differed from one medium to another depending on the medium composition. Spore masses were matched against the seven color wheels of Tresner and Backus, [19] as used in the ISP [17].

Table 3: Cultural characteristics of the *Str.griseofuscus* NV-38.

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
Tryptone yeast extract broth (ISP 1)	Weak	White (ISCC-NBS 263)	Light Gray (ISCC-NBS 264)	None
Yeast -malt extract agar (ISP 2)	Good	Light Gray (ISCC-NBS 264)	greenish (ISCC-NBS 264)	Very deep red purple (SCC-NBS 243)
Oat meal agar (ISP 3)	Good	Light Gray (ISCC-NBS 264)	Light Gray (ISCC-NBS 264)	Very deep red purple (SCC-NBS 239)
Inorganic-trace salt- starch agar (ISP 4)	Good	Light Gray (ISCC-NBS 264)	greenish (ISCC-NBS 264)	Gray red purple (ISCC-NBS 245)
Glycerol asparagine agar (ISP 5)	weak	Hygroscopic grayish	Light Gray (ISCC-NBS 264)	Light yellow brown (SCC-NBS 76)
Peptone yeast extract iron agar (ISP 6)	Weak	Hygroscopic growth	colorless	None
Tyrosine agar (ISP 7)	Weak	Hygroscopic growth	Colorless	None
Starch-nitrate agar	Good	Light Gray (ISCC-NBS 264)	greenish (ISCC-NBS 264)	Slightly purple (ISCC-NBS218)
Nutrient agar	Weak	Hygroscopic growth	Colorless	None
Czapek's agar	Good	White (ISCC-NBS 263)	greenish (ISCC-NBS 264)	Slight red purple (SCC-NBS 237)

Microscopically, it was observed that the morphology of the spore chains of aerial mycelium are of the spiral type (Fig. 2a). According to the shape of the spore chains observed under light microscopy, the isolates were grouped as rectus-flexible (RF), spiral (S) and retinaculum apertum (RA), [12]. The micrograph in Fig. 2b shows that the individual spores are cylindrical with a smooth surface, which was determined according to the categories of Tresner et al. [30], who found that spore surface is one of the descriptive characterizations for each type of culture.

Physiological and biochemical characteristics

Table 4 shows that the isolate does not produce melanin pigment on the media used while NV-38 degraded protein and starch, but not lipid. It liquefied gelatin. Hydrogen sulfide is produced and the results were positive for nitrate reduction, urea test and catalase production, and negative for tyrosine degradation. Other categories were tested and considered to ascertain the species classification of a new isolate strain as recommended by Holt et al. [31].

Fig 2: Light microscope for aerial mycelium showing spiral spore chain (G x 10) (a) and scanning electron micrographs showing smooth spore surface; (b) for *Strptomyces griseofuscus* NV-38 (X7500) using starch nitrate agar medium at 14-21 days.

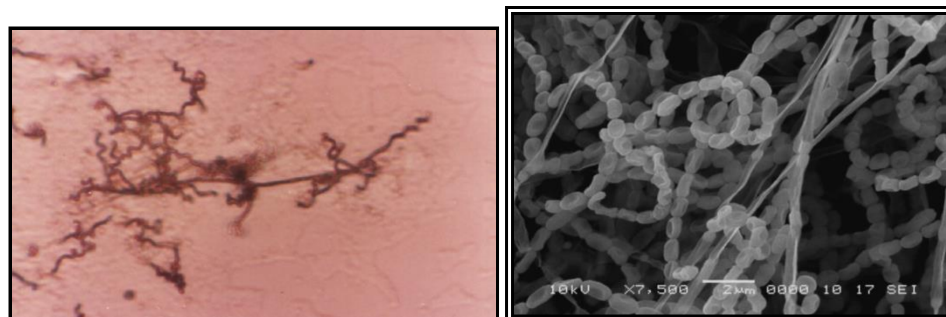


Table 4: Morphological, physiological and biochemical characteristics of *Str. griseofuscus* NV-38.

Character	Results	Character	Results
Morphological characteristics		Nitrogen source utilization	
Spore chain	Spiral	L-Asparagine	+++
Spore mass	Light gray	L-Glutamic	+
Spore surface	Smooth	L-lysine	++[b]
Motility	Non motile	L-Ornithine	+[c]
Color of substrate mycelium	Grayish	L-Tyrosine	+
Diffusible pigment	Purple	L-Valine	++
Cell wall hydrolysis		L-Histidine	+++
Diaminopimelic acid (DAP)	LL-DAP	Glycine	+++
Sugar pattern	ND [a]	L-leucine	+
Physiological characteristics		L-Phenyl alanine	Wg[f]
Melanin pigment:		L-Serine	-
Peptone-yeast extract iron agar	-[b]	L-Methionine	-
Tyrosine agar	-	L-Cysteine	-
Tryptone-yeast extract broth	-		
Hydrolysis of:		Tolerance to NaCl (%)	
Protein	+[c]	1:3	+++
Starch	+	4:6	++
Lipid	-	7:9	+
Catalase production	+	1:3	+++
H_2S production	+	Growth inhibitors	
Nitrate reduction	+	Crystal violet (0.0001%)	+
Tyrosine degradation	-	Crystal violet (0.0002%)	-
Urea test	+	Sodium azide (0.02)	-
Utilisation as C-source:		Phenol (0.1%)	-
D-Glucose	**+++[d]**	Growth temperature °C	
D-Mannose	+++	10	-
D-Mannitol	+++	25:37	+
D (+) trehalose	+++	Growth pH	
L-Rhamnose	+++	5:8	++
D-Fructose	+++	9	+
Meso-Inositol	++[e]	10	Wg
D-Galactose	++	11	-
Sucrose	++	Sensitivity to antibiotic	
Maltose	+++	Rifampicin (50 μg mL^{-1})	+
Starch	+++	Erythromycin (15 μg mL^{-1})	+
Cellulose	-	Penicillin (10 μg mL^{-1}	-
Salicine	-		
L(+)Arabinose	++		
Raffinose	-		

[a]ND= not detected, [b](-) = negative, [c] (+) = moderate , [d](+++) = abundant, [e](++) = good growth, ,[f] (wg)= weak growth

The cell wall of the isolate was confirmed to contain the LL-diaminopimelic acid (LL-DAP) type [18], a typical constituent of *Streptomyces.* Furthermore, the isolate can use most of the sugar tested using ISP-9. However, it cannot use raffinose, salicin and cellulose as a C-source.

Strain NV-38 was growing well on medium containing most of the amino acids used. However, a weak growth was recorded on a medium containing L-Phenyl alanine. No growth at all was noticed on a medium containing L-Serine, L-Methionine, and L-Cysteine as an N-source.

In addition, NV-38 can grow in the presence of crystal violet (0.0001%) and sodium azide (0.01%). Sensitivity to erythromycin was positive, but it was negative toward penicillin. Furthermore, the results showed that a good growth of NV-38 was noticed in a temperature range of 25-37°C, the presence of nearly 9 % NaCl and within a pH range of 5-9.

Comparison with the known strains

The characteristics of this strain were compared to published descriptions of various *Streptomyces* species in the morphological, physiological and biochemical characters [32]. In conclusion, NV-38 belongs to the gray series' group*,* with negative melanin pigment, spiral hyphae and a smooth spore surface. The closest strains to NV-38 are *Str. griseofuscus* and *Str. mutabilis.* These kindred strains have the same aerial and substrate mycelia colors, spore shape, melanin production and physiological characteristics with some differences between them in the utilization of sugars. In particular, NV-38 does not resemble any known strain completely.

Classification and identification of *Streptomycetes* based on morphological and biochemical characterization [33] are not enough in most cases. Therefore, using molecular data, predominantly rRNA gene sequences [34] have been introduced.

Molecular identification of strain NV-38

The 16S rRNA is a powerful tool for phylogenetic analysis and species differentiation of the genus *Streptomyces.* It can be used as a genetic method in parallel to conventional taxonomic methods, including numerical, phenetic and other genetic analyses [35]. Therefore, in this work, phylogenetic analysis based on 16S rRNA gene sequence of the isolate were partially sequenced in parallel to conventional methods.

PCR amplification of the 16S rRNA gene

The 16S rRNA gene of NV-38 was amplified using the *Streptomyces*-specific PCR primers previously mentioned in Materials and methods. The PCR product of the isolate showed a band at 1300 bp (Fig. 3). The specificity of the PCR is affected by factors such as the primers, the properties of the gene regions flanking the target site, the annealing temperature in the PCR reactio and the reaction conditions [34]. The primer pair was used StrepB/StrepF-amplified DNA according to Rintala *et al.* [21].

Phylogenetic analysis

The alignment of the 16S rRNA nucleotide sequence of NV-38 consisted of 385 bp. The 16S rRNA reported gene sequence was matched in the gene bank database through the NCBI BLAST (www.ncbi.nlm.nih.gov). A comparison between the 16S rRNA sequence of this strain with those members in the genomic database bank was performed. This comparison indicated a high level of sequence similarity (99%) with *Str. griseofuscus* NRRL B-1989.

The multiple sequence alignment and phylogenetic tree are represented and illustrated graphically in Fig. 4. The phylogenetic tree was derived from the distance matrices using neighbor-joining method [36]. In conclusion, the phylogenetic analysis coupled with a conventional method of NV-38 indicated that the closest strain is *Streptomyces griseofuscus*. Therefore, *Str. griseofuscus* NV-38 was proposed as its name.

Fig 3: PCR product of 16S rDNA gene for the *Strptomyces griseofuscus* NV-38.; where (M) is marker 100 bp ladder.

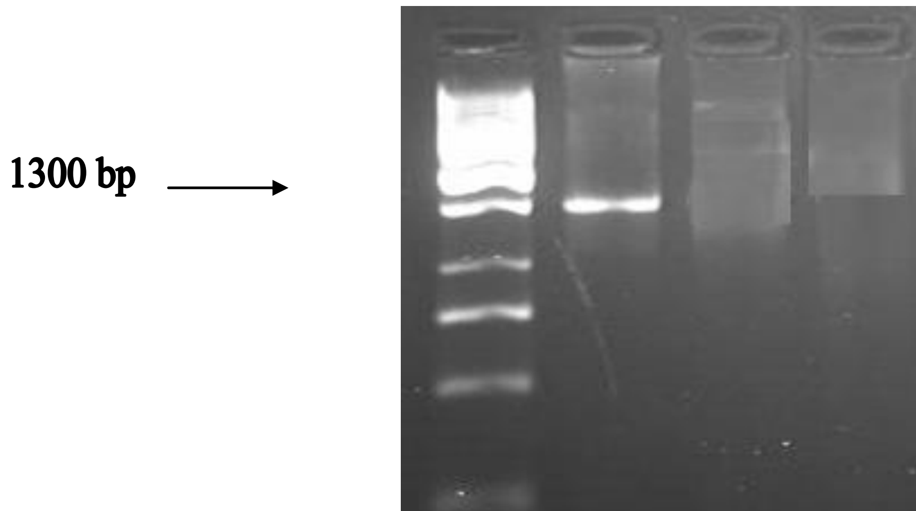
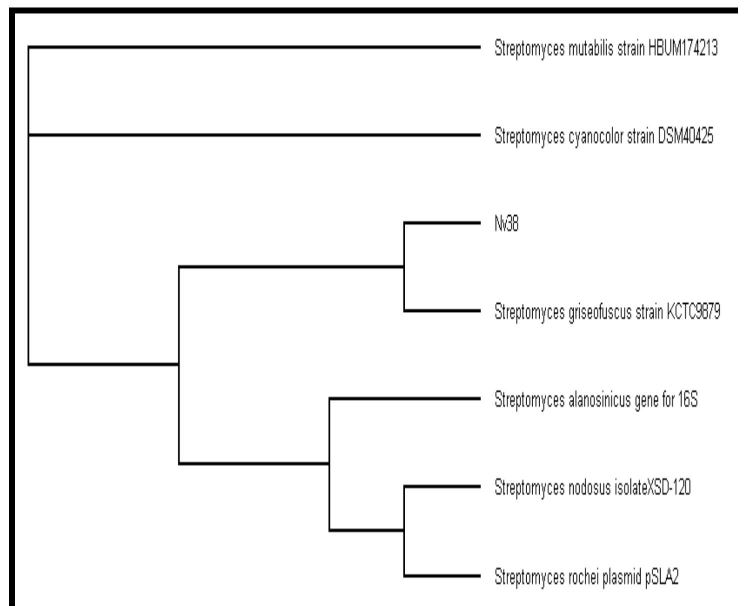


Fig 4: Phylogenetic tree showing the position of *Strptomyces griseofuscus* NV-38, Neighbor-joining method



Nucleotide sequence GenBank ID

The nucleotide sequence of the 16S rRNA gene of *Str. griseofuscus* NV-38 has been deposited in the GenBank ID: HQ839862.

Extraction and purification of active antifungal compound

The results indicated that ethyl acetate is the most suitable organic solvent to extract the antifungal substance, especially when the active filtrate was adjusted to pH 2. Different solvent systems were used to separate the crude antibiotic to separated bands. The best solvent system was methanol: hexane: acetic acid (7: 2: 1 v/v/v). This solvent system separated the crude antibiotic into four separated bands; each band was bioassayed on the *F. moniliforme*. The active band at R_f 0.75 was scratched, collected, and dissolved by methanol, filtrated and evaporated under vacuum and re-purified by developing it in the same solvent system. The pure active substances were also bioassayed on *F. moniliforme.*

Physicochemical characteristics of the antifungal metabolite

Classic color reactions have a great significance in the quantitative estimation, especially those of biological origin. These reactions could be used to detect the presence of certain groups in the molecule of the investigated compounds. The results showed that the purifying antifungal agent has no sugars, amino group, sulfur, di-ketone group, nitro group, chloride, phenolic group, aromatic aldehyde or aromatic dike tones or aromatic amines, while it probably has a free aldehyde or keto sugar group.

The strain *Str. griseofuscus* is well known for producing peptide antibiotics such as TA243, Azinomycin A and B, Cazinophilin and polyene antibiotics such as Bildenden and Bundlins A and B [37].

This is the first study of the antifungal activities of *Streptomycetes* from the New Valley soil habitats. The results indicated that *Streptomyces* NV-38 provides significant inhibitory activity against pathogenic fungi and has potential as a biological control agent. Additionally, further studies will have to be done to evaluate the efficacy of control under fields and laboratory conditions before it becomes an agricultural practice.

ACKNOWLEDGMENT

The authors gratefully thank Prof. Dr. Ahamed El Dewany for providing the molecular biology lab in Chemistry of Natural and Microbial products Department, NRC. We thank the central laboratories in NRC for spectroscopic analysis.

REFERENCES

[1] Ouhdouch Y, Barakate M, Finance C. Eur J Soil Biol 2001; 37:69-74.
[2] Pal KK, Gardener MB. The plant health Instructor 2006; DOI: 10.1094/PhI-2006-1117-02.
[3] Pohanka A. Ph.D thesis 2006 ; 72-73.
[4] Gutleb AC, Morrison E, Murk AJ. a review Environ Toxic and Pharmac 2002; 11:309-320.
[5] Rheeder JP, Marasas WF, Vismer HF. Appl Environ Microbiol 2002; 68:2102-2105.
[6] Silva SC, Fermino SAC, Silva G M. Sci. Agric. (Piracicaba, Braz.) 2008; 65: 50-55.
[7] Berdy J. J antibiot 2005; 58: 1-26.
[8] Bressan W, Biocontrol 2003; 48: 233-240.
[9] Kampfer P, Kroppenstedt RM, Dott W. J Gen Microbiol 1991; 137 : 1831-1891.
[10] Kim WG, Kim JP, Kim CJ, Lee KH, Yoo ID, Benzastatins ABC and D. J antibiot (Tokyo)1996; 49: 26-30.
[11] Nelson PE, Toussoun TA, Marasas WFO. An illustrated Manual for identification 1983; the Pennsylvania State University Press University Park and London.
[12] Samson RA, Hoekstra ES, Frisvad C, Filtenborg O. 6th ed. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands 2000; 64-97.
[13] Oskay M, Tamer AU, Azeri C. Afr J Biotechnol 2004; 3:441-446.
[14] Cooper KE. In “An analytical Microbiology”, Kavanagh FW, (Ed.), Vol. I, II Academic Press, New York and London, 1972.
[15] Locci R. *Streptomycetes* and related genera. In: Williams S.T., Sharpe M.E., Holt J.G., editors. Bergey’s manual of systematic bacteriology. Baltimore: Williams and Wilkins, 1989, pp. 2451-2493.
[16] Elwan SH, El-Nagar MR, Ammar MS. Bulletin of the faculty of science Riyadh University, 1977, 8 : 105-119.
[17] Chapman GS. J Bacteriol 1952; 63: 147.
[18] CowanCowan ST and Steel’s. 2nd Ed. Cambridge, Univ Press 1974.
[19] Jones K. J Bacteriol 1949; 57: 141-145.
[20] Shirlling EB, Gottlieb D. Int J Syst.Bacteriol l966; 16 : 313-340.
[21] Becker B, Lechevalier MP, Gordon RE, Lechevalier HA. Appl Microbiol 1964;1 : 421-423.
[22] Lechevalier MP, Lechevalier HA. Int J Syst.Bacteriol 1970; 20 : 435-443.
[23] Tresner HD, Backus EJ. Appl Microbiol 1963; 11 : 335-338.
[24] Pospiech B, Neumann A. Tren Gene 1995; 11 : 217-218.
[25] Rintala H, Nvalainen A, Ronka E, Suutari M. Mol and Cell Prob 2001; 15:337-347.
[26] Hall TA, BioEdit a user-friendly biological sequence alignment editor and analysis program for windows 95/98. Nucleic acid Simpson 1999 ; 41: 95-98.

ISSN: 0975-8585

[27] Page RDM. Treeview Comp Appl in the Biosc 1996;12 : 357-358.
[28] Kutzner HJ. Springer-Verlag, Berlin, 1986, pp. 2028-2090.
[29] Wang Y, Zhang ZS, Ruan JS, Wang YM, Ali SM. J Ind Microbiol 1999; 23: 178-187.
[30] Lazzarini A, Cavaletti L, Toppo G, Marinelli F. Anton Leeuw 2000; 78:399-405.
[31] Shomurat T, Yoshida J, Amano S, Kojina M, Niida T. J Antibiot 1979; 32: 427-435.
[32] Anderson TM, Bodie EA, Goodman N, Schwartz RD. Appl Environ and Microbiol 1986; 51: 427-428.
[33] Thakur D, Yadav A, Gogoi BK, Bora TC. J de Mycol Médic 2007;17 : 242-249.
[34] Holt JG, Shape NE, Williams ST. Baltimore, London 1989.
[35] Tresner HD, Davies MC, Backus EJ. J Bacteriol 1961; 81: 70-80.
[36] Shirlling EB, Gottlieb D. Int J Sys Bacteriol 1968;18: 69-189.
[37] Von Wintzingerode F, Gb◌el UB, Stackebrandt E. FEMS Microbiol Rev 1997; 21 : 213-229.