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Original Article

Isolation, Screening and Identification of two *Streptomyces* soil isolates exhibiting strong antimicrobial activities against Extended Spectrum Beta Lactamase (ESBL) producers

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ABSTRACT

One of the urgently needed antimicrobials is those antagonizing extended-spectrum beta-lactamase (ESBL) producers as they represent an emerging global health threat. Objective: Screening soil bacterial isolates for anti-ESBL activity and identifying the promising isolate(s). **Methodology and results:** A total number of 173 *Streptomyces* isolates were recovered from various Egyptian soil samples. All recovered isolates were primarily screened for their antagonistic activity against the reference strain, *Klebsiella pneumoniae* ATCC 700603 using agar plug method. Thirty putative *Streptomyces(s)* isolates revealed positive antagonistic activity and were secondarily screened against three clinically pathogenic ESBL producers using agar diffusion method. Of these, two isolates exhibited the highest anti-ESBL activity were selected and identified as *S. fulvissimus* isolate W2 and *S. manipurensis* isolate H21 using 16S ribosomal RNA gene sequencing. Their morphologies, antibiotic susceptibilities and antimicrobial spectra were studied. **Conclusion:** The two selected isolates *S. fulvissimus* isolate W2 and *S. manipurensis* isolate H21 can be used for novel drug discovery as they have potential *in vitro* antagonistic activity against ESBL producers and broad spectrum antimicrobial activities.

Keywords: ESBL; Antimicrobial activities; *Streptomyces manipurensis*; *Streptomyces fulvissimus*

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INTRODUCTION

The development of novel antibiotics could not overcome the emergence of antibiotic resistant bacterial strains [1]. Lately, the development of novel antimicrobial agents has sharply declined [2]. This is because the pharmaceutical companies almost lost interest in the field of novel broad spectrum antimicrobial therapies investigation [3, 4]. Moreover, the guidelines for novel antibiotics restrict their use till evidence of being safe [5]. The bacterial resistance all over the world draws the researchers to explore new drugs to combat with [6]. This problem is much more complicated when infections are caused by extended spectrum beta lactamase producers those confer resistance to β-lactam antibiotics [7].

Extended-spectrum beta lactamases (ESBLs) are enzymes that confer resistance to oxyimino-β-lactams (e.g., cefotaxime, ceftazidime, and ceftriaxone) and the monobactam aztreonam. These enzymes have been found in different members of the family Enterobacteriaceae and most common in *Klebsiella pneumoniae* and *Escherichia coli*. Unfortunately pathogenic ESBL producers are highly associated with nosocomial outbreaks [8].

Streptomyces are industrially important microorganisms due to their capability to produce bioactive secondary metabolites such as antivirals, antifungals, antitumor, anti-hypertensives and mainly antibiotics. Antibiotic production is species specific and almost 80% of the naturally derived antibiotics are obtained from the genus *Streptomyces, Actinomycetes.* [9,10]. It was estimated about 100,000 antibiotics are produced by the genus *Streptomyces* and they remain a rich source of novel antibiotics remain to be identified especially when increase antibiotic resistance amongst pathogens [11]. About 45-55% of 10.000 known antibiotics were produced by *Streptomyces* [12, 13]. *Streptomyces* are Gram-positive bacteria with genetic material (DNA) is GC-rich (70%) in comparison with other bacteria such as *Escherichia coli (50*%). They can grow in different environments, with a filamentous form like fungi [9, 10]. Therefore, aim of this work was to isolate, screen various soil *Streptomyces* isolates that show potential inhibitory activity against different ESBL producers followed by identification and characterization of *Streptomyces* isolate(s) that showed maxiumum antimicrobial activities against ESBL producers.

MATERIALS AND METHODS

Microorganisms

***Klebsiella pneumoniae* ATCC 700603,** a reference strain which produces the Novel Enzyme SHV-18 was used in primary screening for antimicrobial activity.

Pathogenic ESBL producing clinical isolates

Three pathogenic ESBL producing isolates were previously genetically identified and isolated from clinical specimens [14]. They were used in secondary screening for antimicrobial activity Table 1.

Recovery of *Streptomyces* isolates from soil samples: Soil samples were collected from various locations in Egypt. Several diverse habitats in different areas were selected for the recovery of *Streptomyces* isolates. These habitats included: Agricultural fields (36 soil samples); Marine soil from North coast (2 soil samples); Garden field (4 soil samples) and other supplied enriched soil samples from Department of Microbiology and Immunology, Ain Shams University (32 soil samples). Previously purified isolates were kindly provided by Dr. Ahmed Saiid, lecturer of Microbiology in Department of Microbiology and Immunology, Ain Shams University (47 soil isolates). The samples were taken along a depth of 5-25 cm after removing approximately 3 cm of the soil surface. The samples were placed in polyethylene bags, closed tightly and stored in refrigerator [15]. Then they were dried at 50˚C for 10 min. Enrichment of soil samples was done as described by [16]. The air-dried soil (10 g) was mixed in a mortar with 1 g of calcium carbonate; the mixture was incubated for 10 days at 28˚C in two closed concentric inverted sterile Petri dishes, the inner one contained the enriched soil sample while the outer one contained a large moistened filter paper disc to maintain high relative humidity. After enrichment, an appropriate dilution of 1%w/v soil suspension was plated onto the surface of starch nitrate agar for 5-7 days at 28˚C. Visual observation of both morphological and microscopic characteristics using light microscopy, and simple stain properties were performed colonies having the morphological culture characteristics of *Streptomyces* (leathery or fibrous colonies appear as granular powdery with hard texture) were purified by streak plate technique on the starch nitrate agar medium. The plates were incubated at 28˚C for 7 days. Individual colonies from the purified cultures were subcultured onto starch nitrate agar slants. The slants were incubated at 28˚C for 7 days then stored at 4˚C and subcultured every month. After antimicrobial activity screening, the recovered *Streptomyces* isolates were maintained as suspensions of spores and mycelial fragments in 10% glycerol (v/v) at -20˚C.

Screening the recovered bacterial isolates for their anti-ESBL activity: Recovered *Streptomyces* isolates were grown on surface of soybean agar for

5-7 days. Reference strain, *Klebsiella pneumonia* ATCC 700603 (0.5 McFarland) was spread onto surfaces of Mueller Hinton agar (MHA) plates supplied with 25µg/ml penicillin G. Agar plugs (10mm) from *Streptomyces* inoculated plates were removed, placed surface down onto the surfaces of the previously surface inoculated MHA plates. They were incubated at 37˚C for 18-24 h and zones of inhibition were recorded. The experiment was repeated three times and mean was calculated.

Submerged culture: After preliminary screening using agar plug method, further studies in shake flask condition were carried out. This was done for the purpose of antibiotic production in liquid medium. One pure colony of *Streptomyces* prepared by isolation on starch nitrate agar for 7 days was used for inoculating 10 ml of the seed medium contained in 100 ml soybean broth flask. The flask was incubated at 28˚C and 200 rpm for 72 h. An aliquot (1.25 ml) of seed culture was used to inoculate 25 ml of main culture, of the same composition of seed culture, contained in 250 ml flask. The inoculated flask was then incubated at 28˚C and 200 rpm for 72 h. After three days of incubation, the obtained culture broth was centrifuged at 12000 rpm for 15 min to separate mycelium and supernatant, and the cell free culture supernatant (CFCS) obtained was harvested and used in secondary anti-ESBL screening. This was done against reference ESBL producer strain and three previously genetically identified pathogenic ESBL producers [17].

Secondary screening for the positive isolates: The cell free culture supernatants (CFCS) of positive test isolates (30 isolates) were further tested against reference strain *Klebsiella pneumoniae* ATCC 700603 and pathogenic ESBL producers using standard agar well diffusion method**.**

Agar well diffusion technique was carried out as described by [18, 19] with minor modifications. Mueller-Hinton agar was prepared according to the manufacturer's directions and autoclaved. A sterile swab was dipped in the adjusted isolate suspension (0.5 McFarland). The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum. A dry Mueller-Hinton agar plate was surface inoculated by the swab. Wells of ten millimetre diameter were punched in Mueller Hinton agar plates which were surface inoculated with ESBL producing isolates. The tested supernatant (150 µl aliquots) was loaded into each well. The agar plates were kept at 4˚C 1-2 h to allow the diffusion of the produced antimicrobial metabolite to agar medium. The plates were then incubated at 37˚C and the diameters of the inhibition zones were determined in mm after 18-24 h. Each experiment was repeated three times and the mean values of inhibition zones were calculated.

Criteria for isolate selection included the exhibition of potent antimicrobial activity against both reference strain and pathogenic strains with large inhibition zone. Two isolates (W2 and H21) that fulfilled these criteria were selected for further studies.

Morphological characterization of the promising *Streptomyces* isolates: Spore chain morphology and spore surface ornamentation of *Streptomyces* isolates W2 and H21 were viewed after incubation at 28°C for 14 days on starch nitrate agar medium using a scanning electron microscope (SEM) (Quanta FEG 250®, Japan). The imaging was performed by the staff members of the Scanning Electron Microscopy Unit at the National Research Center, Giza, Egypt. Sputter coating: Aluminum stubs were prepared followed by applying conductive carbon tape to them. The cut bottoms of the wells were mounted onto the surface of *Streptomyces* grown plates. The gold to be sputtered was set as the cathode and the mounted bottoms were located on the anode to be coated. The sputter coater (Edward 5150A, England) was operated under vacuum with argon admitted to the chamber by a fine control valve so that the well bottoms got sputter-coated with gold. Imaging: The gold coated bottoms were placed on the holders inside the microscope specimen chamber and an electron beam was directed towards them under vacuum. The signals resulting from the different points on the sample bombarded by electrons are collected by a detector, amplified and displayed as an image on a computer monitor.

Molecular identification of the selected isolates: The two selected *Streptomyces* isolates (W2 and H21) were identified using 16S ribosomal RNA gene sequence analysis. This was carried out at GATC Company, Germany via Sigma Scientific Co., Egypt. The gene coding for 16S ribosomal; RNA was isolated from each isolate using two universal primers of the following sequence, forward primer sequence: F-5'-AGAGTTTGATCCTGGCTCAG-3'; reverse primer sequence: R-5'-GGTTACCTTGTTACGACTT-3' [20]. The isolated genes were sequenced at the Bioneer Company, Germany by the use of ABI 3730xl DNA sequencer. The nucleotides sequence obtained for each isolate was aligned against the different nucleotides sequences available in data base using NCBI nucleotide BLAST program (http://www.ncbiblast.com/) in order to assess the degree of nucleotide/amino acid identity.

Determination of antibiotic susceptibility patterns of *Streptomyces* W2 and H21: Antibiotic sensitivity and resistance patterns of *Streptomyces sp*. W2 and H21 were assayed by the disc diffusion method [21]. A *Streptomyces* inoculum was prepared by growing cells in Soybean broth for 48 h at 30°C. Petri plates were prepared with 25 ml of sterile Mueller-Hinton agar medium. The test culture was swabbed on the top of the solidified media and allowed to dry for 10 min. Different antibiotic loaded discs (Table 6) were placed on the surface of the medium and left for 30 min at room temperature for diffusion of the antibiotics. The plates were incubated for 48 h at 30˚C. After incubation, the organisms were classified as sensitive or resistant to an antibiotic according to the diameter of inhibition zone given in a standard antibiotic disc chart.

Determination of antimicrobial spectra: The antagonistic activities of supernatants of *Streptomyces* isolates W2 and H21 against different strains were determined. It was carried out as described previously using agar well diffusion method [18, 19]. This was done against standard reference strains listed in Table **2** and clinically isolated uropathogenic MDR isolates listed in Table 3.

Screening for antifungal activity: Antifungal activities were evaluated in a similar way to the antibacterial technique but using Malt agar medium. Cups were made using sterile cork borer (6 mm). They were filled by 100 µl of supernatants. Plates were incubated at 28˚C for 24 h for *Candida* spp. and 48 h for *C. neoformans* and moulds. Ketoconazole (100 µg/ml) was used as a positive control for fungi. This was done in triplicate and the means of inhibition zones were measured.

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RESULTS

Recovery and microscopical characterization of soil isolates: A total number of 173 bacterial isolates were recovered from different soil samples collected from various locations in Egypt *Table 4***.** The soil sample suspensions were spread onto the surface of starch nitrate agar plate. Bacterial colonies appearing as colourful chalky/dusty appearance, hard, not gummy and does not easily lift from agar were collected, purified and examined microscopically by simple stain. Simple stain by light microscopy (x1000) using oil immersion lens showed delicate long mycelia with or without fragmentation for the different collected isolates. The obtained microscopical features and colonies characters are diagnostic for *Streptomyces* species.

Screening the recovered bacterial isolates for their anti-ESBL activity: Thirty isolates of the 173 recovered ones showed antimicrobial activities against *Klebsiella pneumoniae* ATCC 700603 by agar plug test as shown in Figure 1 . Accordingly, these 30 isolates were further screened for their antimicrobial activities using their CFCS.

Secondary screening for the positive isolates: In this screening test, the antimicrobial activities were studied against different ESBL producers with different genetic characters. The results revealed that 10 *Streptomyce*s isolates showed antibacterial activities against all tested ESBL producers (Figure 2). Two isolated coded (W2 and H21) were selected for further studies as they showed largest inhibition zones against all tested ESBL producers Table **5**.

Morphological characterization of the promising *Streptomyces* isolates: *Streptomyces* and other actinomycetes can produce many spores called arthrospores. Morphology of the spore chains can be utilized to identify them. Spore chain of *Streptomyces* isolate W2 was straight to rectiflexibile: as incomplete spirals (hooks) and straight spore chains are common. Spirals usually have only 1-3 turns. Rectiflexibile morphology: mature spore chain usually have 10-20 spores per chain. Spore surface was smooth. Spore length and width were 860nm and 500nm, respectively (Figure 3). However, *Streptomyces* isolate H21 spore chains were composed of 10 to 30 spores, straight or slightly coiled and spore surfaces were smooth with a regular line pattern on their surface. Spore length and width were 1µm and 500nm, respectively (Figure 4).

Molecular identification of the selected isolates: The two selected *Streptomyces* isolates (W2 and H21) were identified using 16S ribosomal RNA gene sequence analysis. For *Streptomyces* isolate W2 and *Streptomyces* isolate H21, the alignment pattern and scores obtained by BLAST analysis showed that they were identified as *Streptomyces fulvissimus* (isolate W2) and *Streptomyces manipurensis* (isolate H21) (Figure 5&6). Both forward and reverse sequence files of each *Streptomyces* isolate were analyzed and assembled into a final consensus sequence. The Final consensus sequences of *Streptomyces* isolate W2 and *Streptomyces* isolate H21 were annotated and submitted into the NCBI GenBank database under the accession codes; MH036743 and MH036744, respectively.

Determination of antibiotic susceptibility patterns of *Streptomyces* W2 and H21: An antibiotic sensitivity test was done against the most commonly used antibiotics for bacterial infections

using the disc diffusion method. *Streptomyces fulvissimus* isolate *W2* exhibited sensitivity towards amikacin, ciprofloxacin, doxycycline, erythromycin, ertapenem, gentamicin, levofloxacin, linezolid, meropenem and vancomycin. However, *Streptomyces manipurensis* isolate H21 isolate showed sensitivity to amikacin, azithromycin, cephoxitin, ciprofloxacin, coamoxiclav, doxycycline, erythromycin, ertapenem, gentamicin, levofloxacin, linezolid, meropenem and vancomycin (Table 6).

Studying the antimicrobial spectra: The extent of antimicrobial activity of the two *Streptomyces* isolates against the test organisms is different. *Streptomyces fulvissimus* isolate W2 exhibited strong antibacterial activity (IZ ≥30 mm) against 11out of 16 tested Gram positive and Gram negative organisms (70%). It showed moderate activity against *Candida parasilosis* (IZ: 20-30 mm), moderate activity against *Candida krusei* and *Cryptococcus neoformans* (IZ ≤20 mm) and no antifungal activity against the rest organisms. However, *Streptomyces manipurensis* isolate H21 showed strong activity (>30 mm) against *Proteus vulgaris* ATCC 33420 and *Staphylococcus saprophyticus* ATCC 49907 and moderate activity (IZ: 20-30 mm) against other tested organisms. It showed antifungal activity against all tested fungi (Table **7**&8).

DISCUSSION

Soil bacteria represent important sources for biologically and industrially important products**.** Gram-positive bacteria are more commonly observed in organic rich soil habitats [22]. In our study, different soil samples collected from different habitats of Egypt soils yielded plenty of *Streptomyces* with different characters. All of the recovered 173 *Streptomyces* isolates were screened for their antibacterial activities against reference ESBL producer *Klebsiella pneumoniae* ATCC 700603. The Clinical & Laboratory Standards Institute (CLSI) selected *K. pneumoniae K6 (ATCC 700603*) as an ESBL quality control (QC) strain. Molecular characterization of *K. pneumoniae* K6 revealed that it produces the novel β-lactamase SHV-18 [8]. Anti-ESBL activities of recovered *Streptomyces* isolates were tested by agar plug method. This screening method allowed utilization of small amount of medium for both cultivation and bioactive compounds production. Moneywise, agar plug method enables detection of antibacterial activities of large number of *Streptomyces* in short time with less cost [23].

Thirty isolates (17.34%) showed positive antagonistic activities against reference strain, *Klebsiella pneumoniae* ATCC 700603. The positive *Streptomyces* isolates were further screened using agar well diffusion method against reference strain, *Klebsiella pneumoniae* ATCC 700603 and other clinical ESBL pathogens. These isolates showed different activities from that of primary screening when subjected to submerged culture. Some isolates did not show activity in liquid medium. This finding is similar to that described previously [24]. The strong activity expressed by a large zone of inhibition on agar plates indicated that those most active isolates produce water-soluble antimicrobial metabolites [25]. Ten isolates (5.8%) were active against all pathogenic ESBL producers in agar well diffusion and two isolates exhibited potent antimicrobial activity against all tested ESBL producers with large inhibition zone (>25mm). The isolates coded W2 and H21 were selected for completion of this study. Comparison with 16S ribosomal RNA sequences NCBI database, revealed that *Streptomyces* isolate W2, accounts for 99% identity to *Streptomyces fulvissimus* strain DSM 40593. In case of *Streptomyces* isolate H21, the alignment pattern accounts for 98% identity to *Streptomyces manipurensis strain* MBRL 201. They have been deposited in GenBank (NCBI, USA) under the accession number MH036743 and MH036744, respectively.

Antibiogram experiments of the two selected isolates demonstrated that they confer resistance to all beta lactam antibiotics and some macrolides. This may be due to the presence of multiple biosynthetic clusters, involving different classes of antibiotic compounds. Isolate W2 and H21 inhibited almost all tested Gram positive and Gram negative bacteria suggesting different modes of action of the antibacterial agent(s) produced by them. Moreover, Isolate H21 exerted potential antifungal activity against all tested fungi (yeasts and moulds).

The two selected strains showed some similarity in their antagonistic activity. Thus biosynthetic pathways of the active metabolites of these strains seem to have similarity in nature. A typical cluster of secondary metabolism involves genes responsible for multidomains enzymes that synthesize versatile bioactive metabolites. When bioactive metabolites have antimicrobial activity, this activity is related to their corresponding resistance genes [26].

From the present study, it was concluded that Egyptian soil is a rich source of economically important *Streptomyces*. This was shown by the broad activity spectra of *Streptomyces fulvissimus* isolate W2 and *Streptomyces manipurensis* isolate H21, while the first one exhibited the best performance. They can be used as potential

alternatives to control ESBL pathogens. The results presented in this work paves for the production of the promising antibacterial compounds by the two selected isolates.

CONCLUSION

Soil microorganisms are potential sources of novel potential natural products for medical and commercial exploitation [27]. Egyptian soils have different natures; therefore a vast distribution of antibiotic producing *Streptomyces* in such environment is most likely. The great importance of *Streptomyces* among numerous versatile soil microorganisms is partially due to their diverse and large metabolite production rates and their biotransformation capabilities [9]. The two selected isolates revealed promising *in vitro* antagonistic activities against ESBL producers and broad spectrum antimicrobial activities. Further studies should focus on both isolates aiming to utilize them for production of antimicrobial agent(s) against emerged resistant bacteria.

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Table 1 Details of the pathogenic ESBL producing clinical isolates used in the antimicrobial activity test

Isolate code	Isolate	Detected gene (s)		
		ctx-m	***shv***	***tem***
6	*E. coli*	+	-	-
15	*Klebsiella pneumoniae*	+	+	+
16	*Klebsiella pneumoniae*	-	+	+

(+) the gene could be detected, (-) the gene could not be detected

Table 2 List of standard reference strains used for studying *in vitro* antibacterial spectra

Character	Strain source
Gram negative rod	*Klebsiella pneumonia* ATCC BAA-1705
	Shigella.sonnei ATCC 25931
	Salmonella typhimurium ATCC 13284
	Proteus vulgaris ATCC 33420
	Proteus mirabilis ATCC 12453
	E. coli ATCC 25922
	Klebsiella oxytoca ATCC 700324
Gram positive cocci	*Staphylococcus epidermidis* ATCC 1228
	Staphylococcus aureus ATCC 43300
	Staphylococcus aureus ATCC BAA-977
	Staphylococcus saprophyticus ATCC 49907
Yeasts(unicellular fungi)	*Candida glabarta* ATCC MyA-2950
	Candida Krusei ATCC 14243
	Candida albicans ATCC 10231
	Candida tropicalis ATCC 13803
	Candida lusitaniae ATCC 3449
	Candida parasilosis ATCC 22019
	Cryptococcus neoformans RCMB 0049001
Moulds (multicellular fungi)	*Aspergillus niger* RCMB 002005
	Fusarium oxysporum RCMB 001018
	Penicillium italicum RCMB 001004

RCMB: Regional Center for Mycology and Biotechnology
ATCC: American Type Culture Collection

Table 3 Panel of MDR pathogens for studying *in vitro* antibacterial spectra

Character	ID (species)	Source	Notes on antibiotic resistance pattern	Detected genes
Gram negative rods	*E. coli 55*	male urine	Penicillin Cephalosporins Carbapenems Folate inhibitor Fluoroquinolone Aminoglycosides	*ctx-m* *tem* *aac(6')-Ib* *aac(6')-Ib-cr* *qnrS*
	Klebsiella pneumonia 14	female urine	Penicillin Cephalosporins Carbapenems Fluoroquinolone Aminoglycosides	*ctx-m* *shv* *tem* *aac(6')-Ib* *aac(6')-Ib-cr* *qnrS* *qnrB* *oxa-A*
Gram positive cocci	*Staphylococcus aureus S51*	female urine	Penicillin Tetracycline Folate inhibitor Macrolide	ND
	Staphylococcus aureus S10	male urine	Penicillin Cephalosporins Aminoglycosides Macrolides Fluoroquinolone lincosamide	ND

ND: no genes were detected on plasmids

Table 4 Distribution of *Streptomyces* included in the study according to their origin and preliminary screening for their anti-ESBL activities

origin	No. of soil samples	Type of soil	No. of recovered *Streptomyces* isolates	No. of positive isolates
Assuit	9	Agricultural field	12	2
Beni Suef	1	Agricultural field	4	0
Cairo	2	Agricultural field	8	2
Menofia	24	Agricultural field	58	9
North coast	6	4 Garden field 2 marine fields	9 3	1 2
Previously enriched soil samples	32	Agricultural field	32	3
Previously purified samples	-	Agricultural field	47	11
		Total	**Σ= 173 isolates**	**Σ= 30 isolates**

Table 5 Antimicrobial activities of CFCS using agar well diffusion method

Isolate code	Mean Inhibition zone diameter (mm)			
	***Klebsiella pneumoniae* ATCC 700603**	**ESBL isolate 6**	**ESBL isolate 15**	**ESBL isolate 16**
YGM3	14	16	15	15
YGM4	23	0	0	0
YGM5	22	0	0	0
YOM1	14	0	0	0
YTS2	17	12	13	12
YTS2"	15	0	0	0
W2	**30**	**22**	**24**	**22**
YDM	15	0	0	0
YN2-5	16	13	13	14
H21	**26**	**18**	**17**	**19**
YH-2-3	18	0	0	14
YLM	20	0	0	13
YLS2	18	15	13	15
Y1	17	17	17	16
YN3	15	0	0	14
YN3"	20	0	0	0
44-3	16	0	0	0
32	17	0	0	0
123-2	17	0	0	0
S126	11	0	0	0
S148	19	15	14	16
S10	13	0	0	0
S28-7	12	0	0	0
S109-1	13	0	0	0
S12	15	16	15	15
S70	15	0	0	0
S13	17	0	0	0
S151	14	0	0	0
S15	18	13	13	13
S83	13	0	0	0

Table 6 Antibiotic susceptibility test for *Streptomyces fulvissimus* W2 and *Streptomyces manipurensis* H21

Standard antibiotic	**Source**	**Resistance pattern**	
		Streptomyces fulvissimus W2	*Streptomyces manipurensis* H21
Amikacin (AK) 30 µg/disc	Bioanalyse®, Turkey	S	S
Amoxicillin (AX) 25µg/disc	Oxoid®, UK	R	R
Ampicillin+Sulbactam (SAM) 10+10 µg/disc		R	R
Azithromycin (AZM) 15µg/disc		R	S
Aztreonam (ATM) 30µg/disc, 10 µg/disc		R	R
Cefepime (FEP) 30µg/disc		R	R
Cefotaxime (CTX) 30µg/disc		R	R
Ceftazidime (CAZ) 30µg/disc		R	R
Ceftraixone (CRO) 30µg/disc		R	R
Cephoxitin (FOX)		R	S

30 µg /disc			
Ciprofloxacin (CIP) 5µg/disc		S	S
Clindamycin (DA) 2 µg/disc		R	R
Co-amoxiclav (AMC) 30µg/disc		R	R
Co-triamoxazole (SXT) 25µg/disc		R	R
Doxycycline (DO) 30µg/disc		S	S
Ertapenem(ETP) 10µg/disc	Bioanalyse®, Turkey	S	ND
Erythromycin (E) 15µg/disc	Oxoid®, UK	S	S
Gentamicin (CN) 10 µg/disc		S	S
Imipenem (IPM) 10µg/disc		R	R
Levofloxacin (LEV) 5µg/disc		S	S
Linezolid (LZD) 30 µg/disc	Bioanalyse®, Turkey	S	S
Meropenem (MEM) 10 µg/disc		S	ND
Vancomycin (VA) 30 µg/disc	Oxoid®, UK	S	S

S: sensitive, R: resistant, ND: not done

Table 7 Antibacterial and antifungal activities of *Streptomyces fulvissimus* isolate W2 and *Streptomyces manipurensis* isolate H21

Characteristic	**ID (species) source**	**Mean IZ diameter(mm)**	
		Streptomyces fulvissimus *isolate W2*	Streptomyces manipurensis *isolate H21*
Gram negative rod	*Klebsiella pneumoniae* ATCC 700603	30±0.4	27±0.3
	Klebsiella pneumoniae ATCC BAA-1705	29±0.1	20±0.4
	Shigella sonnei ATCC 25931	30±0	23±0.5
	Salmonella typhimurium ATCC 13284	38±0	27±0.5
	Proteus. vulgaris ATCC 33420	40±0	No growth
	Proteus. mirabilis ATCC 12453	NA	28±0.5
	E. coli ATCC 25922	26±0	19±0.5
	Klebsiella oxytoca ATCC 700324	31±0	21±0.5
	E coli E55 male urine	32±0.5	24±0
	Klebsiella pneumoniae K14 female urine	31±0.5	24±0
Gram positive cocci	*Staphylococcus epidermidis* ATCC 1228	31±0.5	32±0
	Staphylococcus aureus ATCC 43300	29±0	24±0.8

	Staphylococcus aureus ATCC BAA-977	30±0.5	23±0.5
	Staphylococus saprophyticus ATCC 49907	30±0.45	No growth
	Staphylococcus aureus S51 female urine	29±0.5	23±0
	Staphylococcus aureus S10 male urine	30±0.5	25±0
Yeasts	*Candida glabarta* ATCC MyA-2950	NA	23±0.5
	Candida Krusei ATCC 14243	17±0	17±0
	Candida albicans ATCC 10231	NA	22±0
	Candida tropicalis ATCC 13803	NA	24±0.5
	Candida lusitaniae ATCC 3449	NA	32±0
	Candida parasilosis ATCC 22019	27±0	27±0.5

Cup diameter= 10mm. Volume/cup= 150µl. NA: no activity.

Table 8 Antifungal activities of *Streptomyces fulvissimus* isolate W2 and *Streptomyces manipurensis* isolate H21

Pathogenic microorgainsm	**Mean IZ diameter (mm)**		
	Streptomyces fulvissimus isolate W2	*Streptomyces manipurensis* isolate H21	Ketoconazole(control) 100 µg/ml
Aspergillus niger **RCMB 002005**	NA	13	15
Cryptococcus neoformans **RCMB 0049001**	22	15	25
Fusarium oxysporum **RCMB 001018**	NA	14	18
Penicillium italicum **RCMB 001004**	NA	16	19

Cup diameter= 6mm. Volume/cup= 100µl. NA: no activity

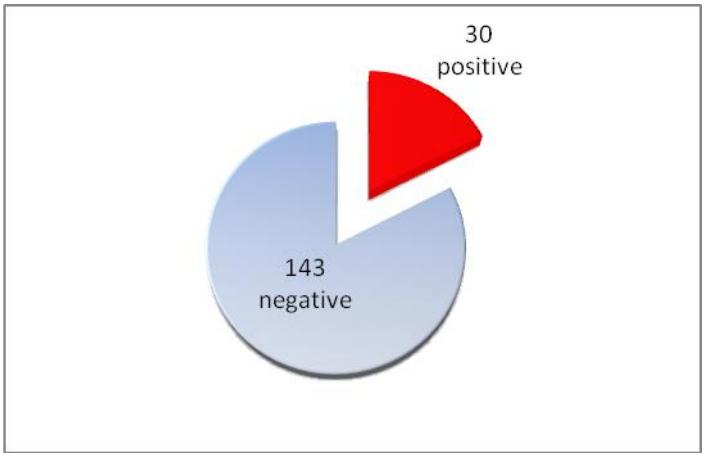

Figure 1 Thirty isolates (17.34%) showed positive antagonistic activities against reference strain, *Klebsiella pneumoniae* ATCC 700603 via agar plug test.

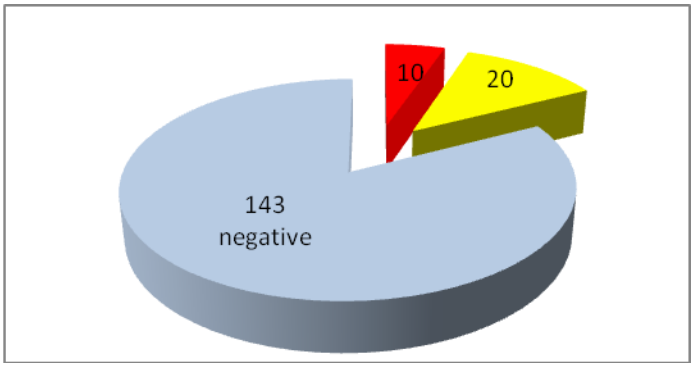

Figure 2 Ten isolates (5.8%) were active against all tested pathogenic ESBL producers in agar well diffusion.

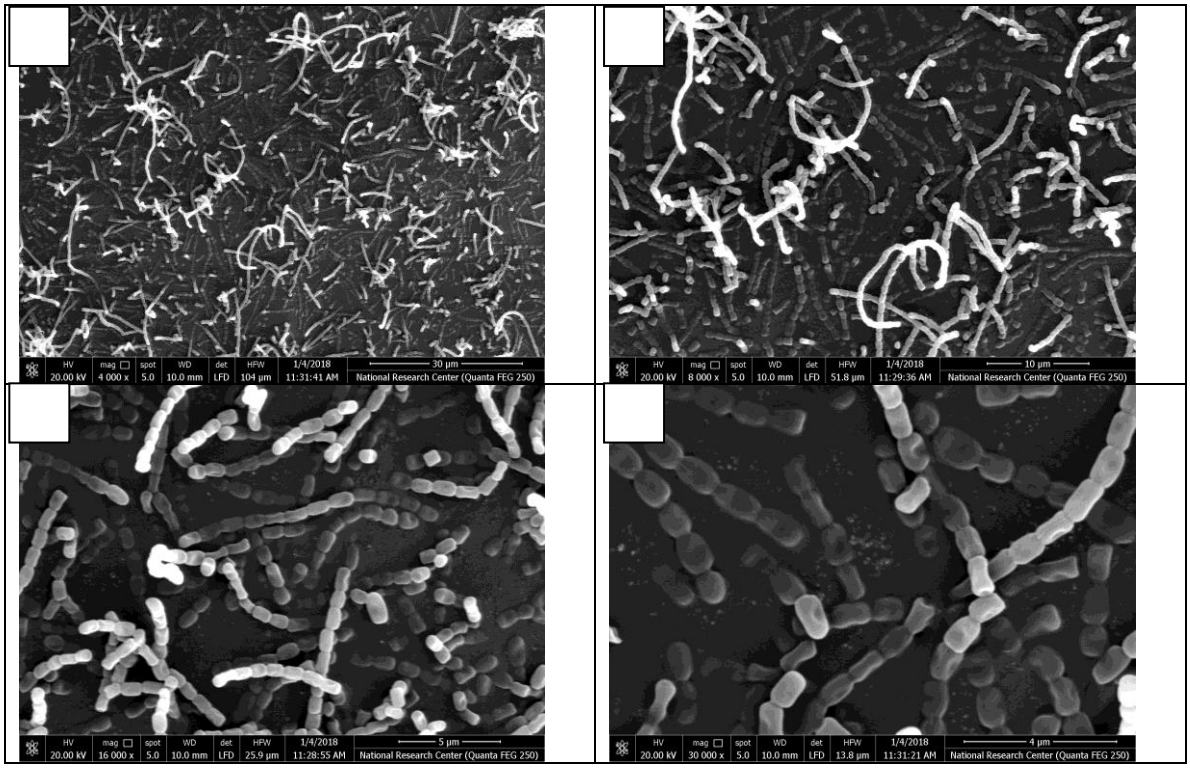

Figure 3 Scanning electron micrograph of the *Streptomyces* isolate W2 illustrating straight to rectiflexible spore chain and smooth surfaced spores growing on starch nitrate agar medium A. 4000X, B. 8000X, C. 16000X, D. 30000X.

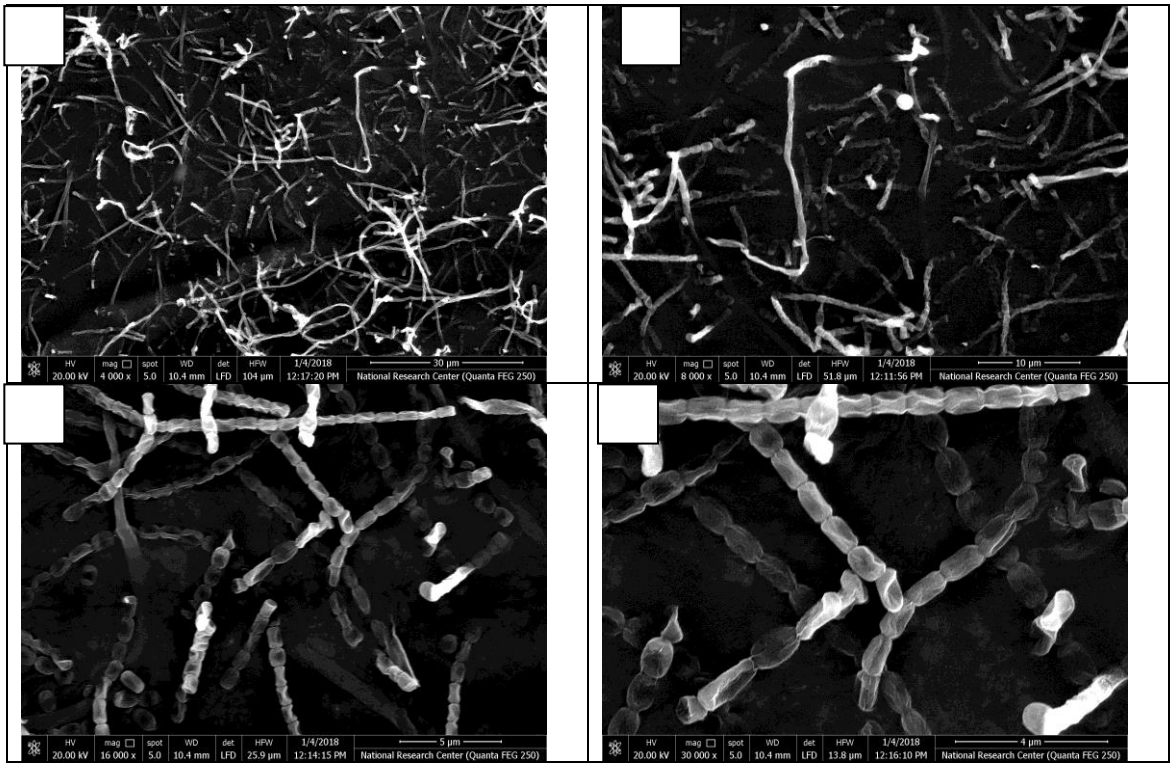

Figure 4 Scanning electron micrograph of the *Streptomyces* isolate H21 illustrating straight to slightly coiled spore chain and smooth surfaced spores growing on starch nitrate agar medium A. 4000X, B. 8000X, C. 16000X, D. 32000X.

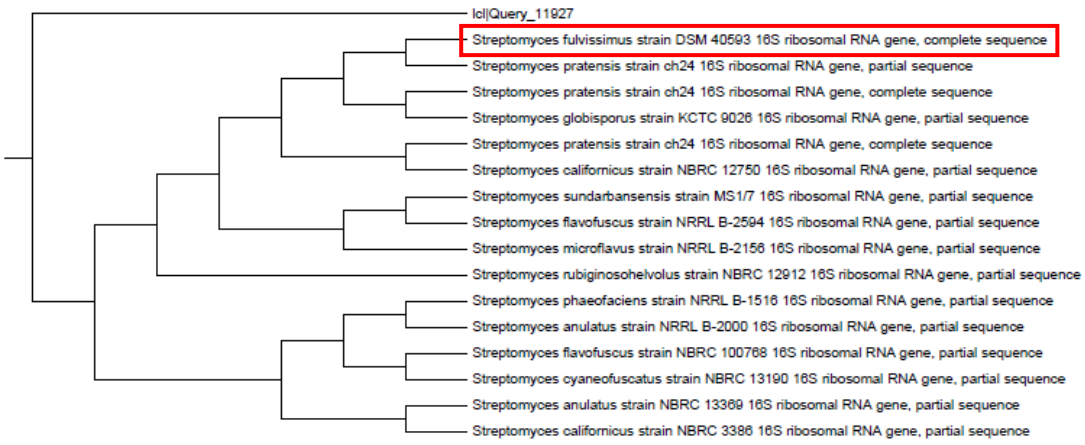

Figure 5 Phylogenetic position of *Streptomyces* isolate code W2 showing 16S rDNA tree of the phylogenetic similarity compared with the sequences of other known *Streptomyces* species.

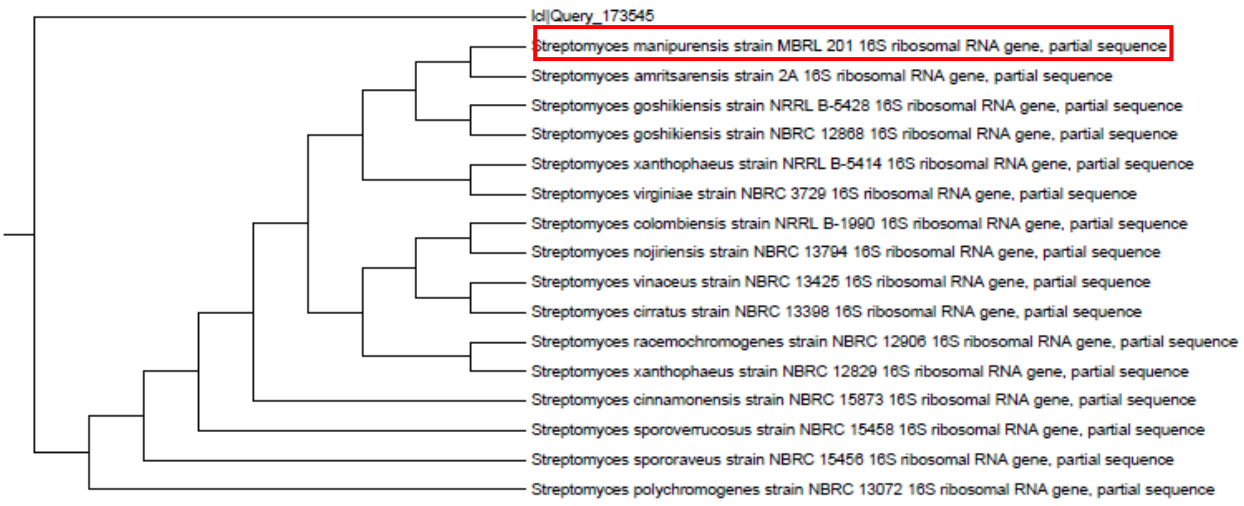

Figure 6 Phylogenetic position of *Streptomyces* isolate code H21 showing 16S rDNA tree of the phylogenetic similarity compared with the sequences of other known *Streptomyces* species.

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