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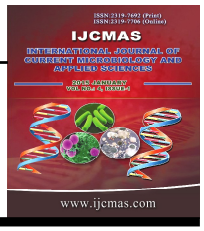

Original Research Article

Testing of Production of Inhibitory Bioactive Compounds by Soil Streptomycetes as Preliminary Screening Programs in UAE for Anti-Cancer and Anti-Bacterial Drugs

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Keywords

Anti-bacterial, Anti-tumor, Antibiotic, Soil, Streptomyces, UAE

A B S T R A C T

Streptomyces is a rich source for a wide variety of useful secondary metabolites and mainly antibiotics. In the present work, a preliminary screening program for isolation of inhibitory bioactive compounds-producing streptomyctes from UAE soils is initiated to search for novel anti-bacterial and anti-tumor antibiotics. Several bacteria and yeasts were tested for their susceptibility to the inhibitory bioactive compounds produced by the 35 soil *Streptomyces* strains that were isolated from UAE soils. Morphological characterization revealed that more than 70% and 60% of the recovered *Streptomyces* strains possessed gray aerial mycelium and distinctive reverse side pigment, respectively. However, more than 75% of the strains were unable to produce soluble pigments. Scanning electron microscopy of the spore surface of the nine most potent strains revealed the majority of them have spiny surface morphology. Using agar diffusion method, data indicated that the majority of the isolates (80%) were able to inhibit the tested bacteria and yeasts with inhibition zone diameter ranged between 11–40 mm and 12–36 mm, respectively. The unusual antibiotic profile of strains No. 6 and 51E stressed their potential to be novel as indicated by the widest inhibition zone diameter. PCR amplification for three morphologically uncharacterized actinomycetes isolates (43, 73, and 74) using specific primers targeting actinomycetes-specific sequences as well *Streptomyces*-specific sequence indicated that they are actinomycetes with ~350 bp single band, and they have the actinomycetes 16S ribosomal DNA-specific sequence. Also, PCR results confirmed that two isolates (73 and 74) to be *Streptomyces* strains with an approximate size of 1000 bp. It can be concluded that various strains of *Streptomyces* produce bioactive compounds which are effective against eukaryotic cells and may be used as a source of anti-cancer agents.

Introduction

Actinomycetes are widely distributed in soils, especially in dry, slightly acidic and rich in organic matter, and represent a high proportion of the soil microbial biomass. Among the genera of actinomycetes, in nature *Streptomyces* is represented by the

largest number of species and varieties, which differ greatly in their morphology, physiology and biochemical activities (Osman *et al.,* 2011). They are important micro-organisms that produce various useful enzymes and secondary metabolites such as immunomodulators, antitumour compounds and antibiotics (Bouizgaene *et al.,* 2009). Having especial environmental characteristics, and being rich in actinomycete population, the microbiology of the United Arab Emirates (UAE) soils has to be explored for new active strains of actinomycetes.

Interestingly, the majority of the antibiotic-producing actinomycetes are found among members of genus *Streptomyces* which lead to a growing economic importance of this group of organisms (Osman *et al.,* 2011). The number and types of actinomycetes present in a particular soil would be greatly influenced by geographical location implicated as soil temperature, type, pH, organic matter content, cultivation, aeration and moisture content. Actinomycetes populations are relatively lower than other soil microbes and contain a predominance of *Streptomyces* (Arifuzzaman *et al.,* 2010).

With the development of antibiotic resistant strains of bacteria, there is a trend to expand programs for novel inhibitory bioactive compounds produced by actinomycestes from unique environments with the possibility of finding novel drugs produced by new species. The detection and identification of possible strains from the genus *Streptomyces* is a valuable approach, as they are known to be the largest antibiotic-producing organisms and has been studied extensively for a very long period of time. Therefore, the main purpose for conducting this research is to screen for inhibitory bioactive compounds-producing soil streptomycetes isolated from soils of United Arab Emirates, and to describe the cultural characteristics of the recovered isolates and their efficacy as a mean of controlling eukaryotic and prokaryotic microorganisms. Here, we explore whether inhibition occurs by action of bioactive compounds produced by soil streptomycetes that can possibly affect cancerous eukaryotic cells of higher organisms.

Materials and Methods

Location, sampling and sample processing:

A soil sample was collected from University of Sharjah Campus/Sharjah-UAE. The soil sample was collected by scraping off an approximately 3 cm of soil surface and taking approximately 250 g of soil sample at 10 cm below the surface. The sample was collected in a sterile container, placed in a sterile bag, transferred to the laboratory and stored at 4 °C until further use. Another two soil samples were selected from other habitats for this study to indicate the value of conducting further screening programs and identify the recovered such organisms as members of streptomycetes by molecular approach. These samples were from Al Kharaen/Sharjah and Bida'a Zayed/Abu Dhabi, which were treated similarly as indicated before.

Isolation technique:

Ten grams of pre-treated soil sample was suspended in 90 mL distilled water, incubated in orbital floor shaker incubator (MaXQ 5000, Thermo Scientific-Canada) at 28±1 °C with shaking at 140 rpm for 30 min. Mixtures were allowed to settle then serial ten-fold dilutions were prepared. 0.1 ml was taken from each dilution and spread evenly over the surface of starch casein nitrate agar (SCNA) (El-Nakeeb and

Lechevalier, 1963; Kuster and Williams, 1964) plates (in triplicate) with sterile L-shaped glass rod and incubated at 28° C ± 1 for 7 days. Plated dilutions that gave 20–200 colonies at 28°C were chosen for further isolations. Typical streptomycetes colonies recovered on SCNA plates were selected on morphological basis (Shirling and Gottlieb, 1966). Colonies were picked out, and then repeatedly streaked on SCNA plates to purify bacterial colonies that showed streptomycetal-like appearance.

Characterization of *Streptomyces*

Streptomyces colonies were characterized morphologically and physiologically according to the international *Streptomyces* project (ISP) (Shirling and Gottlieb, 1966). General morphology was determined by examination of the surface of crosshatched cultures grown on oatmeal agar plates, and incubated in the dark at 27°C for 21 days. Color of aerial spore mass of colonies was determined according to the scale adopted by Prauser (Prauser, 1964) and isolates were grouped into separate color series according to the system proposed by Nonomura (1974). Melanin reactions were detected by growing the isolates on Peptone Yeast Extract Iron Agar medium (ISP media No.6) (Shirling and Gottlieb, 1966).

Antimicrobial activity by Agar disc diffusion method

This was tested by the Bauer–Kirby method (Bauer *et al.,* 1966) against five pathogenic bacteria (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebseilla sp., Saccharomyces sp., and Candida sp.*). Each tested microbe was grown in 250 ml Erlenmeyer flask containing 50 ml of nutrient broth (Himedia/India) (pH 7.2) with overnight shaking at 100 rpm and 37°C. Turbidity of organisms in the broth was adjusted to be equal to or greater than 0.5 McFarland turbidity standards (1.5 x 10^8 cfu/ml). The test organisms were homogeneously inoculated by a sterile cotton swab on the surface of two freshly prepared Mueller-Hinton agar (Himedia, India) then 3 agar discs (5 mm in diameter) were cut out from each streptomycete culture that has been grown on oatmeal agar (ISP-3) (Shirling and Gottlieb, 1966); and then transferred by a flame-sterilized needle to be placed onto the surface of Mueller-Hinton agar plates. Plates that were seeded only by the tested pathogens were considered as negative controls. Plates were then incubated at 28°C± 1. The streptomycetes isolates that showed an inhibition zone with a diameter more than or equal to 5 mm were considered as active producers. Inhibition zones were visually detected after 24 h.

Electron microscopy examination

The spores' surface of nine of the active streptomycetes isolates was examined under scanning electron microscope at magnifications of 5000X–25000X. The stub was placed over a 21-day old culture of each isolate grown on oatmeal agar. The stub was then placed in a sputter coater (Quorum Technology Mini Sputter Coater, SC7620, Target: Gold / Palladium (80% & 20%) for 2 min. Approximately 100 Å of Gold (80%) / Palladium (20%) deposited. The gold sputterer was set at 1 kv, plasma current of 18 mA and chamber pressure of 10-2 mbar. After coating, the specimen was viewed with a Tescan VEGA XM variable pressure SEM at an Accelerating Voltage: Max. 30kV. Secondary electron image was viewed on a computer screen supported with EDS (Oxford Instruments X-Max 50 EDS detector (LN2 free system) and Resolution of 125 eV. The spore surface structures were classified to: smooth (sm), spiny (sp),

warty (wa) and hairy (ha) (Nonomura, 1974).

Molecular characterization of the isolates

a. Extraction of genomic DNA from pure actinomycetes isolates

The genomic DNA used for the PCR was prepared from the single colonies grown on SCNA medium for 3 days. Genomic DNA extraction was conducted using PureLink® Genomic DNA Mini Kit (Invitrogen, USA) according to the manufacturer instructions. The isolated DNA was checked for purity and quantified according to standard procedures (Sambrook *et al.,* 1989).

b. Actinomycetes-specific PCR primers

For molecular identification of the recovered most active metabolite-producing actinomycetes, two sets of primers were used in this study. The first set (AM45F and AM45R) had the following sequences: 5'-GTG AGT CCC CAG ATC ACC CCG AAG-3' and 5'-GTG GGC AAT CTG CCC TTG CAC TCT-3' respectively, and they amplified a *Streptomyces*-specific target sequence of 1000 bp. The second set (Actino-16S-F (AO3) and Actino-16S-R (AO4)) had the following sequences: 5'-AAA TGG AGG AAG GTG GGG AT-3' and 5'-AGG AGG TGA TCC AAC CGC A-3', respectively, and they amplified a target sequence of 350 bp that is common in the 16-S ribosomal DNA of *Actinomycetes*. Primers were synthesized by Invitrogen Life Technologies (USA).

c. *Streptomyces*- specific and Actinomycetes-specific PCR primers

Amplification of 16S rRNA gene fragment of the active antibiotic-producing isolates. The reaction mixture for PCR amplification was prepared in 50 µl volume containing the following: Actino-F; 0.2 µM, Actino-R; 0.2 µM, 1X Taq OCR master mix (Qiagen, USA), 0.25 µg template DNA and Nuclease-Free water (Qiagen, USA) was used to bring the reaction volume to 50 µl.

PCR amplification was carried out in 0.2 ml thin walled, nucleases free PCR tubes (Treff Lab, Switzerland) using iCycler thermocycler (Bio-Rad, USA) programmed as follows: a hot start of 94°C for 3 min, followed by 30 cycles of amplification at 94 °C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and extra extension at 72°C for 5 min. Finally, the tubes were held at 4°C for direct use, or stored at -20°C until needed.

d. Electrophoresis and photography

PCR products were checked for DNA by standard electrophoresis procedure (Sambrook *et al.*, 1989) with 1% (w/v) agarose gels (PROMEGA, USA) and detected by staining with ethidium bromide (EB) (Acros Organic, USA) at 0.5µg/ml final concentration. Electrophoresis was carried out at 110 Volts for 45 minutes. The size of the PCR products was estimated using 1 Kb DNA ladder (Promega, USA). Gels were viewed and photographed using Gel Doc-It-310 (Imaging System, UVP-USA).

Result and Discussion

Isolation of actinomycetes

By employing the enrichment method, a total of 35 different actinomycetes-like colonies were recovered from the soil sample that was collected from UoS Campus/Sharjah-UAE. All of these isolates were selected based on their colony morphology on starch casein nitrate agar plates (SCNA) after 10 days of incubation at

27°C. The colony morphology indicated that they were small (1–10 mm diameter), discrete and leathery, initially with relatively smooth surface but later developed a weft of aerial mycelium that appeared granular, powdery and velvety.

One isolate (43) from Al Kharaen/Sharjah and two isolates (73 and 74) from Bida's Zayed were obtained (Data not shown). These isolates were neither further morphologically characterized nor tested for antibiotic activity, but chosen as a test for molecular identification.

Characterization of Streptomycetes from soil

A total of 35 different Streptomycetes isolates were recovered from UOS campus soil and the majority of them (70%) were with gray aerial mycelium, and (72%) had reverse side pigment (Table 1). Results indicated that more than 60 and 70% of the recovered *Streptomyces* strains possessed distinctive reverse side pigment and gray aerial mycelium, respectively. However, more than 75% of the strains were unable to produce soluble pigments (Table 1).

Antimicrobial activity

Streptomycetes isolates were screened for their ability to produce inhibitory bioactive compounds against four tested bacteria (*Staphylococcus aureus, Bacillus subtilis, E. coli*, and *Klebsiella sp.*) and two yeasts (*Saccharomyces* sp. and *Candida* sp.). Results indicated that most of the isolates showed inhibitory activity against at least one or more tested bacteria and yeasts. Some isolates didn't show any antibiotic activity (Table 2).

When streptomycetes isolates were tested for their activity on different Gram negative and positive bacteria, data indicated that 23 isolates (~ 64%) have an activity against one or more tested bacteria with an inhibition zone diameter ranged between 11 and 40 mm (Table 2). However, 30 isolates (~ 83%) have an activity against one or more tested yeasts with and an inhibition zone diameter ranged between 12 and 36 mm (Table 2). Details about the activity of each single isolate on the tested bacteria and yeasts are shown in (Table 2).

Results indicated that isolates 6, 15, 24, 27, 46, 48E, 49E, 50, and 51E were able to inhibit all tested bacteria with the ability of isolate 6 to exhibit maximal inhibitions against *S. aureus* and *E. coli* with an inhibition zone diameter of 40 and 25 mm, respectively (Figure 1). *S. aureus* was the most sensitive and *B. subtilis* was the most resistant to the compounds produced by the isolates with an inhibition zone diameter of 14 mm (Table 2). The same potent isolates were found to inhibit both tested yeasts with the ability of isolate 51E to exhibit maximal inhibition against *Saccharomyces* sp. and *Candida* sp. with inhibition zone diameter of 36 and 24 mm, respectively (Figure 2). The two yeasts showed considerable similar sensitivity to the inhibitory compounds produced by the *Streptomyces* isolates; however, *Candida* showed overall wider zones of inhibition as compared to *Saccharomyces* (Table 2). Of all the tested isolates, *Candida* showed an average zone of inhibition of 23 mm, compared to an average of 17.6 mm for *Saccharomyces*.

Electron microscopy

The most potent isolates underwent further characterization using SEM. A secondary electron image was generated on a computer screen allowing for the identification of spore surface morphology**.** Scanning electron microscopy to attain a more in-

depth visualization of the spore surface of the most potent strains revealed spiny surface morphology (Table 3) for the majority of the isolates. Spore surface morphology was identified as smooth for isolates 15, 24, 27 and spiny for isolates 6, 46, 48E, 49E, 50E, 51E (Table 3 and Figure 3).

Analysis of the 16S rRNA gene fragment of actinomycetes isolates

Performing the PCR reactions using the first set of primers (AM45-F and AM45-R) showed a single band for isolates 73 and 74 with an approximate size of 1000 bp (Fig. 4), indicating that strains 73 and 74 both have the *Streptomyces*-specific sequence. By using the second set of primers (Actino-16S-F (AO3) and Actino-16S-R (AO4), data showed a single band with an approximate size of 350 bp (Fig. 5), indicating that all 3 isolates have the *Actinomycetes* 16S ribosomal DNA-specific sequence.

Streptomyces isolates were purified from soil samples which underwent enrichment treatment with calcium carbonate under humid conditions. The preliminary treatment of soil samples proved to be efficient in increasing the number of available isolates which otherwise would have been inaccessible (Alferova and Terekhova, 1988). Alferova and Terekhova (1988) advised that this isolation procedure be used in combination with other selective methods for isolation of actinomycetes from soil samples in screening of cultures producing new bioactive compounds.

Detection and identification of microorganisms producing novel antibiotics has been intensively pursued for many years (Blatz, 1998). Several *Streptomyces* screening projects have been conducted in different areas (Saadoun and Al-Momany, 1997, 1998, 2000, Saadoun and Gharaibeh, 2002, 2003, Saadoun *et al.,* 1998, 1999, 2000, 2008) and have founded the distribution and existence of *Streptomyces* for some regions. The screening and isolation of streptomycetes from soil habitats performed in this work show that these microorganisms have a great potential to produce antimicrobial compounds.

Cultural and morphological characterization was examined of 35 *Streptomyces* isolates recovered from UOS-campus soil sample and were distributed into series according to the color of their aerial mycelium. Gray series was found to represent (69%) of the total number of isolates; however, the lowest occurrence was noted for the blue series (3%). Similar results were previously reported by many authors for Moroccan *Streptomyces* isolates (Barakate *et al.,* 2002), Jordanian *Streptomyces* isolates (Saadoun and Gharaibeh, 2003) and Bangladesh *Streptomyces* isolate (Rahman *et al.,* 2011). Furthermore, the cultural and morphological properties of all isolates in the region almost similar to what was obtained in UAE soil. Morphological characterization using scanning electron microscopic had played a major role in distinguishing the isolates spore surface. During the study, it was recorded that of the nine most potent isolates, isolate 6 had smooth spore surface while three had spiny spores surface.

Many anticancer drugs have been developed using compounds of *Streptomyces* origin, but these drugs are generally toxic to normal eukaryotic cells and tissues as well. There is great interest in discovering novel compounds produced by *Streptomyces* which have higher selective toxicity towards cancerous cells. Yeasts such as *Candida* and *Saccharomyces* are good models for eukaryotic cells because they share a

common ancestor. Features such as molecular mechanisms, biological networks and sub-cellular organizations are conserved throughout all eukaryotes.

This research revealed that our *Streptomyces* isolates do have an inhibitory effect on these models and this is indicative of results that can be expected in eukaryotic cells of higher organisms (Castrillo and Oliver, 2011). Although both tested eukaryotic microorganisms are types of yeasts possessing fungal characteristics, yet they have differences which may contribute to their varying levels of susceptibility to *Streptomyces* (Garcia *et al.,* 1997). These isolates could potentially be used in preliminary screening and development of anticancer drugs following the testing of their effects on cancerous cell lines.

The PCR reaction performed using primers specific for a *Streptomyces* region (AM45F and AM45R) revealed a single clear band with an approximate size of 1000 bp for strains 73 and 74 supporting the results obtained by the microbial and cultural examination of those two strains which suggested the possibility that those two strains might be *Streptomyces*. However, the second pair of primers (Actino-16S-F (AO3) and Actino-16S-R (AO4)) showed a single clear band with an approximate size of 350 bp in all 3 isolates, which strongly suggests that all isolates are indeed Actinomycetes.

Overall, the result of the molecular characterization using PCR amplification and PCR-specific primers correlates with what was obtained from the microbial and cultural characterization, in which it was concluded that all 3 isolates (43, 73, and 74) are actinomycetes and isolates 73 and 74 being *Streptomyces*.

Table.1 Morphological and culture characteristics of soil *Streptomyces* isolates and their inhibitory effect against tested bacteria and yeasts

	Color Series				
	Gray	Blue	Variable*	NAM**	Total
Number of isolates	24 (69)***	1(3)	6 (17)	4 (11)	35 (100)
Pigment Production					
Reversed side	20	1	3	0	26 (74)
Soluble pigment	6	1	2	0	8 (23)
Antibiosis					
S. aureus	18	1	3	1	23 (66)
B. subtilis	8	1	1	0	10 (28)
E. coli	13	0	2	0	15 (43)
Klebsiella Sp.	13	0	2	0	15 (43)
Saccharomyces sp.	16	1	5	1	23 (66)
Candida sp.	22	1	3	1	27 (77)

*Variable color: pink, crame or olive
**NAM: No Aerial Mycelium
***Numbers in parentheses represent the percentage of the total

Table.2 Activity of all recovered soil streptomycetes against the tested bacteria and yeasts as assayed by Bauer-Kirby method

Inhibition Zone Diameter (mm)						
	Bacteria				Yeasts	
Strain	*S. aureus*	*B. subtilis*	*E. coli*	*Klebsiella Sp.*	*Saccharomyces*	*Candida*
1	17	-	16	15	15	22
2	-	-	-	-	12	-
3	-	-	-	-	-	-
4	15	-	-	-	-	15
5	-	-	-	-	13	19
6*	40	18	25	23	23	31
7	33	-	-	11	-	30
8	16	-	-	-	-	12
11	20	-	14	12	17	20
15	40	14	21	20	18	30
17	13	-	-	-	-	-
18	-	-	-	-	-	-
19	35	-	11	-	18	28
21	15	-	15	15	13	22
23	-	-	-	-	-	-
24	22	18	17	17	19	24
25	-	-	-	-	14	-
26	-	-	-	-	-	-
27	20	18	16	16	18	22
28	23	-	16	15	20	24
29	20	-	-	-	14	19
30	-	-	-	-	-	13
31	-	-	-	-	-	-
36	-	-	-	-	-	-
39	22	20	-	-	20	21
41	-	-	-	-	-	13
42	-	-	-	-	-	17
43	15	-	15	13	18	22
44	21	-	-	-	15	23
45	11	-	-	-	14	15
46	30	11	11	11	15	24
48E	25	15	13	12	20	30
49E	26	13	14	14	21	32
50E	24	14	13	11	22	30
51E	33	17	17	16	24	36

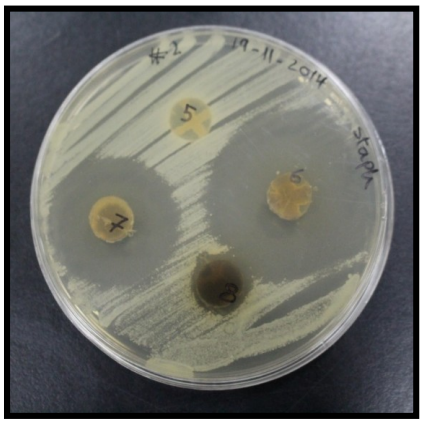
*Antibiotic Activity of the most potent *Streptomyces* isolates as indicated by inhibition of all tested microorganisms with the largest inhibition zones.

Table.3 Characterization of the most potent antibiotic active *Streptomyces* producer isolates

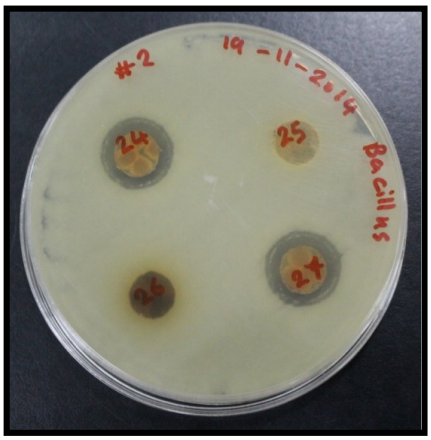
Isolates	Cultural characters						Antibiosis					
	AM	ME	RS	SP	Spore chain	Spore surface	1	2	3	4	5	6
6	Gray	ND	+	-	ND	Spiny	31	23	23	25	18	40
15	Gray	ND	+	+	ND	Smooth	30	18	20	21	14	40
24	Gray	ND	+	-	ND	Smooth	24	19	17	17	18	22
27	Gray	ND	+	+	ND	Smooth	22	18	16	16	18	20
46	Gray	ND	+	-	ND	Spiny	24	15	11	11	11	30
48E	Gray	ND	-	-	ND	Spiny	30	20	12	13	15	25
49E	Gray	ND	+	+	ND	Spiny	32	21	14	14	13	26
50E	Gray	ND	+	+	ND	Spiny	30	22	11	13	14	24
51E	Gray	ND	+	+	ND	Spiny	36	24	16	17	17	33

Cultural characters: AM: aerial mycelium; ME: melanin pigments; RS: reverse side pigments; SP: soluble pigments; 1: *S. aureus*; 2: *B. Subtilis*; 3: *E. coli*; 4: *Klebsiella sp.*; 5: *Saccharomyces sp.*; 6: *Candida sp.*

Fig.1 Agar diffusion test of representative of most potent streptomycetes isolates 6 on *S. aureus* (a), 24 and 27 on *B. subtilis* (b), 24 and 27 on *E. coli* (c), and 48E, 49E, 50E and 51E on *Klebsiella* sp.(d)



(a)



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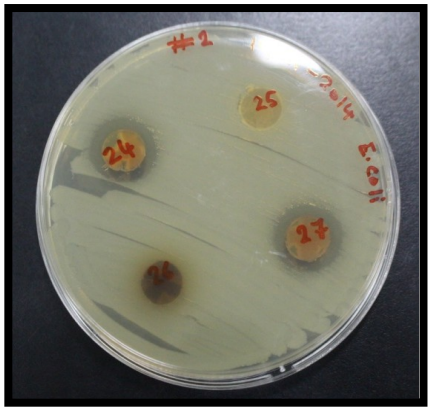
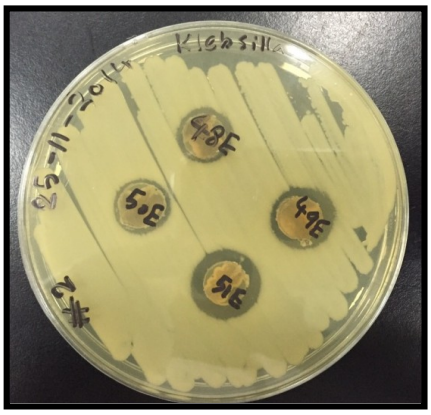


(c)



(d)

Fig.2 Agar diffusion test representing the most potent streptomycetes isolates 6 on *Candida* (a), 24 and 27 on *Candida* (b), 24 and 27 on *Saccharomyces* (c), and 48E, 49E, 50E and 51E on *Saccharomyces*.(d)

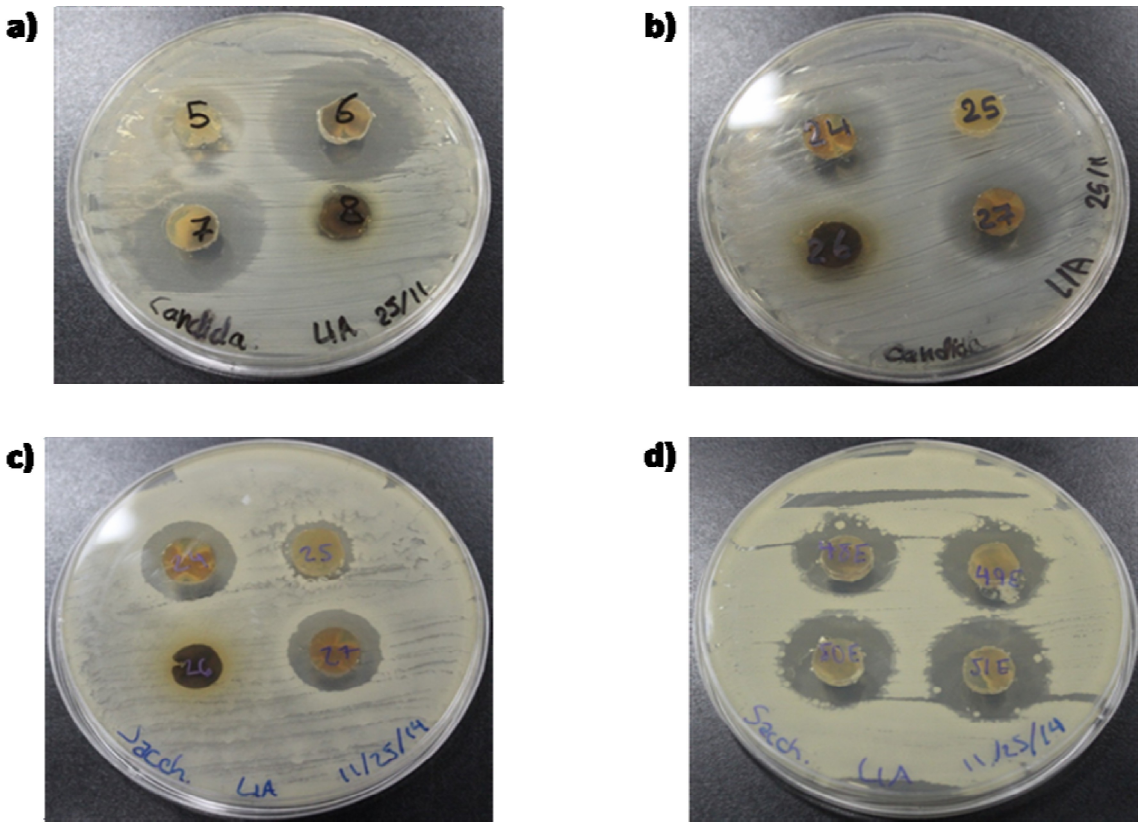
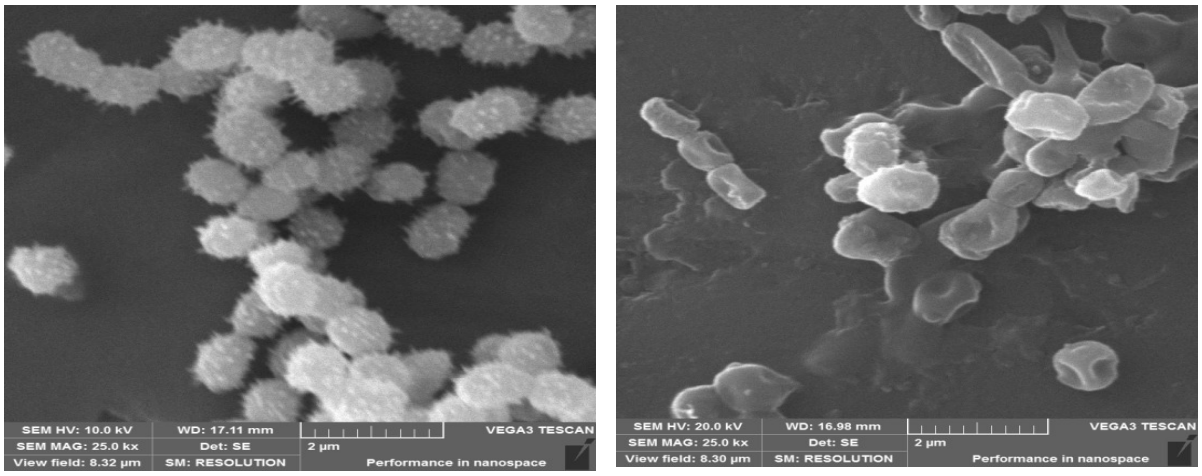
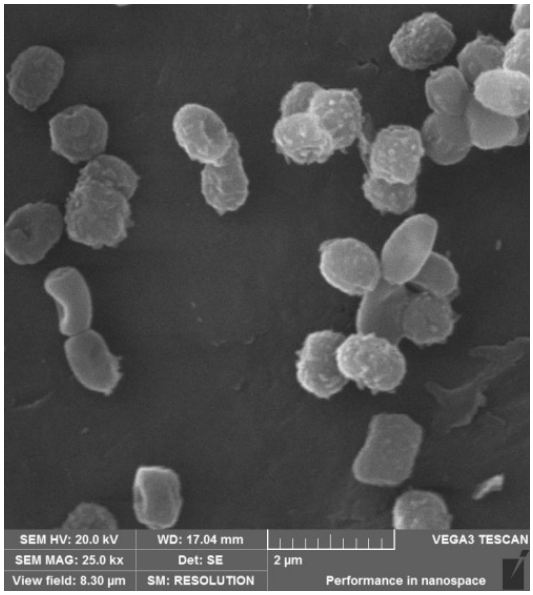


Fig. 3. Spore surface morphology of representative most potent streptomycetes isolates as examined by scanning electron microscope at 25000 X. Isolate 6 (a): spiny, isolate 15 (b): smooth, isolate 51E (c): spiny



(a) (b)



(c): spiny

Fig.4 1% agarose gel electrophoresis of PCR amplification of the 1000 bp *Streptomyces*-specific region of the two antibiotic-producing *Streptomyces* isolates using their DNA as a template and (AM45-F) and (AM45_R) primers. Lane 1: 1 Kb DNA ladder; lanes 2-4: active antibiotic-producing acinomycetes isolates 43, 73, and 74

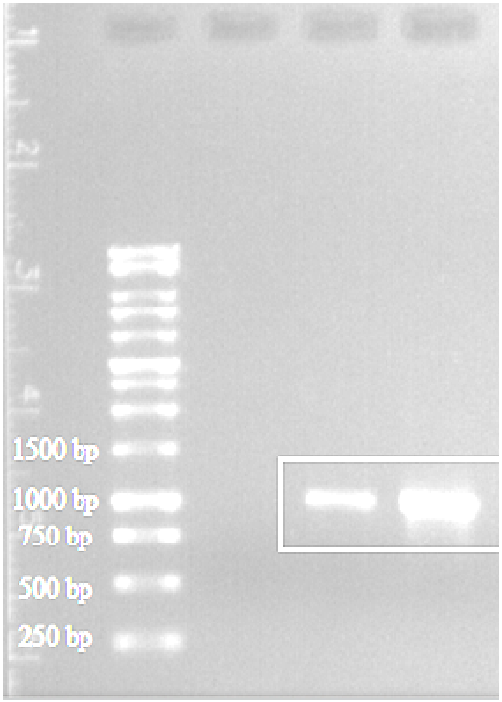


Fig.5 1% agarose gel electrophoresis of PCR amplification of the 350 bp *Streptomyces*-specific region of the three antibiotic-producing actinomycetes using their DNA as a template and (Actino-16S-F (AO3) and Actino-16S-R (AO4) primers. Lane 1: 1 Kb DNA ladder; lanes 2-4: active antibiotic-producing acinomycetes isolates 43, 73, and 74

The two primer sets AM45-F/AM45-R and AO3-F/AO3-R showed specificity in detecting *Streptomyces*, demonstrating the reliable use of these primer pairs. The primer pairs were able to identify the most potent soil actinomycetes isolates more simply, accurately and rapidly, confirming the results of the conventional description and identification of the recovered actinomycetes colonies on the agar medium. Also, they can be applied for differentiation in screening projects, taxonomic characterization and phylogenetic analysis.

The unusual antibiotic profile of the potent strains 6 and 51E underlined their potential as a source of novel antibiotics and might be used as a source of anti-cancer agents as they are effective against eukaryotic organisms. Therefore, further studies are encouraged to observe if these active strains are capable of producing novel compounds other than the common antibiotics that are inhibiting tested bacteria and yeasts and identify these compounds. Furthermore, it is conceivable that through changing their growth conditions, the activity of these isolates may increase several fold. Molecular characterization and optimizing the culture conditions for the production of bioactive compounds by the most potent strains are highly recommended.

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