Antimicrobial Agent Producing Microbes from some Soils' Rhizosphere in Al-Madinah Al-Munawwarah, KSA

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Abstract: In the present investigation, a trial was done to find a new antimicrobial agent producing microbe from soil microbiota of local habitats to control the problem of multiple drug resistance. Isolation of different microorganisms from some soils' rhizosphere in Al-Madinah Al-Munawwarah, viz. corn (*Zea mays*), datepalm (*Phoenix dactylifera*), catnip (*Mentha piperita*), sunflower (Helianthus), balessan (*Amyris gileadensis*), nabk-tree (*Ziziphus Spina-Christi Willd*), basil (*Marrubium vulgare*) was carried out. All microbial isolates were then screened for their antagonistic activity against the most resistant eight target bacteria isolated from caesarean section site infections (*E.coli*, *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Citrobacter* spp., *Acinetobacter* spp., methicillin resistant *Staphylococcus aureus* MRSA, and coagulase negative Staphylococcus). Among the total 86 fungal and bacterial isolates, only 15 of them (17.44%) were capable of biosynthesizing antimicrobial metabolites. One of the actinomycetes that was obtained from catnip rhizosphere, Al-Ouayna district in Al-Madinah Al-Munawwarah**,** found to exhibit the highest antimicrobial activity and it matched with *Streptomyces ramulosus* in the morphological, physiological and biochemical characters. Thus, it was given the suggested name *Streptomyces ramulosus*, A-MM-24. Therefore, microorganisms isolated from Al-Madinah Al-Munawwarah's soil could be an interesting source of antimicrobial bioactive substances. In addition, they are promising enough to deserve further purification, characterization and separation of the active metabolites from them. [Journal of American Science 2010;6(10):915-925]. (ISSN: 1545-1003).

Key words: Antimicrobial agent producing microbe**,** Al-Madinah Al-Munawwarah, resistant bacteria, soil microbiota, *Streptomyces ramulosus* A-MM-24.

1. Introduction:

Antibiotics are one of the pillars of modern medicine (Ball *et al.*, 2004), but the rate of loss of efficacy of old antibiotics is outstripping their replacement with new ones for many species of pathogenic bacteria (Hancock, 2007). The emergence of antibiotic resistant bacteria is a problem of growing significance in dermatological and surgical wound infections (Colsky *et al.*, 1998; Giacometti *et al*., 2000). In general, the most important resistance problems in the management of wounds have been observed with *S. aureus* and coagulase-negative staphylococci among the Gram-positive species and with *E.coli*, *Klebsiella pneumoniae* and *P. aeruginosa* among the Gram-negative species (Filius and Gyssens, 2002).

Considerable research is being done in order to find new chemotherapeutic agents isolated from soil (Rondon *et al.*, 2000; Crowe and Olsson, 2001; Courtis *et al.*, 2003). Soil microbial communities are among the most complex, diverse and important assemblages of organisms in the biosphere; and they participate in various biological activities. Accordingly, they are an important source for the search of novel antimicrobial agents and molecules with biotechnological importance (Hackl *et al*., 2004).

One of the areas in soil where one can find abundance in microbial populations is the rhizosphere. It is a thin layer of soil adhering to a root system which is rich in microbial diversity. The magnitude of this area depends on the plant and the size of the roots that the plant posses (Rondon *et al*., 1999; Rondon *et al*., 2000 and Dakora & Phillips, 2002).

Many groups of microorganisms like Gram-positive, Gram-negative bacteria and fungi have the ability of synthesizing antimicrobial agents and the top cultivable antimicrobial agent producers present in soils are the actinomycetes (Pandey *et al*., 2002).

Accordingly, in the present investigation, we tried to find a new antimicrobial agent producing microbe from soil microbiota of local habitat. In addition, we aimed at selection and identification of the most potent antimicrobial producing one to control some multi-resistant isolates from caesarean section site infections (CSSIs).

2. Materials and Methods

I. Isolation and antimicrobial activity of some microorganisms from different habitats of Al-Madinah Al-Munawwarah

I.1. Soil sampling

Fourteen soil samples were collected from seven soils' rhizosphere, viz. corn (*Zea mays*), datepalm (*Phoenix dactylifera*), catnip (*Mentha piperita*), sunflower (*Helianthus*), balessan (*Amyris gileadensis*), nabk-tree (*Ziziphus Spina-Christi Willd*), basil (*Marrubium vulgare*) in Al-Madinah Al-Munawwarah and its surroundings at Al-Aziziah and Al-Owinah. All samples were collected aseptically in sterile plastic bags.

I.2. Preparation of soil samples

The collected soil samples were sieved to remove various contaminants and divided into two parts. A part was left as it is for isolation of bacteria and fungi. The other part was air-dried, mixed with $CaCO_3$ and incubated for several days at 28 °C (Abu-Elainin, 2004). The air drying and mixing the samples with $CaCO_3$ will reduce the vegetative bacterial cells and allow many actinomycete spores to survive (Tsao *et al.*, 1960).

I.3. Isolation of antimicrobial agent producing microbes

I.3.1. Media used for isolation

Starch nitrate agar medium (Tadashi, 1975). It was used in isolation and maintenance of actinomycetes.

Casein starch agar medium (Kuster and Williams, 1964). It was used in the isolation of actinomycetes.

Yeast extract malt extract medium (ISP-2) (Pridham et al., 1957). It was used in the isolation and characterization of actinomycetes.

Nutrient agar medium. It was used in the isolation and maintenance of other bacteria.

Czapek-Dox agar medium. This medium was used in the isolation and maintenance of fungi.

I.3.2. Isolation methods

It was done using the soil dilution plate technique described by Johnson *et al.* (1959) and consecutive transfers and technical purification steps were carried out.

I.3.3. Screening of the antimicrobial activity of the different soil isolates

Selecting of the most resistant target strains from CSSI

In-vitro antimicrobial activities of the different soil microbes were tested against the most resistant bacterial isolates from caesarean section site infections (CSSIs).

Sixty seven isolates were obtained from cases with CSSIs in a previous study by Abo-Shadi & Al-Mutrafy (2007). 73.1% of the total isolates were Gram-negative bacilli and the rest Gram-positive cocci. According to antibiotic susceptibility testing data of all these isolates, we chose the most resistant isolate from each species. They were found to be eight isolates, six Gram-negative *(E.coli*, I_5; *Klebsiella* spp., $I_{11;}$ *Pseudomonas* spp., I_6; *Proteus* spp., I_1; *Citrobacter* spp., I_1; *Acinetobacter* spp., I_2); and two Gram-positive isolates (methicillin resistant *Staphylococcus aureus* MRSA, I_3; and coagulase negative Staphylococcus CONS, I_2). Tables 1 & 2 show antibiotic susceptibility testing data of the selected target organisms.

Preparation of the target suspension

This procedure was done according to Revira-Revira (2005). The eight target isolates were sub-cultured on slants of nutrient agar for 24 hours. Physiological saline solution (0.85%) was used in order to prepare suspension of each target resistant bacteria with a 0.08-0.1 absorbance. Absorbance of the target suspensions was determined by using a spectrophotometer set at 625 nm. The turbidity of this solution is equivalent to a 0.5 McFarland turbidity standard.

In vitro antibiosis

Agar well method. This method was followed in assay of any soil isolate. Duplicate plates were used for each target organism. The detection of clear inhibition zones around the wells on the inoculated plates is an indication of antimicrobial activities.

Cork borer method. It was followed in assay of any actinomycete isolate.

Streak susceptibility testing was followed according to Madigan *et al*. (2003); Revira-Revira (2005) for the actinomycete isolates.

I.3.4. Selection of the most potent antimicrobial agent producing microorganism (AAPM). All microbial isolates obtained from different soils were again screened for production of antimicrobial agents in order to select the most potent one, and the chosen strain was then subjected to characterization.

II. Characterization of the most potent actinomycete isolates producing antimicrobial agent

It was carried out in the National Research Centre (NRC), Cairo, Egypt. Identification process was performed to the species levels according to specific characteristics.

II.1 Morphological studies.

Morphological characteristics of aerial hyphae, spore mass, spore surface, color of aerial and substrate mycelia and soluble pigments production were conducted by growing the organism on ISP-media. For this purpose the following media were used: Starch nitrate agar, glycerol asparagine agar (Pridham and Lyons, 1961), inorganic salt starch agar (Kuster, 1959), yeast malt extract agar and oatmeal agar medium (Kuster, 1959).

The color of organism under investigation was identified using the ISCC-NBS color-name charts II illustrated with centroid detection of the aerial, substrate mycelia and soluble pigments (Kenneth and Deane, 1955).

II.2. Physiological and biochemical characteristics.

To study growth parameters as the salt tolerance and temperature, spores were inoculated in starch nitrate broth (50 ml) amended with 1, 3, 5, 7 and 9% NaCl and incubated at 30 ˚C for 7 days. Similarly, starch nitrate agar slants inoculated with the actinomycete isolates were allowed to incubate at different growth temperatures till 40 ˚C.

Many characteristics were studied. Lecithinase was detected using egg yolk medium according to the method of Nitsh and Kutzner (1969); lipase (Elwan *et al.,* 1977); protease (Chapman, 1952); pectinase (Hankin *et al.,* 1971); casein medium (Gorden *et al*, 1974); cellulytic activity (Ammar *et al*., 1998); urease activity (Cowan 1974), indole production (Cowan, 1974); α-amylase (Ammar *et al.,* 1998), sensitivity to potassium cyanide (Cowan, 1974) and catalase test (Jones, 1949). Esculin broth and xanthine have been conducted according to Gordon *et al.* (1974), and nitrate reduction was performed according to the method of Gordon (1966). Melanin pigment (Pridham *et al.,* 1957); and hydrogen sulphide production was carried out according to Cowan (1974). The utilization of citrate (Cowan, 1974), different carbon and nitrogen sources was carried out according to Pridham and Gottlieb (1948).

Determination of Diaminopimelic acid (DAP) and sugar pattern was carried out according to Becker et al. (1964), and Lechevalier and Lechevaier (1970).

In addition, the actinomycete isolate under study was tested for its ability to grow in the presence of different antibiotics. The antibiotic sensitivity assay was done by agar diffusion assay. Antibiotic discs were placed on the surface of agar plates pre-inoculated with spores of isolates to be tested and zones of inhibition were measured after incubation at 30 ˚C for 3 days. Clear zones of inhibition around the discs were the indication of antibiotic sensitivity of the isolates. Antibiotic discs of amoxicillin (30 µg), carbenicillin (100 µg), gentamycin (10 µg), cephradine (25 µg), chloramphinicol (30 µg), cloxacillin (1 µg), doxycycline (30 µg), erythromycin (15 µg), keflex (30 µg), and augmentin (30 µg) were used.

3. Results and Discussion:

The increase in the frequency of multi-resistant pathogenic bacteria is created an urgent demand in the pharmaceutical industry for more rational approaches and strategies to the screening of new antibiotics with a broad spectrum of activity, which resist the inactivation processes exploited by microbial enzymes (Saadoun and Gharaibeh, 2003; Motta *et al.,* 2004).

The species belonging to the genus *Streptomyces* constitute 50% of the total population of soil actinomycetes and 75-80% of the commercially and medicinally useful antibiotics have been derived from this genus (Mellouli *et al.,* 2003). Screening and isolation of promising actinomycetes with potential antibiotics is still a thrust area of research and it is suggested that the exploration of materials from different areas and habitats have a vital role to play in the search for new microbes and novel metabolites and is urgent to counter the threats posed by the fast emerging phenomenon of antibiotic resistance (Saadoun and Gharaibeh, 2003). However, it appears that these niches, which should be first screened for bacteria that generate new antibiotics, are not exotic places, but rather, established collections of *Streptomyces* species (El-Naggar *et al.,* 2006). Thus, in the present work, different microorganisms were isolated from Saudi soils and then screened with regard to their potential to generate antibiotics.

For the purpose of obtaining antimicrobial agents against resistant target bacteria from CSSI, isolation of different microorganisms from the rhizosphere of different soil localities was carried out. Table 3 shows the different soil localities at Al-Madinah Al-Munawwarah from which, isolation of different organisms was carried out.

Interactions that take place in the rhizosphere can be beneficial for the plant and also for the microbial community present. The Exudates released by plants have various effects in the surrounding ecosystem as altering the physical-chemical properties of soil by inhibiting the growth of other plants, enhancing symbiotic relationships, and selecting the type of microbiota that can colonize the area. Also, the microflora present in the rhizosphere can produce antagonistic molecules that will inhibit or kill the pathogens present (Rondon *et al.,* 1999; Rondon *et al.,* 2000; Jaben *et al.,* 2004).

Thus, in the rhizosphere, plants select for a specific composition of bacterial communities (Kowalchuk *et al.,* 2002 and Kuske *et al.,* 2002) depending on the type and amount of organic root exudates and of nutrients released from senescent or dead roots (Rangel-Castro *et al.,* 2005).

Table 1. Antimicrobial susceptibility pattern of the selected target Gram-negative bacterial isolates from CSSI cases.

Isolate name	AMP	AUG	FOX	Cefuroxime	Cefataxine	Cefitriaxone	CAZ	AK	GM	Tienam	ATM	Norfl	PIP	KF	TS
E.coli, **I_5**	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R
Klebsiella **spp.,I_{11}**	R	R	S	S	S	S	S	S	R	S	S	S	R	R	R
Pseudomonas **spp.,I_6**	R	R	S	R	R	S	R	S	S	S	R	R	R	R	S
Proteus **spp.,I_1**	R	S	R	R	S	R	S	S	S	S	R	S	R	R	R
Citrobacter **spp., I_1**	R	R	S	S	S	S	S	S	S	S	S	S	R	R	S
Acintobacter **spp.,I_2**	R	S	S	S	S	S	S	S	S	S	R	S	R	R	S

AMP= Ampicillin , AUG= Augmentin, FOX=Cefoxitin, CAZ=Ceftazidime, AK= Amikacin, ATM= Aztreonam, PIP=Piperacillin, KF= Cephalothin, TS=Cotrimoxazole, Norfl=Norfloxacin, GM= gentamycin. I= isolate.

Table 2. Antimicrobial susceptibility pattern of the selected target Gram-positive bacterial isolates from CSSI cases.

Isolate name	P	Erythromycin	KF	TS	Clindamycin	Vancomycin	AMP	AUG	AK
S. aureus,**I_3**	R	R	R	R	R	S	R	R	R
CONS,I_2	R	R	R	R	R	S	R	R	R

P=Penicillin, KF= Cephalothin, TS= Cotrimozole, AMP= Ampicillin, AUG= Augmentin, AK=Amikacin, I= isolate.

Table 3. Soil samples from different localities for isolation of AAPM.

Soil sample	District	Plant rhizosphere
S_1	Al-Ouayna	Sunflower
S_2	Al-Ouayna	Datepalm
S_3	Al-Ouayna	Nabk-tree
S_4	Al-Ouayna	Catnip
S_5	Al-Ouayna	Catnip
S_6	Al-Ouayna	Datepalm
S_7	Al-Aziziah	Balessan
S_8	Al-Aziziah	Nabk-tree
S_9	Al-Aziziah	Date palm
S_{10}	Al-Aziziah	Basil
S_{11}	Al-Aziziah	Corn
S_{12}	Al-Aziziah	Catnip
S_{13}	Al-Aziziah	Date palm
S_{14}	Al-Aziziah	Basil

S= soil sample

In view of the present data, different fungi and bacteria were isolated from different soils on different cultural media. Their antimicrobial activity against the eight target resistant CSSIs isolates was tested. The total AAPM from the fourteen soil samples were 86 isolates (24 fungal, 30 bacterial and 32 actinomycete isolates) after all purification steps.

The microorganisms isolated and evidenced antibacterial potential against target organisms under study were only 4 out of the total 24 fungal isolates (16.67%) (Table 4), 7 isolates out of the total 32 actinomycete strains (21.88%) (Table 5), and only 4 out of the thirty other bacterial isolates (13.33%) (Table 6). The most potent producer strain was then selected and identified. Only actinomycete strain A_{24} S_4 was found to exhibit the broadest spectrum of antimicrobial activity esp. against MRSA, I_3 and *E.coli*, I_5 in comparison with other AAPMs under study. So, it was selected as the most potent AAPM. This actinomycete isolate A_{24} S_4 obtained from catnip rhizosphere (*Menthae piperitae*) in Al-Ouayna district, Al-Madinah Al-Munawwarah, was subjected for characterization.

Actinomycetes antagonism has been reported for a wide variety of fungal and bacterial pathogens. Several authors have screened soil samples collected from different parts all over the world for AAPMs as Brazil in study of Huddleston *et al.* (1997); Morocco (Yedir *et al.,* 2001); India (Haque *et al.*, 1992; Peela *et al.*, 2005; Thakur *et al.*, 2007, Yadav *et al.;* 2009); China (Li *et al.;* 2008) and Egypt (Abu-Elainin, 2004; El-Naggar *et al.*, 2006). Unfortunately, we couldn't find any research done in this respect in KSA to compare with except Al-Zahrani (2007) who collected soil samples from various locations in Jazan region.

In the present study, the prevalence of soil actinomycetes that exhibited various degrees of antibacterial activities was 21.88% (7 out of 32). This ratio is higher than Yedir *et al.* (2001) (10%). On the other hand, it is lower than ratio of Peela *et al.* (2005) (68.75%) and Thakur *et al.* (2007) (59.09%). This discrepancy can be attributed to the multi resistance of our target organisms.

Morphological characterization of actinomycete isolate A_{24} S_4

Scanning electron micrograph of this isolate was represented in Figure 1. Furthermore, the cultural characteristics of this local actinomycete strain are provided in Table 7. The spores evidenced smooth surfaces, and were morphologically cylindrical.

Biochemical characterization of actinomycete isolate A_{24} S_4

Regarding biochemical characterization, the actinomycete isolate $A_{24}S_4$ could hydrolyze protein, starch, lipid, pectin, cellulose, lecithin and casein. Degradation of esculin and xanthin, H_2S production, and gelatin liquefaction were positive. Nitrate reduction was also positive. Thus, this strain is a nitrate reductase producer and hence this isolate may play a vital role in soil fertility of agricultural soil. Yadav *et al.* (2009) in their study also found that the actinomycete strain A160 was nitrate reductase producer.

On the other hand, catalase, production of melanin pigment on different media, indole production, urea degradation, citrate and KCN utilization were negative.

Concerning utilization of carbon sources, A_{24} S_4 strain utilized mannose, mannitol, glucose, fructose, maltose, arabinose, rhamnose, raffinose, xylose sodium acetate, starch, but did not utilize sucrose, galactose, lactose and meso-inositol. Utilization of a variety of carbon sources by this actinomycete isolate will help in adaptation to a variety of inoculation sites and wide soil types (Yadav *et al.*, 2009).

Concerning utilization of nitrogen sources, the strain understudy utilized phenylalanine, arginine, serine, glycine, tryptophan, alanine, lysine, leucine, cystine and asparagine, but did not utilize valine & histidine.

The strain was found to grow up to 10% of NaCl concentration. The strains of Yadav *et al.* (2009) showed high salt tolerance up to 7% of NaCl concentration and they reported that this is an important trait for their proliferation, colonization and for biological control of disease in crops in agricultural field for coastal area with high salt nature. Vasavada *et al.* (2006) also isolated a *S. sannanensis* strain RJT-1 from the alkali soil of Rajkot, India which grew in 9% NaCl.

The growth was inhibited in the presence of phenol (0.01%) and crystal violet (0.001%) and no inhibition was detected in sodium azide (0.01%). Good growth was detected within a temperature range of 20 to 40 °C, with no growth above 40 °C. The cell wall hydrolysate contained LL-diaminopimelic acid and sugar pattern were not detected.

Furthermore, A_{24} S_4 strain was resistant to amoxicillin, Keflex, cephardine, doxycycline, augmentin and chloramphinicol, erythromycin, cloxacillin, gentamycin and carbenicillin. Some earlier workers have also been reported on antibiotic resistance of actinomycetes (Kuske *et al.*, 1997; Yadav *et al.*, 2009). This resistance against an array of antibiotics is an added advantage for their survival in environment by combating against other microbes that produce antibiotics and allow their survival and

colonization significantly for long duration in the inoculated soil (Yadav *et al.*, 2009).

The cultural and physiological properties of the isolated local strain, as well as its carbon and nitrogen compound utilization characteristics were compared to those of the actinomycetes as described in the recommended international Keys (Lechevalier *et al.;* 1989; Hensyl, 1994). It could be stated that actinomycetes isolate belongs to the genus *Streptomyces*; and it is suggestive of being related to *Streptomyces ramulosus*, and thus given the name & the code number *Streptomyces ramulosus*, A-MM-24.

In an extensive isolation study of Chinese soils, Xu *et al.* (1996) demonstrated that streptomycetes were the most numerous actinomycete group in soil. Actinomycetes are Gram-positive bacteria characterized by the formation of aerial mycelium on solid media, presence of spores and high G + C content of DNA (60-70 mol %). They are important class of bacteria since they produce numerous natural products such as antibiotics and enzymes (De Schrijver and De Mot, 1999). On the basis of morphological and chemical criteria actinomycetes have been grouped into different genera. *Streptomycetes* is an actinomycetes with cell wall type I, belonging to the family *Streptomycetaceae* (Shirling and Gottlieb, 1968).

The first time to discover *S. ramulosus* was carried out by Ettlinger *et al.* in 1958 from German soil. Also, *S. ramulosus* has been found again in 1983 (Chen *et al.*, 1983).

In a taxonomic study of the Genus *Streptomyces* on the basis of levels of AT-L30 N-terminal amino acid sequence homology, Ochi (1995) constructed a phylogenetic tree on the basis of the levels of similarity of the amino acid sequences and revealed the existence of six clusters within the genus. The third cluster contains *Streptomyces ramulosus* and *Streptomyces ochraceiscleroticus*. More recently, Khalifa (2008) isolated *S. ramulosus* from Egyptian soil.

In conclusion, the present data focusing on obtaining microbial local isolates which have the ability to produce antimicrobial agent. An interesting scope for further research would be to improve antimicrobial agent production by the strain *Streptomyces ramulosus*, A-MM-24, with purification and characterization of the antibiotic obtained from this isolate.

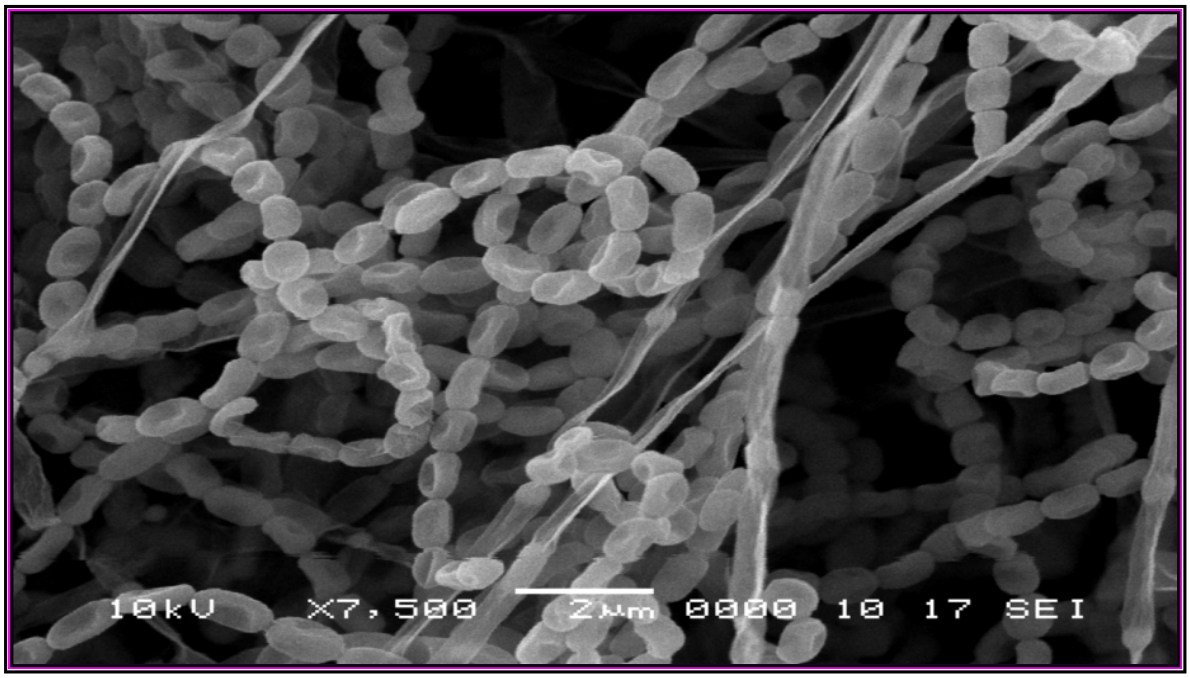

Figure 1. Scanning electron micrograph of the actinomycete isolate A_{24} growing on starch nitrate agar medium, showing spiral shaped spore chain and smooth spore surfaces (X 7, 500)

Table 4. Antimicrobial activities of fungal isolates from different soil samples from Al-Madinah Al-Munawwarah.

Fungal isolates	Target organisms							
	Klebsiella spp., I_{11}	*Pseudomonas* spp., I_6	*Proteus* spp., I_1	*E.coli*, I_5	*Citrobacter* spp., I_1	*Acinetobacter* spp., I_2	MRSA, I_3	CONS, I_2
F_1S_4	–	–	+	–	+	+	–	–
F_2S_5	–	–	–	–	–	–	–	–
F_3S_5	–	–	–	+	–	+	–	–
F_4S_4	–	–	+	+	+	–	–	+
F_5S_2	–	–	–	+	+	–	–	+
F_6S_4	–	–	–	–	–	–	–	–
F_7S_5	–	–	–	–	–	–	–	–
F_8S_3	–	–	–	–	–	–	–	–
F_9S_4	–	–	–	–	–	–	–	–
$F_{10}S_1$	–	–	–	–	–	–	–	–
$F_{11}S_2$	–	–	–	–	–	–	–	–
$F_{12}S_9$	–	–	–	–	–	–	–	–
$F_{13}S_{12}$	–	–	–	–	–	–	–	–
$F1_4S_1$	–	–	–	–	–	–	–	–
$F_{15}S_2$	–	–	–	–	–	–	–	–
$F_{16}S_9$	–	–	–	–	–	–	–	–
$F_{17}S_{10}$	–	–	–	–	–	–	–	–
$F_{18}S_{10}$	–	–	–	–	–	–	–	–
$F_{19}S_{10}$	–	–	–	–	–	–	–	–
$F_{20}S_8$	–	–	–	–	–	–	–	–
$F_{21}S_8$	–	–	–	–	–	–	–	–
$F_{22}S_7$	–	–	–	–	–	–	–	–
$F_{23}S_7$	–	–	–	–	–	–	–	–
$F_{24}S_{11}$	–	–	–	–	–	–	–	–

F=fungul & S= soil sample

Table 5. Antimicrobial activities of different actinomycete isolates from different soil samples from Al-Madinah Al-Munawwarah.

Actinomycete isolates	Target organisms							
	Klebsiella spp., I_{11}	*Pseudomonas* spp., I_6	*Proteus* spp., I_1	*E.coli*, I_5	*Citrobacter* spp., I_1	*Acinetobacter* spp., I_2	MRSA, I_3	CONS, I_2
A_1S_7	–	–	–	–	+	+	–	+
A_2S_1	–	–	-	–	+	–	–	+
A_3S_{14}	+	–	–	–	+	+	–	–
A_4S_9	–	–	–	–	+	–	–	–
A_5S_{14}	–	–	–	–	–	–	–	–
A_6S_8	–	–	–	–	–	–	+	–
A_7S_8	–	–	–	–	–	–	–	–
A_8S_{10}	–	–	–	–	–	–	–	–
A_9S_3	–	–	–	–	–	–	–	–
$A_{10}S_{14}$	–	–	–	–	–	–	–	–
$A_{11}S_4$	–	–	–	–	–	–	–	–
$A_{12}S_{10}$	–	–	–	–	–	–	–	–
$A_{13}S_{10}$	–	–	–	–	–	–	–	–

$A_{14}S_8$	–	–	–	–	–	–	–	–
$A_{15}S_{11}$	–	–	–	–	–	–	–	–
$A_{16}S_{11}$	–	–	–	–	–	–	–	–
$A_{17}S_9$	–	–	–	–	–	–	–	–
$A_{18}S_5$	–	–	–	–	–	–	–	–
$A_{19}S_3$	–	–	–	–	–	–	–	–
$A_{20}S_3$	–	–	–	–	–	–	–	–
$A_{21}S_2$	–	–	–	–	–	–	–	–
$A_{22}S_1$	–	–	–	–	–	–	–	–
$A_{23}S_2$	–	–	–	–	–	–	–	–
$A_{24}S_4$	–	+	–	+	+	–	+	+
$A_{25}S_5$	–	–	–	–	–	–	–	–
$A_{26}S_7$	–	–	–	–	–	–	–	–
$A_{27}S_4$	–	–	–	–	–	–	–	–
$A_{28}S_1$	–	–	–	–	–	–	–	–
$A_{30}S_1$	–	–	–	–	–	–	–	–
$_{31}S_6$	–	–	–	–	+	–	–	–
$A_{32}S_{13}$	–	–	–	–	–	–	–	–
$A_{33}S_{14}$	–	–	–	–	–	–	–	–

A= Actinomycete & S= soil sample

Table 6. Antimicrobial activities of the different bacterial isolates from different soil samples from Al-Madinah Al-Munawwarah.

Bacterial isolates	Target organisms							
	Klebsiella spp., I_{11}	*Pseudomonas* spp., I_6	*Proteus* spp., I_1	*E.coli*, I_5	*Citrobacter* spp., I_1	*Acinetobacter* spp., I_2	MRSA, I_3	CONS, I_2
B_1S_4	–	–	–	–	–	–	–	–
B_2S_I	–	–	–	–	–	–	–	–
B_3S_1	–	–	+	–	–	–	–	–
B_4S_1	–	–	–	–	–	–	–	–
B_5S_1	–	–	–	–	–	–	–	+
B_6S_3	–	–	+	–	–	–	–	–
B_7S_4	–	–	+	–	–	–	–	+
B_8S_3	–	–	–	–	–	–	–	–
B_9S_3	–	–	+	+	–	–	+	–
$B_{10}S_2$	–	–	+	–	–	–	+	+
$B_{11}S_3$	–	–	–	–	–	–	–	–
$B_{12}S_1$	–	–	+	–	–	–	+	+
$B_{13}S_3$	–	–	–	–	–	–	–	–
$B_{14}S_3$	–	–	–	–	–	–	–	–
$B_{15}S_1$	–	–	–	–	–	–	–	–
$B_{16}S_3$	–	–	–	–	–	–	–	–
$B_{17}S_5$	–	–	–	–	–	–	–	–
$B_{18}S_4$	–	–	–	–	–	–	–	–
$B_{19}S_1$	–	–	–	–	–	–	–	–
$B_{20}S_4$	–	–	–	–	–	–	–	+
$B_{21}S_7$	–	–	–	–	–	–	–	+
$B_{22}S_{11}$	–	–	–	–	–	–	–	–

$B_{23}S_{13}$	–	–	–	–	–	–	–	–
$B_{24}S_{12}$	–	–	–	–	–	–	–	–
$B_{25}S_{10}$	–	–	–	–	–	–	–	+
$B_{26}S_{10}$	–	–	–	–	–	–	–	–
$B_{27}S_{7}$	–	–	–	–	–	–	–	–
$B_{28}S_{9}$	–	–	–	–	–	–	–	–
$B_{29}S_{9}$	–	–	–	–	–	–	–	–
$B_{30}S_{4}$	–	–	–	–	–	–	–	–

B= Bacterial & S= soil sample

Table 7. Cultural characteristics of the most active antimicrobial agent producing actinomycete strain A_{24} S_4.

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
1-Tryptone yeast extract broth (ISP-1)	-	-	-	-
2-Yeast extract malt extract agar (ISP-2)	Good	265-1. gray Medium gray	76-1-y-br Light yellowish Brown	77—m.ybr moderate yellowish brown
3- Oat-meal agar (ISP-3)	-	-	-	-
4-Inorganic salts starch agar medium (ISP-4)	Moderate	8-gy. pink Grayish pink	73-p.OY Pall orange yellow	67-brill.OY Brill. Orange yellow
5-Glycerol – asparagine agar (ISP-5)	-	-	-	-
6-Peptone yeast extract iron agar (ISP-6)	Good	265-1. gray medium gray	76-1-y-br Light yellowish Brown	58-m. Br Moderate brown
7-Tyrosine agar (ISP-7)	Good	264-1. gray light gray	57-I. Br Light brown	77—m.ybr moderate yellowish brown

The color of organism under investigation was consulted using the ISCC-NBS Colour-Name Charts II illustrated with cenroid color.

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5. References:

1. Abo-Shadi MA, Al-Mutrafy AO (2007). Bacteriological study on caesarean section surgical site infection. Egypt J. Biomed. Sci.; 24: 83-93.
2. Abu-Elainin IMM (2004). Microbiological studies on certain Actinomycetes isolated from some Egyptian localities, MSc, Al-Azhar Univ., Egypt.
3. Al-Zahrani SHM *(2007).* Studies on the Antimicrobial Activity of *Streptomyces* sp. Isolated from Jazan. JKAU Sci.; 19: 127-138.
4. Ammar MS, EL-Essaway M, Yassin M, Sherif YM (1998). Hydrolytic enzymes of fungi isolated from certain Egyptian antiquites objects while utilizing the industrial wastes of sugar and integrated industries company (SIIC). Egypt J. Biotechnol.; 3: 60-90.
5. Ball AP, Bartlett JG, Craig WA, Drusano GL, Felmingham D, Garau JA, Klugman K P, Low DE, Mandell LA, Rubinstein E, Tillotson GS (2004). Future trends in antimicrobial chemotherapy: expert opinion on the 43rd ICAAC. J. Chemother.; 16: 419-436.
6. Becker B, Lechevalier MP, Gordon RE, Lechevalier HA (1964). Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. Appl Microbiol Biol.; 12: 421-423.
7. Chapman GS (1952). A simple method for making multiple tests on microorganism. J. Bacteriol.; 63:147.
8. Chen Y, Zeeck A, Chen Z, Zähner H (1983). Metabolic products of microorganisms. 222. beta-Oxotryptamine derivatives isolated from *Streptomyces ramulosus.* J Antibiot.; 36 (7): 913-5.
9. Colsky AS, Kirsner RS, Kerdel FA (1998). Analysis of antibiotic susceptibilities of skin wound flora in hospitalized dermatology patients: The crisis of antibiotic resistance has come to the surface. Arch. Dermatol.; 134 (8): 1006-9.
10. Courtis S, Cappellano C, Ball M, Francois F, Helynck F, Martizez A, Kolvek S, Hopke J, Osburne M, August P, Nalin R, Guerineau M, Jeannin P, Simonet P, Prenodet J (2003). Recombinant environmental libraries provide access to microbial diversity for drug discovery from natural products. Appl. Environ. Microbiol.; 69: 49-55.
11. Cowan ST (1974). Cowan and Steel's Manual for the identification of medical bacteria. Cambridge Univ. press.
12. Crowe J, Olsson S (2001). Induction of laccase activity in *R. solani* by antagonistic *Pseudomonas flouescens* strains and a range of chemical treatments. Appl. Environ. Microbiol.; 67: 2088-2094.

13. Dakora F, Phillips D (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil; 245: 35-47.
14. De Schrijver A, and De Mot R (1999). Degradation of pesticides by actinomycetes. Crit Rev Microbiol.; 25: 85-119.
15. El-Naggar MY, El-Assar SA, Abdul-Gawad SM (2006). Meroparamycin production by newly isolated *Streptomyces* sp. strain MAR01: Taxonomy, fermentation, purification and structural elucidation. J Microbiol.; 44 (4): 432-438.
16. Elwan SH, El-Naggar, MR, Ammar MS (1977). Characteristics of lipase (s) in the growth filtrate dialysate of *B. stearothermophilus* grown at 55ºC using tributyrin-cup plate assay. Bull Sci Riyadh Univ.; 8: 115-119.
17. Ettlinger E, Gaumannr H, Keller-Schierlefi NK, Radolferl NV, Zahner H (1958). Ober die Isolierungand Charakterisierung von Acetomycin. Helv Chim Acta 41: 216-219.
18. Filius PMG, Gyssens IC (2002). Impact of increasing antimicrobial resistance on wound management. *Am. J. Clin. Dermatol.;* 3 (1): 1-7.
19. Giacometti A, Cirioni O, Schimizzi AM, Del Prete MS, Barchiesi F, D'Errico MM, Petrelli E, Scalise G (2000). Epidemiology and microbiology of surgical wound infections. J. Clin. Microbiol.; 38 (2): 918-922.
20. Gordon RE, Barnett DA, Handerhan JE, Pang CH (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the Nocardin strain. Intern J System Bacteriol.; 24: 54-63.
21. Gordon RE (1966). Some criteria for the recognition of *Nocardia madura* (Vincent) Blanchord. J Gen Microbiol.; 45: 355-364.
22. Hackl E, Boltenstern S, Bodrossy L, Sessitsch A (2004). Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. Appl. Environ. Microbiol.; 7: 5057-5065.
23. Hancock REW (2007).The end of an era? Nat. Rev. Drug. Discov.; 6: 28.
24. Hankin L, Zuker M, Sands DC (1971). Improved solid medium for the detection and enumeration of proteolytic bacteria. Appl Microbiol.; 22: 205-509.
25. Haque SF, Sen SK, Pal SC (1992). Screening and identification of antibiotic producing strains of Streptomyces. Hindustan Antibiot Bull.; 34(3-4): 76-84.
26. Hensyl, WR (1994). Bergey's Manual of Systematic Bacteriology, 9th Ed., John, G., Stanley, H., Williams, T. (Eds.) Williams and Wilkins, Baltimore,USA.
27. Huddleston AS, Cresswell N, Neves MCP, Beringer JE, Baumberg S, Thomas DI, Wellington EMH (1997). Molecular detection of streptomycin-producing streptomycetes in Brazilian soils. Appl Environ Microbiol.; 63(4): 1288-1297.
28. Jaben N, Rasool S, Ahmad S, Ajaz M, Saeed S (2004). Isolation, identification and bacteriocin production by indigenous diseased plant and soil associated bacteria. Pakistan J Biol Sci.; 7: 1893-1897.
29. Johnson LF, Curl EA, Bond JH (1959). Methods for studying soil microflora- plant disease relationships, Burgess, Minneopolis.
30. Jones K (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristics. J Bacteriol.; 57: 141-145.
31. Kenneth LK, Deane BJ (1955). Color universal language and dictionary of names. United States Department of Commerce. National Bureau of standards. Washington, D.C, 20234.
32. Khalifa MA (2008). Bioprocess development for the biosynthesis of bioactive compounds from microbial origin. M. Sc. Thesis, Faculty of science (Boys), Al-Azhar University, Cairo, Egypt.
33. Kowalchuk GA, Buma DS, de Boer W, Klinkhamer PG, van Veen JA (2002). Effect of a above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie Leeuwenhoek; 81: 509-520.
34. Kuske CR, Barns SM, Busch JD (1997). Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions. Appl Environ Microbiol.; 63: 3614-21.
35. Kuske CR, Ticknor LO, Miller ME, Dunbar JM (2002). Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Appl. Environ. Microbiol.; 68: 1854–1863.
36. Kuster E, Williams S (1964). Selection of media for isolation of streptomycetes. Nature; 202: 928-929.
37. Kuster E (1959). Outline of a comparative study of criteria used in characterization of the actinomycetes. Intern. Bull Bact. Nomen. Taxon.; 98-104.
38. Lechevalier MP, Lechevalier HA (1970). Chemical composition as criterion in the classification of aerobic actinomycetes. J Syst Bacteriol.; 20: 435-443.
39. Lechevalier HA, Willium ST, Sharpe ME, Holt JG (1989). The Actinomycetes: A practical guide to genetic identification of actinomycetes. p. 2344-3330. *In*, Bergy's Manual of Determinative Bacteriology. The Williams & Wilkins Co., Baltimore, Maryland, USA.
40. Li J, Zhao GZ, Chen HH Wang HB, Qin S, Zhu WY, Xu LH, Jiang CL, Li WJ (2008). Antitumour and antimicrobial activities of endophytic streptomycetes from pharmaceutical plants in rainforest. Lett Appl Microbiol.; 47(6): 574-80.
41. Madigan MT, Martinko JM, Parker J (2003). Brock Biology of Microorganisms. Pearson Education International.
42. Mellouli L, Mehdi RB, Sioud S, Salem M, Bejar S (2003). Isolation, purification and partial characterization of antibacterial activities produced by a newly isolated Streptomyces sp. US24 strain. Res Microbiol.; 154: 345-52.
43. Motta AS, Cladera-Olivera F, Brandelli A (2004). Screening for antimicrobial activity among bacteria isolated from the Amazon Basin. Brazilian J Microbiol.; 35: 307-310.
44. Nisch B, Kutzner HJ (1969). Egg-yolk agar as a diagnostic medium for Streptomycetes. Exp 25: 113.

45. Ochi K (1995). A taxonomic study of the genus *Streptomyces* by analysis of ribosomal protein AT-L30. Intern J System Bacteriol.; 45 (3): 507-514.
46. Pandey B, Ghimire P, Prasad V, Agrawal V (2002). Studies of the antimicrobial activity of the actinomycetes isolated from the Khumby region of Nepal. Department of Bacteriology. University of Wisconsin- Madison. Madison. Wisconsin.
47. Peela S, Bapiraju Kurada VVSN, Terli R (2005). Studies on antagonistic marine actinomycetes from Bay of Bengal. World J Microbiol Biotechnol.; 21: 583-5.
48. Pridham TG, Anderson P, Foley C, Lindenfelser LA, Hesselting CW, Benedict RC (1957). A selection of media for maintenance and taxonomic study of Streptomyces. Antibiot Annu.; 947-53.
49. Pridham TG, Gottlieb D (1948). The Utilization of carbon compound by some actionmycetes as an aid for species determination. J Bacteriol.; 56: 107-114.
50. Pridham TG, Lyons AJ (1961). Streptomyces albus (Rossi-Doria) Waksman et Henrici: taxonomic study of strains labeled *Streptomyces albus*. J. Bacteriol.; 81: 431-441.
51. Rangel-Castro JI, Killham K, Ostle N, Nicol GW, Anderson IC, Scrimgeour CM, Ineson P, Meharg A, Prosser J (2005). Stable isotope probing analysis of the influence of liming on root exudate utilization by soil microorganisms. Environ. Microbiol.; 7: 828–838.
52. Rivera-Rivera MJ (2005). Isolation and characterization of antimicrobial agent producing microbes and generation of metagenomic libraries from diverse forest soils in Puerto Rico. MSc Thesis (Biology). University of Puerto Rico Mayagüez Campus.
53. Rondon M, August P, Bettermann AD, Brady SF, Grossman TH, Liles MR, Loiacono KA, Lynch BA, MacNeil C, Minor C, Tiong M, Osborne J, Clardy J, Handelsman J, Goodman R (2000). Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl. Environ. Microbiol.; 66: 2541-2547.
54. Rondon M, Raffel S, Goodman R, Handelsman J (1999). Toward functional genomics in bacteria: analysis of gene expression in *Escherichia coli* from a bacterial artificial chromosome library of *Bacillus cereus*. Microbiol.; 96: 6451-6455.
55. Saadoun I, Gharaibeh R (2003). The Streptomyces flora of Badia region of Jordan and its potential as a source of antibiotics active against antibiotic-resistant bacteria. J Arid Environ.; 53: 365-371.
56. Shirling EB, Gottlieb D (1968). Cooperative description of type culture of Streptomyces II species description from first study. Int J Syst Bacteriol.; 18: 69-189.
57. Tadashi A (1975). Culture media for actinomycetes. The Society for actinomycetes. Japan National Agricultural Library; 1: 1-31.
58. Thakur D, Yadav A, Gogoi BK, Bora TC (2007). Isolation and screening of Streptomyces in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. Journal de Mycologie Médicale; 17: 242-249.
59. Tsao PH, Leben C, Keitt GW (1960). An enrichment method for isolating actinomycetes that produce diffusible antifungal antibiotics. Phytopathology; 50: 88-89.
60. Vasavada SH, Thumar JT, Singh SP (2006). Secretion of a potent antibiotic by salt-tolerant and alkaliphilic actinomycetes *Streptomyces sannensis*. Curr Sci.; 91: 393-1397.
61. Xu L, Li Q, Jiang C (1996). Diversity of soil actinomycetes in Yunnan, China. Appl Environ Microbiol.; 62: 244-248.
62. Yadav AK, Kumar R, Saikia R, Bora TC, Arora DK (2009). Novel copper resistant and antimicrobial Streptomyces isolated from Bay of Bengal, India. Journal of Medical Mycology; 19 (4): 234-240.
63. Yedir OY, Barakate M, Finance C (2001). Actinomycetes of Moroccan habitats: Isolation and screening for antifungal activities. Europ J Soil Biol.; 37 (2): 69-74.

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