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Isolation, Characterization and Bioactivities of Soil actinomycetes From the World Heritage Site (WHS) of Saint Katherine

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SOIL ACTINOMYCETES were isolated from 10 different sites in the World Heritage Site (WHS) of St. Katherine: Wadi El Deer, City center, Wadi El Talaa, Wadi El sheikh, Wadi El Arbean, Wadi El sheikh Awad, Wadi El Rutg, Om Kaisoum, Wadi Gebal, Gabal El Monagah. Simplified methods for isolation and characterization of actinomycetes recovered are described. Successful recovery was achieved on the media (S.C, 1/10 S.C and MGA). A total of 359 isolates were obtained and identified according to the standard macroscopic, microscopic as well as chemotaxonomy methods. A high percentage was obtained for *Streptomyces*, *Nocardoides, Kitasatosporia* spp. and unidentified spp., representing 30.4, 23.7, 11.4 and 29.3 %, respectively. The metabolic extracts from 250 randomly selected isolates exhibited various antimicrobial activities towards one reference culture (*E. coli* NCMB 11943) and two clinical cultures (*Staphylococcus aureus*, *Candida albicans*). Variable activities were obtained with different actinomycete isolates; the highest activity was against the Gram +ve *Staphylococcus aureus*, followed by *Candida albicans* and the Gram -ve *Escherichia coli*.Organic extracts from 243 isolates were effective in causing more than 40% Ehrlich ascites carcinoma (EAC) cell death after 120 min. *Micromonspora*, *Pseudonocardia* and *Streptomyces spp.* metabolic extracts caused the highest percentage of EAC cell death that averaged 70.58, 63.3 and 61.5 %, respectively. Actinomycetes isolated from WHS Heritage Site of Saint Katherine are suggested to be a potential source of bioactive metabolites. In conclusion, desert actinomycetes represent a promising source for antimicrobial and antitumor bioactive agents. More attention should be paid to desert soils as unique habitat for actinomycetes, though further scientific evidence needs to be produced to verify the importance of these actinomycetes and their metabolites.

Keywords: Soil,Actinomycetes, Antitumor, Characterization, Isolation

Actinomycetes is considered as highly attractive cell factories, or bioreactors, for most applications in different fields. They are exceptionally rich, diverse and easily accessible sources for production of bioactive secondary metabolites. These secondary metabolites are usually species specific and embrace a very wide range of structural types with various biological activities. Many of these secondary metabolites are of fundamental industrial applications, such as vitamins, immunomodifiers, pigments, enzymes, enzymes – inhibitors, etc., and others have vital role in agricultural and environmental applications (Maheshwari *et al*., 2012). Members of the class Actinobacteria, and especially

Streptomyces spp. have long been recognized as prolific sources of useful bioactive metabolites, providing more than half of the naturally occurring antibiotics discovered to date and continuing to be a source of new bioactive metabolites (Berdy, 2005). On the other hand *Sterptomyces* spp. has been related to some respiratory diseases which are caused often, by inhalation of its spores which present in bioaerosols (Kagen *et al*., 1981 and Van den Bogart *et al*., 1993). Toxins production by this genus can also result in health damage (Paananen *et al.*, 2000). Some actinomycetes are pathogenic, such as Mycobacteriales. However, many others are extremely useful due to their ability to produce compounds with pharmaceutical properties (antibiotic, antifungal, antitumor, immunosuppressive). Actinomycetes are distributed extensively in soil and provide many important bioactive compounds of high commercial value, including many of medical importance (Takizawa *et al*. 1993 and Baltz, 2007). They also had a prominent position and have proved a major profilic source of most antibiotics, such as streptomycin, macracidmycin, kedarcidin and others. Of all the antibiotics discovered till now, 75% are produced by actinomycetes. (Chaudhary, 2013).

For many years, arid desert soils were considered economically unimportant, and any ecological research, including the examination of microbial characteristics, was sporadic. During the past two decades, however, the economic and agricultural utilization of arid lands has emerged as a critical element in maintaining and improving the world's food supply; consequently, biological and environmental research on these soils has increased. Desert soils are usually characterised by high soil pH and often by high salinity. Both of which influence the activities of soil microorganisms. Arid areas may include saline surface deposits and hypersaline bodies of water, inhabited by unique microbial population (Brock, 1979; Williams 1981). At the turn of the 20th century, most desert soils were considered abiotic and sterile. In 1912, Lipman first demonstrated that desert soils were inhabited by numerous microorganisms when he described microbiological and characteristics of California desert.

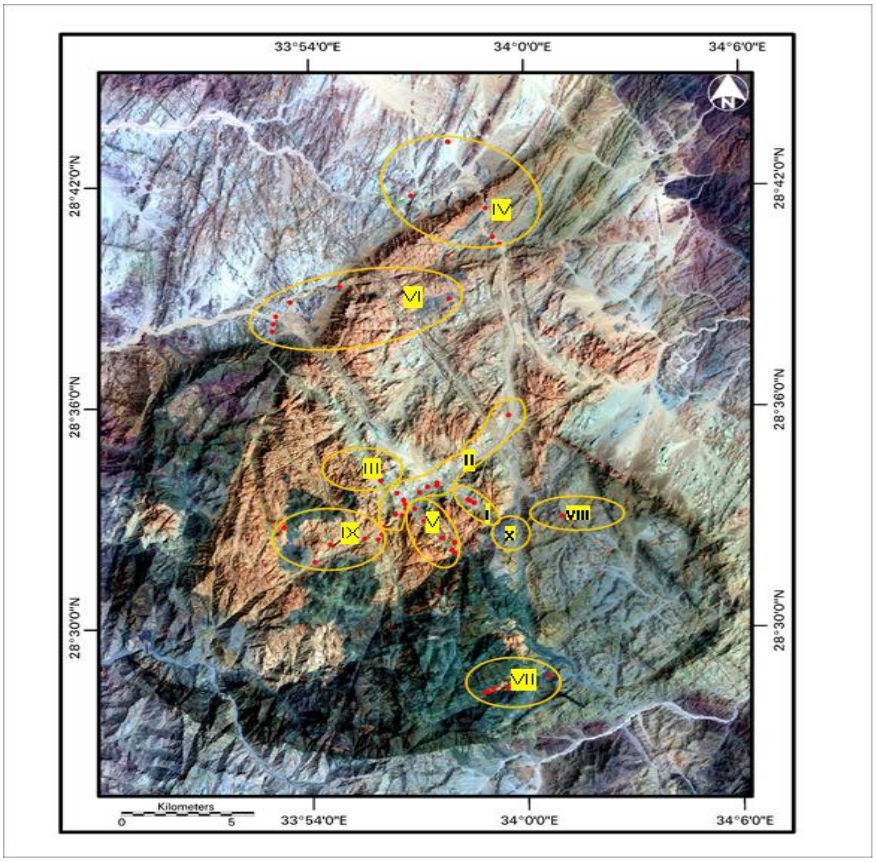
The WHS is characterized by a unique topography of high mountain range, associated with marked plant and animal biodiversity merging from the varying climatic conditions. Köppen-Geiger climate classification system classifies its climate as hot desert, with very low humidity (Egypt Climate Index, 2013). However, the site is still unexploited for microbial diversity and could be a valuable resource for novel actinomycetes of biotechnological value.

The aim of this work was to investigate the ability of actinomycetes isolated from desert habitat of the World Heritage Site (WHS) of Saint Katherine to produce bioactive compounds that may have antimicrobial and antitumor activities, under laboratory conditions.

Materials and Methods

Site description and Soils Sampli

Soil Samples were collected in plastic bags during the early summer of 2005. Fifty soil samples were collected from 10 geographical sites. Each sample was collected in triplicates (10-15 cm) depth and a composite sample was used for actinomycetes isolation. with characteristic topography of high mountain range, associated with marked biodiversity merging from the varying climatic condition of WHS. The WHS Protectorate is an area of 601 km2 located within the 4350 km2 of Saint Katherine protectorate which is centered on the monastery of Saint Katherine.

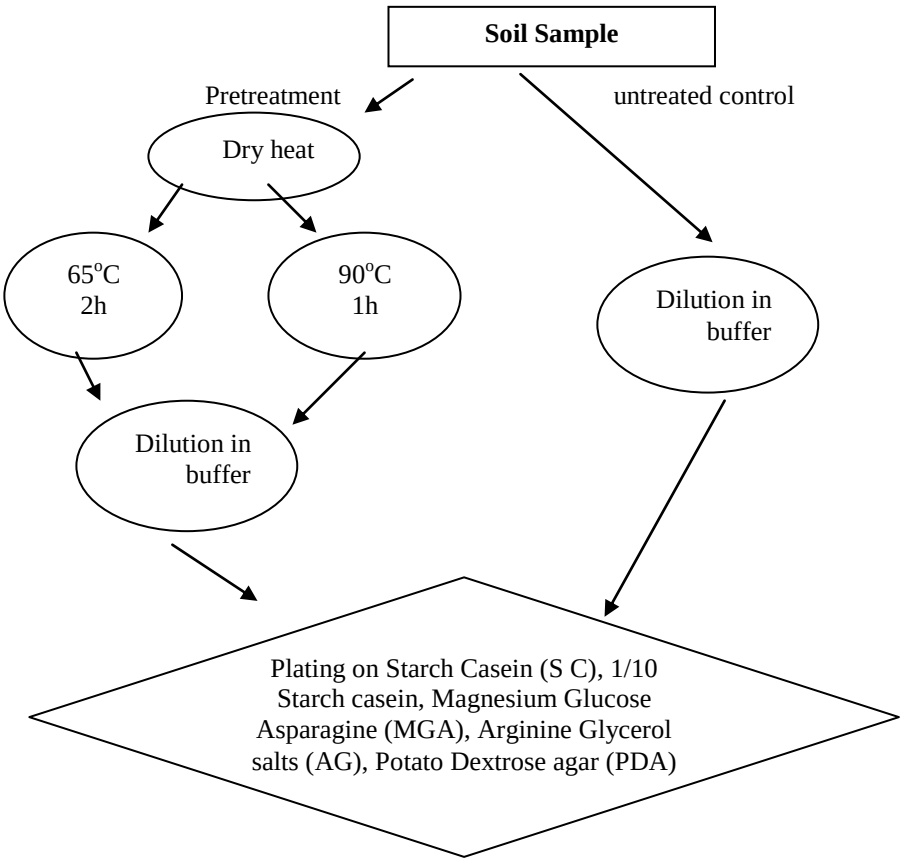


Soil analysis

Soil analysis was carried out in the Agriculture Research centers, Faculty of Agriculture, Suez Canal University. The investigated physico-chemical properties in this study were porosity, electric conductivity, pH, chloride, total nitrogen, nitrate, and organic matter.

Isolation and characterization of soil actinomycetes:

All procedures were performed in a laminar flow hood using standard aseptic techniques. Soil samples were processed with modification of the methods described by Demain and Davies (1999). Cyclohexamide antibiotic (50 mg/L) was used in all media to suppress fungi, permitting rare species to grow.



Proposed strategies for selection of optimal conditions for isolation of soil actinomycetes from the WHS soils.

Enumeration of soil actinomycetes

Soil samples (10-15 cm depth) were collected aseptically from the targeted localities and kept in sterile polyethylene bags at 4° C until analysis. Actinomycetes were isolated after serial dilution in phosphate buffer on SC agar supplemented with cycloheximide (50 µg/ mL) using spread plate technique and applied for heat pretreatment technique for selecting the best isolation technique. Counts were recorded after incubation for 2-3 weeks at 28° C.

Representatives of each colony form were picked-off from the isolation plates and streaked on SC medium to obtain pure cultures. They were then preserved as spore suspensions by in 20% glycerol at 20ºC (Kieser *et al*., 2000). Isolates were characterized and identified up to the genus level based on the standard methods (Holt *et al.,* 1994). These were macromorphology, micromorphology, and chemotaxonomy (detection of diaminopimelic acid (DAP) isomers in the whole cell hydrolysate and whole cell diagnostic sugar pattern)

Fermentation procedure

Preserved spore suspensions of actinomycete isolates were inoculated into 30 mL of SC broth and incubated in a shaker incubator for 5-7 days at 28° C, 100 rpm (Kieser *et al*., 2000). Cultures were extracted using equal concentrations of ethyl acetate, for three successive times with vigorous shaking for 30 min. The ethyl acetate fractions were evaporated under vacuum into a preweighed vial and then redissolved in ethyl acetate giving a final concentration of 10 mg/mL.

Antimicrobial Screening:

Metabolic organic extracts from the 900 isolates were tested by disc diffusion method at 0.1 mg/ disc against human pathogenic microorganisms (Castillo *et al*. 2002). The tested microorganisms were one reference bacterial strain *Echerichia col*i NCMB 11943 and two clinical cultures *Staphylococcus aureus* and *Candida albicans* (obtained from Suez Canal University, Dermatology clinic lab.). The growth medium used for bacterial culturing was nutrient broth and for the fungal culture was Sabouraud dextrose liquid medium.

Antitumor Screening

Induction of EAC in mice

Female mice (Swiss albino) of 20-25 gm body weight were injected with a fixed number of viable cells of Ehrlich ascites carcinoma EAC supplied from National Cancer Institute, Cairo University; (5 x 10^6 cells/20 gm body weight in 500 µl sterile saline); into the abdominal cavity of each mouse by intraperitoneal (i.p.) injection using sterile syringe. These mice were kept in well- ventilated cages under standard conditions at room temperature, pressure, and humidity. The animals were provided with common diet and water ad libitum for about 10--15 days. After this period the tumor cells multiplied relatively freely within the peritoneal cavity. Tumor growth in each animal was monitored by recording daily weight change of mice and measuring of abdominal volume. EAC cells were withdrawn by sterile disposable syringe to detect the volume of tumor cells/mouse and then diluted with physiological buffer for further investigations. The viability of the cells was 99% as judged by trypan blue assay.

In vitro trypan blue assay

Different concentrations of each actinomycetes metabolic extracts out of 240 extracts (50 to 500 µg/ml) were prepared in clean vials. Then each extract was treated with EAC cells (12.2 x 10^6 cells/ml phosphate buffer). The treated

extracts were incubated for 2 hr at 37° c. Trypan blue cell count was conducted to evaluate the total number of viable and apoptotic cells according to the method of Mclimans *et al*. (1957). Trypan blue works by leaking into cells with damaged cell wall and dyeing their nucleus with blue color. After the incubation period, 2 µl of 50 % trypan blue was added to 18 µl of the EAC mixture and microscopically counted using hemocytometer. Cells that showed signs of staining with blue were recorded as dead.

Results

Soil characteristics for the ten locations are presented in Table 1. The EC showed variation in the different locations of the study area with a maximum of 0.92 dSm^{-1} in Wadi El Rutg and minimum of 0.15 dSm^{-1} in Wadi El Sheikh. The soil of the study area, generally, had neutral to alkaline pH, with a maximum of 8.17 in Wadi El Sheikh and a minimum of 7.48 in Wadi El Rutg. The value of chloride concentration in Wadi El Arbaen was notably higher (85.2 mg/kg) than other soil samples (ranged from 14.00 – 53.25 mg/kg). Notably, the values of total nitrogen, nitrates, and organic matter in soil samples of Wadi El Sheikh Awad and Wadi El-Tofaha were low, compared to the other soils; being in the average of 50 mg/kg, 13.5 mg/kg, and 0.101 %, respectively. While, the values of total nitrogen, nitrates, and organic matter ranged from 80 to 350 mg/kg, 18 to 22 mg/kg, and 0.163 to 0.691 %, respectively, in the soils from the other locations.

TABLE 1. Chemical characters of soil samples representing the different investigated locations within the St. Katherine WHS

Location no.	Location name	ALT (m)	EC (dSm^{-1})	pH	Chloride (mg/kg)	Total Nitrogen (mg/kg)	Nitrate (mg/kg)	Organic matter (%)	Porosity (%)	Actinomyctes cfu/g
I	Wadi El deer	1567-600	0.31	7.79	17.75	120	18.0	0.244	18	48
II	City center	1545-628	0.32	7.75	14.00	50	14.0	0.102	18	26.1
III	Wadi El Talaa	1627	0.35	7.93	53.25	115	18.0	0.229	20	7
IV	Wadi El Sheikh	1177-307	0.15	8.17	21.3	80	17.0	0.163	25	47
V	Wadi El Arbaen	1635-750	0.56	7.68	85.2	150	19.0	0.295	15	39.2
VI	Wadi El Sheikh awad	1102- 95	0.20	7.78	28.4	50	13.0	0.100	16	76.8
VII	Wadi El Rutg	1585-647	0.92	7.48	21.3	350	18.0	0.691	11	9
VIII	Om Kaithoum	1641	0.28	7.97	21.3	200	19.0	0.406	13	11
IX	Wadi Gebal	1641-892	0.31	7.81	19.53	155	20.0	0.270	13	42
X	Gabal El Monagah	1600	0.30	7.82	21.3	190	22.0	0.386	2	57

Isolation and phenotypic characterization of actinomycetes from WHS soil:

An initial optimization step was done on 2 random samples to investigate the suitable dilution and treatment technique for the isolation. Direct isolation from diluted soil samples on agar media has resulted in overgrowth of fast growing bacteria and fungi, while dilution technique after soil pretreatment using two different conditions of dry heating showed successful results (Fig 1 a,b). The 65 ˚C for two hours gave selective growth and complete apparent colony growth (i.e mature colonies) of actinomycetes on the isolation plates, while the 90 ˚C for two hours showed successful growth but with lack of aerial mycelia. The best actinomycetes growth from soil sample of location no. II was obtained on SC medium, while from soil sample of location no. IV the best growth was on SC and MGA media.

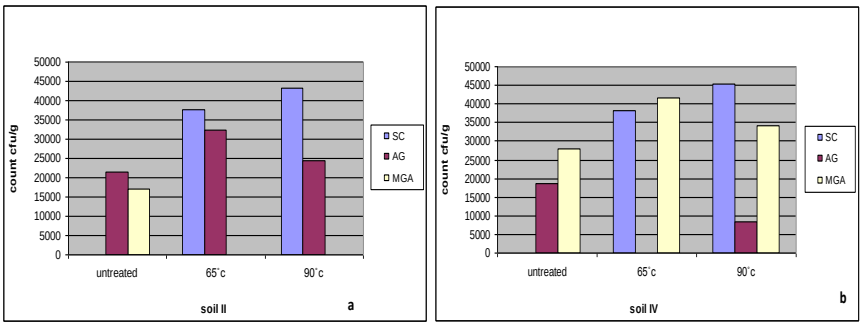


Fig. 1. Dry heating for selecting the best treatment technique before isolation, (a) soil sample of location II and (b) soil sample of location IV.

The second step of optimization was to select the best media for isolation. Ten random samples representative for the ten locations of WHS, were heat treated (65 ˚C/ 2h) and used for isolation on 5 different media: starch casein (SC), 1/10 starch casein, modified Magnesium Glucose Asparagin (MGA), potato dextrose agar medium (PDA) and Arginine Glycerol salts medium (AG). As shown in Fig. 2, MGA, SC and 1/10 SC gave varying counts of actinomycetes. While, AG and PDA media showed non remarkable counts and limited colony variations of actinomycetes on plates, therefore, their results were not represented in Fig 2.

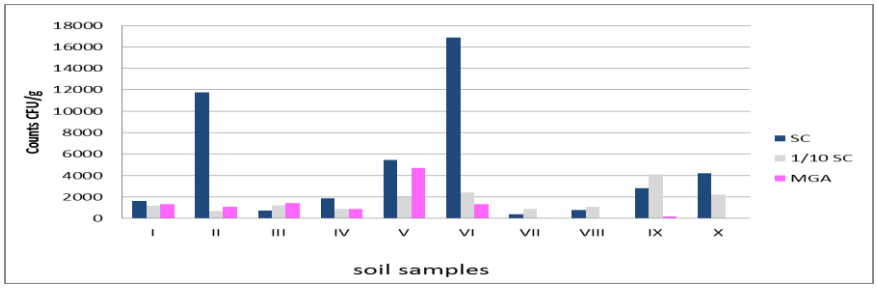
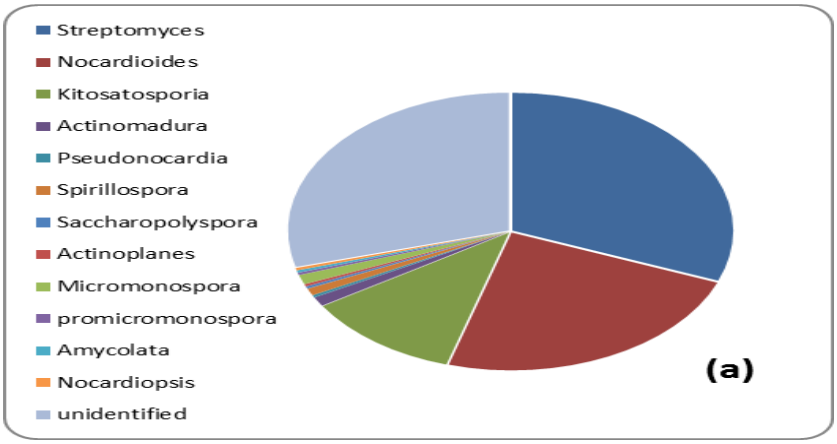


Fig. 2. Counts of actinomycetes on different media using random samples from the ten localities of WHS.

In this study 359 actinomycete isolates were recovered from WHS soil. Marked differences in the micromorphology of these isolates under study adding to their chemotaxonomic criteria resulted in assigning of 70.75 % of the isolates into 12 genera (Fig. 3 a). While the remaining 105 isolates (29.25%) could not be identified, using the standard chemotaxonomic and morphological criteria (Fig 3 b). The major cluster groups were *Streptomyces, Nocardioides and Kitasatosporia* genera. *Streptomyces* strains were the cluster group with the largest number of isolates (30.4%). *Nocardioides* strains also represented about 23.67 %. *Kitasatosporia* strains were recovered with the percentage of 11.4%. In addition, rare genera such as *Spirellospora*, *Nocardiopisis*, *Saccharopolyspora*, *Amycolata*, *Micromonospora*, *Pseudonocardia*, *Actinomadura*, *Promicromonospora* and *Actinoplanes* were recorded.



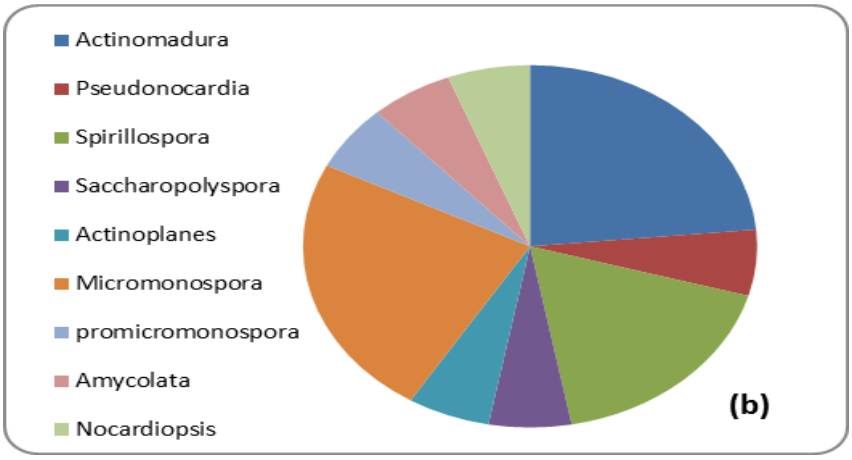


Fig. 3. Presumptively identified actinomycete genera in St. Katherine WHS soils. (a) propotion of the 12 genera. (b) propotion of minior groups.

Antimicrobial activities of actinomycete metabolic extracts

The metabolic extracts from representative 250 isolates were screened for antimicrobial activity. They exhibited various activities towards the reference culture (*E. coli* NCMB 11943) and two clinical cultures (*Staphylococcus aureus* and *Candida albicans*) obtained from Suez Canal University, Dermatology clinic lab. The 6 isolates belonging to the genera *Spirellospora* (3 isolates), *Saccharopolyspora* (1 isolate), *Promicromonospora* (1 isolate), and *Actinoplanes* (1 isolate), didn't show antimicrobial activities as illustrated in Table 2. Generally, the highest activity was against the Gram +ve *Staphylococcus aureus* followed by *Candida albicans* and the Gram –ve *Escherichia coli*.

TABLE 2. Antimicrobial activity of selected actinomycetes against *E. coli, S. aureus* and *C. albicans.*

Genera	No.of isolates	No. of positive isolates		
		E. coli	*S. aureus*	*C. albicans*
Streptomyces	77	30	38	27
Nocardioides	41	12	23	22
Kitasatosporia	28	7	8	11
Actinomadura	3	2	2	2
Pseudonocardia	2	2	2	2
Micromonospora	1	1	1	1
Unknown	94	43	55	48
Total	250	97	129	113

Antitumor activities of actinomycete metabolic extracts:

Organic extracts from 243 isolates of the 250 actinomycetes were effective in causing more than 40% EAC cell death after 120 min (Table 3). *Micromonospora , Pseudonocardia* and *Streptomyces spp*. metabolic extracts caused the highly percentage of EAC cell death that was calculated as 70.58 , 63.3 and 61.5 %, respectively. There were only 4 genera showed no effect with EAC cells identified as *Promicromonospora, Actinoplanes, Spirillospora, ,*

Saccharopolyspora spp.

Dead EAC cells percentage = (total number of EAC cells – number of living EAC) / total number of EAC cells * 100. The Trypan blue stain was used as indicator for counting the dead cells. The living cells were transparent and the dead ones were blue.

Discussion

In Egypt, the desert habitats represent about 94% of the land and are less studied (Hozzein, 2000; Hozzein *et al.*, 2007). Because of being one of the Egyptian desert habitats, it is interested to screen the WHS actinomycetes as a new source for production of novel active compounds. The present study

highlights the ability of these actinomycetes isolated to produce bioactive compounds that have antimicrobial and antitumor effects. Soil samples were collected from ten locations in the WHS and investigated for the diversity of actinomycetes. Soil characters and analysis for the ten areas indicated a poor organic nature of the study area.

TABLE 3. Ehrlich Ascites Carcinoma (EAC) inhibition by metabolic extracts from different actinomycetes genera.

Actinomycetes isolates	% of dead EAC cells
Streptomyces (76)	61.49
Nocardoides (41)	56.7
Micromonospora (1)	70.58
Kitasatosporia (28)	57.2
Pseudonocardia (2)	63.33
Actinomadura (1)	57.13
Unknown spp. (94)	52.05
Promicromonospora (1)	----
Actinoplanes (1)	----
Spirellospora (1)	----
Saccharopolyspora (1)	----

Actinomycete counts ranged between $7x10^3$ - $47x10^3$ cfu/g. Results were in agreement with those previously reported for other sites in Saint Katherine desert where counts ranged between 5 x 10^3 - 59 x 10^3 cfu / g (Mansour, 2003), showed significant differences among the studies soil ecosystems. Rapidly growing bacteria and spreading of fungi often mask the presence of actinomycetes; this is one of the main reasons why actinomycetes isolation from the soil is difficult. Therefore, pretreatment of the sample or the applications of selective antibiotic are needed (Matsukuma *et al.*, 1994). In this study, cycloheximide was effective to inhibit fungal growth; similar result was reported by Castillo *et al*. (2007). Also, using a range of different media can increase the number and range of recovered actinomycetes (Goodfellow and Williams, 1983; McCarthy and Williams, 1992). Different isolation media were used in this study and only three media (SC, 1/10 SC and MGA) showed efficient recovery of high counts and morphologically diverse actinomycetes colonies. Starch casein medium, in particular, showed efficient recovery of soil actinomycete. Also, the low nutrient content media 1/10 SC was effective in the current study and prevented fast growing fungi and unicellular bacteria. McCarthy and Williams (1992) have described an autochthonous behavior - *i.e*. sustained growth at low nutrient concentrations - amongst actinomycetes, which may explain the successful selectivity of the low nutrient medium for actinomycetes.

Streptomyces isolates were found to represent 30.4 % of the isolates, and was recovered from all locations except Wadi El-Rutg and Gabal Al- Monagah where there were relatively high values of organic matters, nitrates, total nitrogen and low porosity. It is worth noting that Wadi El Tofaha, Wadi El-Sheikh Awad and Wadi El Sheikh showed low values of nitrates, total nitrogen and organic matters, with high count of actinomycetes and high frequency. Also, Wadi El Tofaha showed highest percentage of diversity of *Streptomyces*, *Nocardioides* and unidentified isolates, which may be a valuable finding for further investigations.

Actinomycetes are one of the predominant members of soil microbial communities and they have beneficial roles in soil nutrients cycling and agricultural productivity (Elliot and Lynch, 1995). With the exception of pasture, soil factors such as pH and salinity do not significantly affect actinomycetes population in the different soil ecosystems studied. The effect of salinity on microbial biomass and activity is not uniform and the observed differences may be due to the composition of the pool of soluble ions as well as the presence or absence of plant and agricultural activities. In this study there is a difference in the number of actinomycetes that was observed comparing with electrical conductivity differences. This observation does not support the conclusion of Chowdhury *et al*. (2011) that the combined effects of matric and osmotic potential in saline soils is more important for microbial biomass than electrical conductivity.

Actinomycetes participate in many important biochemical processes in the soil. In addition, many species have the capacity to elaborate potent antimicrobial substances. Screening for antimicrobial activity indicated a prominent antibacterial effect, worthy of further investigation for isolates belonging to 6 genera of total 12 identified genera, which were *Streptomyces*, *Nocardioides*, *Kitasatosporia*, *Micromonspora*, *Pseudonocardia*, *Actinomadura*, adding to 94 unidentified isolates. The antimicrobial activity of *Streptomyces* was previously reported in Zin *et. al*, 2011 and Abussaud *et. al*, 2013. Also the antimicrobial activity of *Nocardioides* was studied and reported by El Refai, *et. al*, 2011. The genus *Kitasatosporia* also was studied for producing antifungal compound and reported by Young Ryun Chung *et al*. (1999) which may agree with this study result; where 50% of *Kitasatosporia* isolates showed positive activity. Antitumor screening of the isolates in this study revealed the ability of 243 isolates, of 250 isolates, to inhibit Ehrlich ascites carcinoma cells (EAC cells). *Micromonospora, Pseudonocardia and Streptomyces spp*. metabolic extracts caused high percentage of EAC cell death that averaged 70.58, 63.3 and 61.5% respectively.

In conclusion, WHS soil actinomycetes represent a promising source for antimicrobial and antitumor bioactive agents. More attention should be paid to desert soils as unique habitat for actinomycetes, to verify their important metabolites.

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عزل وتعريف ودراسة النشاط الحيوى لاكتينوميستيك التربة فى منطقة التراث العالمى بمحمية سانت كانرين

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تم في هذا البحث التعرف على قدرة الأكتينوميسيتات المعزولة من التربة الصحراوية في موقع التراث العالمي بسانت كاترين على إنتاج المركبات الأيضية الفعالة بيولوجيا والتي لها تأثيرات مضادة للميكروبات ومضادة للأورام. وقد أختيرت منطقة التراث العالمي بسانت كاترين لتكون موقع الاهتمام لهذه الدراسة لما تتميزبه من طبيعة فريده من سلاسل الجبال والطبيعه المناخية المتنوعة والمصحوبة بتنوع بيولوجي ملحوظ . وتعتبر التربة في هذه المنطقه بيئة متميزة من حيث الخصائص الفيزيائية والكيميائية لها. وشملت الدراسة عدد ١٠ مواقع وهي : (وادي الدير ، وادي تفاحة ، وادي الطالعه ، وادي الشيخ ، وادي الأربعين ، وادي الشيخ عواد ، وادي الرتج ، أم قيثوم ، وادي جبال ، وجبل المناجاة).

قد أشارت خصائص وتحليل التربة لهذه المواقع إلى فقر الماده العضوية بها مع انخفاض نسبة الرطوبة نتيجه للظروف الجافه او شبه الجافه للمنطقه والتي تسمح للأكتينوميسيتات بالنمو بها . وقد تراوح عددها مابين ٧ x ١٠ ^{٣} – ٤٧ x ١٠ ^{٣} وحدة تكوين خلية/ جم.

خلال هذه الدراسة تم عزل ٣٥٩ عزلة وتعريفها بالطرق القياسية والمورفولوجية والكيميائية ، منها ٦٢,٤ % يمثلون ١١ جنس و٣٧,٦ % لم يستطاع تعريفها بتقنيات التصنيف المورفولوجي والكيميائي وربما تمثل تلك العزلات سلالات جديدة ذات أهمية لإستكمال دراستها . وتم اختيار ٢٥٠ عزلة عشوائيا لدراسة النشاط الحيوي لإنتاج مركبات أيضية لها تأثيرات مضادة للميكروبات والأورام.
تم عزل أنواع من جنس ستربتوميسيس *Streptomyces* من جميع أماكن الدراسة ماعدا وادي الرتج وجبل المناجاة ، وهذا الجنس يمثل حوالي ٣٠,٤ % من مجمل السلالات المعزولة.

وشمل الجزء التالي من الدراسة أجزاء مسح لنشاط الأكتينوميسيتات المعزولة كمصدر للمضادات الميكروبية ومضادات الأورام . وقد أوضحت النتائج أن عدد ٦

عزلات منتمية للأجناس سبيريللوسبورا *Spirellospora* وسكاروبوليسبورا *Saccharopolyspora* وأكتينوبلانس *Actinoplances* وبروميكرومونوسبورا *Promicromonospora* لم تظهر نشاط ميكروبي ضد البكتيريا العنقودية *Staphylococcus aureus* والإيشريشيا كولاي *E.coli* والكانديدا ألبيكانز *Candida albicans*. بينما النتائج الايجابية قد ظهرت واضحه مع الانواع ستربتوميسيس *Streptomyces* ونوكايدويدز *Nocardioides* وكيتاساتوسبوريا *Kitasatosporia* وأكتينومايدورا *Actinomadura* وسودونوكارديا *Pseudonocardia*.

وتم إجراء إختبار لقدرة العزلات علي إنتاج مواد مضاده للأورام على عدد ٢٥٠ عزلة بإستخدام تقنية صبغة التريبان الزرقاء Trypan blue assay قد كشفت النتائج على قدرة ٩٧,٢ % من العزلات على منع خلايا السرطان من النمو وموت ٤٠ % منها بعد ١٢٠ دقيقة. وقد تسببت المواد الفعالة المستخلصة من الانواع (*Micromonospora* و *Pseudonocardia* و *Streptomyces*) في اعلى نسبة موت للخلايا السرطانية (٧٠,٥٨ و ٦٣,٣ و ٦١,٥ % على التوالي).