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ORIGINAL PAPER

A new preferential medium for enumeration and isolation of desert actinomycetes

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Abstract In order to facilitate the discovery of novel actinomycetes from the Egyptian deserts, which can be useful as new sources for bioactive metabolites, different media for enumeration and isolation of desert actinomycetes have been tested. For this purpose, 30 soil samples from different six sites representing the Western and Eastern deserts of Egypt were collected. The two deserts are considered hyper-arid and the soil characteristics were determined. The media used were glucose–yeast extract agar, soil extract agar and a new minimal medium (MM) containing glucose, yeast extract and mineral salts. The effects of the soil characteristics on the total viable actinomycete counts on the three media were evaluated. The results showed that the highest actinomycete count in samples from five out of six sites was obtained on MM. Also MM was more selective for actinomycetes and significantly decreased the number of fungal colonies and to a lower extent the number of bacterial colonies. Moreover, it supported the development of different and diverse groups of actinomycetes. From the results obtained in this study, MM is a new useful medium for enumeration and selective isolation of actinomycetes from the desert soils.

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Introduction

One of the efficient ways of discovering novel bioactive metabolites is through isolating new microorganisms, especially actinomycetes which produce about 70% of the known bioactive metabolites (Miyadoh 1993). Therefore, investigation of new ecosystems for isolation of actinomycetes is crucial for the discovery of novel actinomycetes and subsequently for natural product-based drug discovery. Recently, several studies reported the investigation of different habitats for isolation of novel actinomycetes as rich sources of bioactive compounds (Magarvey et al. 2004; Oskay et al. 2004; You et al. 2005; Badji et al. 2006; Dolotkeldieva and Totubaeva 2006; Li and Liu 2006).

In Egypt, the desert covers approximately 94% of the total land area and it is considered a part of the Sahara-Arabian Deserts (McGinnies et al. 1968; Mares 1999). Though the plant and animal species have been reasonably studied and documented, information on microbial diversity in the Egyptian deserts still scarce. Actinomycetes from the Egyptian deserts have been studied by few scientists (Mansour and Mashaly 1986; Mansour 2003; Hozzein et al. 2004) and still of interest to explore their diversity and biological activities.

Recently, we started intensive study on actinomycete diversity in the Egyptian deserts and their antimicrobial activities to benefit from our natural resources. In the course of searching for a more effective medium for the isolation of desert actinomycetes, a new minimal medium has been developed and found to be efficient in isolation of high numbers and diverse isolates from the Egyptian desert soils.

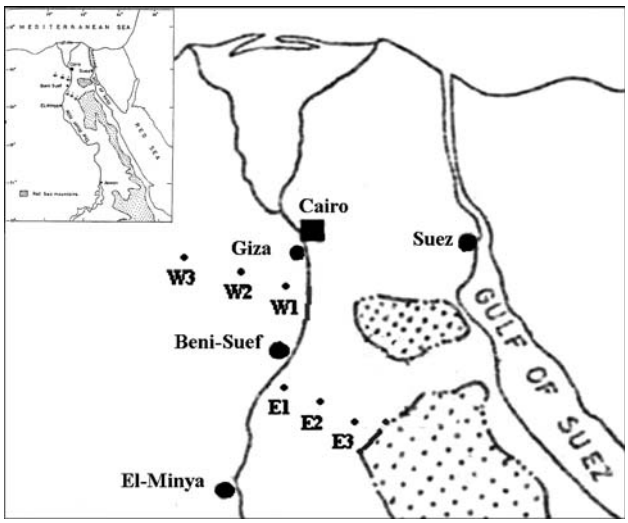

Fig. 1 A map showing the sites from which the desert soil samples were collected

Materials and methods

Sites of sampling

Three sites representing different ecosystems in both the Eastern Desert (E1, E2 and E3) and Western Desert (W1, W2 and W3) in Beni-Suef and Giza Governorates of Egypt were selected for the present study (Fig. 1). Wadi Sannur, which represents the Eastern Desert, is one of the largest drainage systems which extends east of the Nile Valley to the Red sea coast. Dahshour Desert, which representing the Western Desert, is an arid region extends from the historical area of Dahshour and Sakara westward to Farafra Oasis in the Western Desert.

Sampling and sample processing

Thirty soil samples, average of four samples from each site, were collected during summer 2005 in clean sterile plastic bags. Each soil sample was taken at a depth of 5–20 cm with a collecting spatula, mainly from the rhizosphere of the dominating plants if present. The samples were spread over sheets of paper for air drying for 7 days at room temperature, and then passed through a 2 mm sieve to remove gravel and debris. A composite of the samples from each site was made by mixing the samples thoroughly.

Characteristics of the study area

Climate

Meteorological parameters of the study areas including temperature, rainfall and relative humidity are given here as obtained from Beni-Suef and Giza weather stations.

Chemical analysis

Samples were suspended in distilled water at 1:5, shaken vigorously to ensure uniformity, and then allowed to settle down for about 10 min. Hydrogen ion concentration was measured by using a Beckman digital pH meter. Organic matter content was determined by using the wet combustion method of Walkley and Black after Tan (1996). Total carbonates were determined by the rapid titration method according to Grimshaw et al. (1989), bicarbonates by titration with standard potassium bisulfate and chlorides by titration with silver nitrate as described by Piper (1944). Total soluble salts were determined by evaporation of the soil extract and expressed as a percentage of the soil weight according to Jackson (1967).

Microbiological analysis

The soil dilution plate technique (Johnson et al. 1959) was used for this purpose. Serial dilutions of the air-dried soil samples were made by aseptically adding 1 g of soil to 9 ml of sterilized distilled water (10^{-1}), mixed by shaking and further tenfold dilutions were made down to 10^{-6}. Three different agar media were tested for their efficiency for enumeration and isolation of desert actinomycetes, namely, glucose–yeast extract (Gordon and Mihm 1962), soil extract (Atlas 1997) and a minimal medium (MM) of the following composition (g/l): glucose, 0.5; yeast extract, 0.5; K_2HPO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.5 and NaCl, 0.5. All media were supplemented with 18 g agar and MM was supplemented with 1 ml of a microelement stock solution (Labeda and Shearer 1990). Tenth milliliter aliquots of each soil dilution were spread over the surface of the isolation plates, which were then incubated for 21 days at 30°C. Three replicates were used for each dilution and the most suitable dilutions for counting were selected. The average colony count of bacteria, actinomycetes and fungi formed on a plate was calculated. Colonies were recognized by their characteristic cultural and morphological features, and sometimes after microscopic observations. Data are expressed as a colony-forming units (c.f.u./g) of soil dry weight.

Data analyses

Total actinomycete counts in correlation to soil characteristics of the studied areas were statistically evaluated by Pearson correlation coefficient (Zar 1984).

Results and discussion

In qualitative and quantitative terms, the microbial flora is governed by the surrounding ecological conditions (Alexander 1983). The primary ecological factors include the organic matter content, pH and the temperature. So, we reported here the ecological characteristics of the studied areas to show their effects on the microbial flora.

Averages of the meteorological parameters prevailing during the past 3 years in the two studied regions are given in Table 1 as obtained from the nearest weather stations. These parameters clearly revealed that both areas belong to the hyper-arid zone with mean annual rainfall less than 2 mm/year which occurred during the months November–May (winter rainfall). Both regions are characterized by a hot and almost rainless climate, especially during the summer. The absolute maximum temperature was recorded in July and reached 46.4°C (data not shown).

All the soil samples collected from the different sites were sandy and slightly alkaline. The soil characteristics are given in Table 2. Organic matter contents and percentages of bicarbonates, chlorides and total soluble salts were relatively higher in samples from Dahshour desert (W1–W3) compared to samples from Wadi Sannur (E1–E3). The results are generally in accordance with results from previous studies on the Egyptian desert soils (Mansour and Mashaly 1986; Ali et al. 2002; Hozzein 2003; Mansour 2003).

Selective isolation of soil actinomycetes is important for understanding their ecological properties and for finding novel strains which can produce useful bioactive secondary metabolites. Therefore, various media and techniques have been developed for selective isolation of actinomycetes in general, rare actinomycetes or certain genera (Hsu and Lockwood 1975; You and Park 1996; Shearer 1987; Hayakawa et al. 1991, 2000). Although, some work has been done previously on desert actinomycetes (Diab and Al Zaidan 1976; Zhadambaa et al. 2003; Takahashi and Omura 2003), no studies have reported the effectiveness of special selective media for actinomycetes from desert habitats. So, the suitability of some media for enumeration and isolation of desert actinomycetes was investigated by inoculating a series of dilutions of soils onto three different media to select the most appropriate medium for the development of actinomycetes. The media used were glucose–yeast extract (GYE), which was recommended by

Table 1 Averages of the meteorological parameters during the past 3 years in the two studied regions, Wadi-Sannur representing the Eastern Desert (E) and Dahshour desert representing the Western Desert (W), as obtained from Beni-Suef and Giza weather stations

Month	Area	Temperature (°C)			Total rainfall (mm)	Relative humidity (%)
		Mean maximum	Mean minimum	Mean		
January	E	21.0	6.5	13.75	2	56
	W	19.9	6.2	12.3	4.3	58
February	E	22.3	7.9	14.5	0.9	48
	W	21.5	6.9	14	3.5	54
March	E	25.4	9.9	17.6	0.7	41
	W	24.4	9	17.2	2.8	47
April	E	30.2	13.8	22	0.2	36
	W	28.6	12.1	20.3	1	43
May	E	33.9	17.4	25.8	0.1	35
	W	32.4	15.7	24.3	0.4	42
June	E	37	20.3	28.7	0	36
	W	34.8	18.9	27.1	0	43
July	E	39.4	22.8	31.1	0	43
	W	35.3	20.6	27.3	0	54
August	E	36.6	21.5	28.9	0	47
	W	34.7	20.7	27.4	0	56
September	E	34.9	20.2	27.4	0	47
	W	32.6	18.7	25.8	0	57
October	E	31.2	16.6	23.6	0	49
	W	30.5	16.2	23.3	2.1	58
November	E	26.5	12.9	19.7	2.5	57
	W	25.8	12.1	18.5	5.7	64
December	E	21.3	8.8	15.05	1.4	57
	W	21.3	8	14.2	3.8	60

Table 2 The chemical properties of the soil samples collected from different sites representing the Eastern Desert (E1–E3) and the Western Desert (W1–W3) of Egypt

Site	pH	Organic carbon (%)	Total CO_3^{-2} (%)	HCO_3^- (%)	Cl^- (%)	Total soluble salts (%)
E1	7.43	0.075	23	0.049	0.11	1.71
E2	7.46	0.09	32.5	0.033	0.18	1.96
E3	7.65	0.15	25.5	0.041	0.03	0.83
W1	7.45	0.40	19	0.057	2.69	8.8
W2	7.49	0.23	13	0.082	0.32	3.3
W3	7.56	0.32	15.5	0.082	0.25	2.54

many investigators to be the most suitable medium that gives a high ratio of actinomycetes and also has the advantage that it can be easily prepared (Agate and Bhat 1963), soil extract and a new minimal medium (MM). The later two media were selected as low-nutrient media which resemble the poor environments in desert soils that may reveal novel actinomycete strains.

The average numbers of microbial colonies grown on the three different media are shown in Table 3. It is obvious from the results that the highest actinomycete count in samples from all sites was obtained on MM except in site E2 where the soil agar medium gave the highest count. This might be due to the presence of actinomycetes in micro-environments in the soil; especially this site has the highest percentage of carbonates. The actinomycete count was very low in all media with samples from W1 comparing to other sites. This might be attributed to that; this site was markedly characterized with very high percentages of chlorides and total soluble salts.

Surprisingly, the lowest actinomycete counts were obtained with GYE which is one of the common used media for isolation of actinomycetes. It has been reported that microorganisms which proliferate and grow under the most extreme conditions are obligately adapted to their particular environment and fail to grow at lower intensities of the same environmental factor (Horikoshi and Grant 1991). This is may be the reason why desert actinomycetes prefer the low-nutrient media which resemble their poor environments.

The recorded results (Tables 2, 3) revealed that the total viable actinomycete counts on the three media were positively affected by the percentage of bicarbonates, the pH of the soil and the organic matter content. A higher count of actinomycetes on MM was correlated with percentage of bicarbonates ($r = 0.673$), pH of the soil ($r = 0.641$) and less correlation was found with the organic matter content ($r = 0.348$). These results are in agreement with previous studies which stated that the organic matter content and the soil pH greatly affected the actinomycete content in desert habitats (Buyanovsky et al. 1982; Skujinš 1984; Ali et al. 2002; Hozzein 2003). Variations in the percentages of carbonates and chlorides as well as total soluble salts were found to be insignificant.

The MM plates showed the following advantages when compared with the currently used media for isolation of desert actinomycetes. It was more selective for actinomycetes, as the ratios of actinomycetes to the total viable microorganisms showed its highest percentages in five out of the six studied sites on MM and it reached 60.91% in W3 (Fig. 2b). These ratios are much higher than those reported by Bhatnagar and Bhatnagar (2005) for desert actinomycetes. It is also worth mentioning that the use of MM significantly decreased the number of fungal colonies and to a lower extent the number of bacterial colonies as shown in Table 3 and Fig. 2a. Therefore, we think if an antibacterial agent to be included in this medium the selectivity for actinomycetes will increase greatly.

Table 3 Average of microbial counts (c.f.u./g of dry soil $\times 10^{-3}$) from different six sites representing the Eastern (E1–E3) and the Western (W1–W3) deserts of Egypt on the three used media

Site	GYE			Soil extract			MM		
	A[a]	B	F	A	B	F	A	B	F
E1	1.6 ± 0.02	2 ± 0.07	0.65 ± 0.01	0.85 ± 0.01	12.3 ± 1.1	0.65 ± 0.0	4.7 ± 0.3	4 ± 0.4	0.65 ± 0.01
E2	0.1 ± 0.01	0.5 ± 0.02	0.65 ± 0.04	24.6 ± 1.8	33.9 ± 2.05	1.97 ± 0.08	2.3 ± 0.1	4.3 ± 0.4	1.3 ± 0.03
E3	0.5 ± 0.03	0.2 ± 0.01	0.6 ± 0.03	4.7 ± 0.7	42.3 ± 2.3	3.1 ± 0.4	16.5 ± 1.1	15.19 ± 1.3	1.3 ± 0.01
W1	0 ± 0	0.5 ± 0.01	1.15 ± 0.02	0.125 ± 0.0	8.3 ± 1.4	0.975 ± 0.02	3.4 ± 0.3	8 ± 0.8	0.33 ± 0.01
W2	1.4 ± 0.08	4.3 ± 0.4	1.05 ± 0.06	6.97 ± 0.08	26 ± 1.9	2.7 ± 0.05	15 ± 0.09	10.09 ± 0.7	0 ± 0
W3	3.4 ± 0.2	11.6 ± 0.09	1.1 ± 0.01	22.5 ± 1.5	19.3 ± 2.1	0.675 ± 0.02	34.6 ± 2.2	22.2 ± 2.0	0 ± 0

[a] A, actinomycetes; B, bacteria; F, fungi

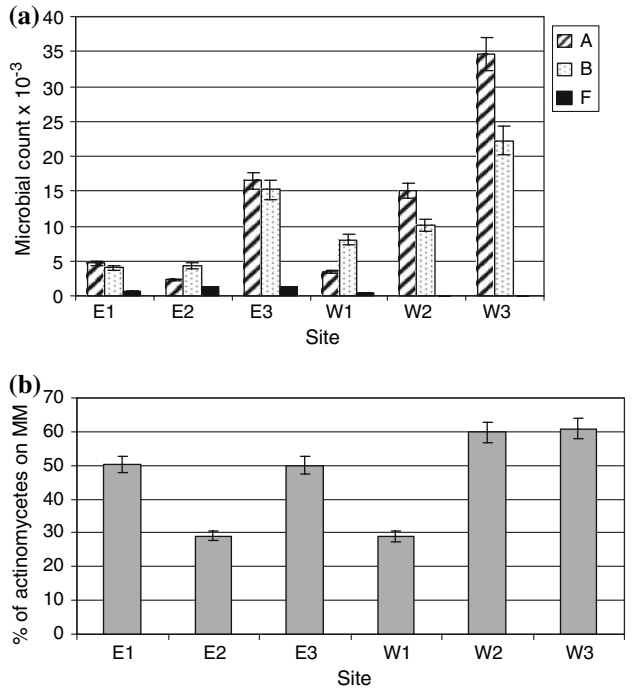

Fig. 2 (**a**) Average of microbial counts (c.f.u./g of dry soil $\times 10^{-3}$) from the six sites on MM showing its selectivity towards actinomycetes in most sites. (**b**) Ratios of actinomycetes to the total viable microorganisms appeared on MM plates showing high selectivity of the medium to actinomycetes in most samples

Also, MM produced significantly greater numbers of actinomycetes than did the other media used except in site E2. Of course there is no ideal or general medium for isolation of all groups of actinomycetes, but ratios of actinomycete counts on MM to the total actinomycete counts on the three media which reached 96.45% in W1 (Fig. 3) clearly proved that this medium is very successful for enumeration and isolation of desert actinomycetes. Moreover, it supported the isolation of different and diverse groups of actinomycetes as judged by the different morphological features and pigmentation. Actinomycete colonies developed well and formed spores abundantly on the aerial or substrate mycelia. The genera *Streptomyces*, *Nocardiopsis*, *Actinomadura*, *Amycolatopsis*, *Micromonospora* and *Nocardia* were identified on the MM plates, while, on other media only the genera *Streptomyces*, *Nocardiopsis* and *Nocardia* could be recognized. From the results mentioned above, MM is a new useful medium for enumeration and selective isolation of actinomycetes from the desert soils.

The present study also proved that the desert habitats are eminently suitable ecosystems for isolation of many novel actinomycetes which could be good source for potentially useful active metabolites and/or biotechnological applications. Therefore, much interest must be oriented to those poorly studied microorganisms. The diversity, classification

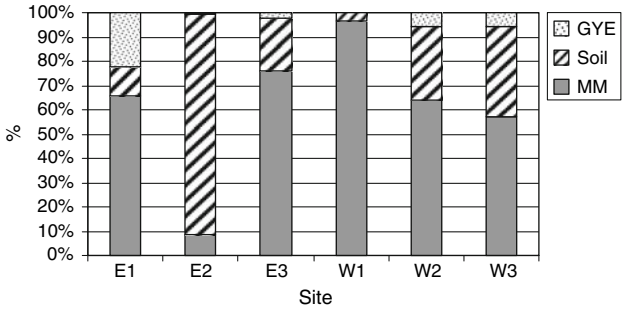

Fig. 3 Percentage of actinomycete colonies appeared on MM compared to the other two used media which clearly proved the efficiency of MM in enumeration and isolation of desert actinomycetes

and biological activities of the isolated actinomycetes are under study.

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