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The Occurrence and Distribution of Soil Actinomycetes in Saint Catherine Area, South Sinai, Egypt

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Abstract: An extensive study was carried out to investigate the occurrence and distribution of actinomycetes isolated from arid area, Saint Catherine, South Sinai, Egypt. Ten different soil samples were collected from different sites. Two hundred and eight actinomycetes cultures were isolated and identified. The existence of high population of actinomycetes, represented by total per gm soil, was significantly correlated ($P<0.01$) with organic matter and soil moisture percentages. The predominant genus in all soil samples was *Streptomyces*, which represented by 74 isolates. The second most common organism (50 isolates) was genus *Nocardia*. The other isolates were identified as *Actinomadura, Nocardiopsis, Pesudonocardia, Rhodococcus, Micromonospora* and *Streptosporangium.* Frequency of each genus was varied in each sample. *Micromonospora* isolates recorded in significantly higher count when the soil moisture increased. Genera diversity was varied which recorded the highest value in site 1 and site 8. Variation in the distribution of actinomycetes, expressed in frequency, richness and diversity of genera, indicate that climate in combination with soil properties play an important role in creation a specific niches for survival and propagation of actinomycetes.

Key words: Actinomycetes, distribution, genera- diversity, occurrence, saint catherine

Introduction

Actinomycetes have been recognized primarily on their morphological criteria. They are usually considered to be bacteria with ability to form branching hyphae at some stage of their development. They are ubiquitous in the world that surrounds us, but they seem to prefer the solid constituents of the earth: litter, humus, dung, soils and even rock surfaces. They are also found in reduced numbers in earth's water, but most of these aqueous forms, although not all are considered to be "wash-ins" from some terrestrial source. Actinomycetes are also found in air that surrounds us. They can be dispersed through the fragmentation of their hyphal biomass. These are carried by wind, water and other agencies to all part of the earth where they grow at the expense of the organic residues present in the substratum on which they land.

Actinomycetes are commonly believed to have a role in man's life, in particular for his own purpose where they can be used for the production of antibiotic, nutrient cycle, production of certain enzymes have a role in digestion of some compounds such as protein in a form of keratin, or for the transformation of steroids into other more desirable derivatives or for the production of vitamins (Lechevalier, 1981). They are thought to be of most significance in the degradation of polymers naturally occurring in plant litter and soil (Lacey, 1973; Lechevalier, 1981). There is also increasing evidence of their ability to degrade man-made compounds that may reach soil as contaminants (Lechevalier, 1981).

The distribution of actinomycetes in terrestrial environments especially those of the extreme conditions like frozen soil, hot and dry deserts of India, Africa and the Americas (Lechevalier, 1981), the petroleum- or heavy metal-polluted soils and certain highly saline environments have been studied by few scientists (Hedrick *et al.* 1968; Jordan and Lechevalier, 1975) and still of interest to explore the different genera of actinomycetes can survive and be adapted to such habitats.

In Sinai desert, which covers approximately 6% of the total land, area of Egypt is considered one of these extreme habitats. It is a part of the Sahara-Arabian deserts (McGinnies *et al.*, 1968; Danin, 1983). It is characterized by an arid to extremely arid climate with Mediterranean influences. Most of Sinai receive less than 50mm annually, however the southern part of Sinai, the average precipitation is around 65-100mm (Moustafa and Klopatek, 1995).

Under these harsh environmental conditions, the occurrence different genera of actinomycetes and their frequencies have not been reported yet. Therefore, the objective of this study is focussed to explore the different

genera that can exist in such habitats. The predominant, frequency and diversity of isolated genera in different soil sites are also considered.

Materials and Methods

Soil samples: Soil samples were collected from ten different sites located in Saint Catherine area. Fig. 1 shows the different sites (Wadi Garagnia, El-Kwiza, Wadi Tala'a, Zawateen, Wadi El-Deir and Kahf El-Gola) from which soil samples were microbiology examined. Physical and chemical properties of soil collected were analyzed and recorded in Table 1.

Isolation technique: Dilution technique was used for isolation of actinomycetes from different collected soil samples. The samples were diluted to give final concentrations 10^{-3}, 10^{-4} and 10^{-5}. The best dilution was considered in count. Isolation of actinomycetes was carried out in accordance with Standard Method (1981) using starch casein agar. Addition of antimicrobial agent to the medium was done to suppress the number of eumycetes. The antimicrobial agent used was cycloheximide at concentration 50 μg ml^{-1}. Colonies were isolated and purified by Streak Plate Technique.

Maintenance of isolates: Pure cultures were grown at 32°C on Asparagin-glucose agar (Asparagin, 0.5 g; glucose, 10g; k_2Hp0_4, 0.5; agar, 20 g; distilled H_20, 1000, pH 7.4) and C-1medium (Nonomura and Ohara, 1969). The culture were grown for two weeks and then stored at 5-10°C.

Identification of isolated cultures: For identification and classification of actinomycetes isolates the following criteria were studied: morphological, cultural and physiological characterizations.

Morphology: The morphology of actinomycetes strains was examined using slide culture technique (Bergey's Manual of determinative Bacteriology) (Williams *et al.*, 1989). After growth, the slide was taken, left in air to dry, stained with either Acid Fast stain or Gram stain and then examined under light microscope.

Culture behavior: The growth behavior of actinomycetes strains were examined at weekly intervals on different media, C1 agar, Asparagin-glucose agar, yeast extract agar (Gordon and Mihm, 1959), glucose peptone agar and Czapeks agar (Waksman, 1950). The presence of aerial mycelium, the color of aerial and substrate mycelium and formation of soluble pigments were recorded.

Physiological tests: The physiological and biochemical properties were tested for all actinomycetes isolates. The tests were carried out in duplicates. Among these are utilization of different carbon source and acid production as a result was determined following the method of Gordon (1968). A variety of carbohydrates were used, mannitol, maltose, glucose, inositol, rhamnose. Decomposition of casein, tyrosine and hydrolysis of starch were determined by the method of Gordon (1968). Nitrate reduction was determined according to the method described by Gordon (1968) and MacFaddin (1980). Gelatin hydrolysis and melanin production (Waksman, 1950) were also preformed.

Data analyses: Total count gm^{-1} soil and genera distribution in correlation to physical and chemical characterization of soil in studied areas were statistically evaluated using Pearson correlation analysis (Zar, 1984). Dominance, and frequency of actinomycetes were calculated for each site. Frequency of each genera in all sites was calculated. Richness and diversity of genera occurred were also calculated for all sites. According to Smith (1986) the following equations were used:

$$\text{Dominance} = \frac{\Sigma\, ni\,(ni-1)}{N\,(N-1)}$$

where ni = total number of certain genus.

N = Total No. of individual isolates of all genera in certain site.

$$\text{Frequency for certain genus per certain site} = \frac{\text{No. of individual of occurred genus in certain site}}{\text{Total No. of isolates of this site}} \times 100$$

$$\text{Frequency for certain genus in all sites} = \frac{\text{No. of individual of specific genus x in all sites}}{\text{Total No. of isolates of all sites}} \times 100$$

Richness = Total number of genera in certain community.

$$\text{Diversity} = \frac{S - 1}{\text{Log } n}$$

Where
S = number of genera in each site.
n = total number of all individuals isolated in all sites.

Results

Total count and genera occurred: The plate counts showed that actinomycetes occurred in high population in soil samples collected from ten dry area in Saint Catherine, South Sinai. A total of 208 actinomycetes cultures were isolated from all sites. Table 2 is illustrated the total count for each site and the distribution of genera that occurred. Site 3 recorded the highest colony count of actinomycetes per gm soil followed by site 1, 2 and 10 respectively. However, site 4 followed by site 5 recorded the lowest number of colony count per gm of soil. Higher count of actinomyceted was highly correlated (r = 0.892, P<0.01) with per cent of organic matter of the soil studies. Per cent of moisture content of soil studies was also positively correlated with actinomycetes count (r = 0.843, P<0.05).

According to morphological and physiological characteristics eight genera have been identified, *Streptomyce, Actinomadura, Nocardia, Nocardiopsis, Pesudonocardia, Rhodococcus, Micromonospora and Streptosporangium* (Fig. 6). The occurrence and distribution of these genera were recorded (Table 2).

Dominance of genera: From the isolated cultures of actinomycetes in each site, genus *Streptomyces* was represented the most dominant genus (Fig. 2). In site 2, 7 and 3 respectively, genus *Streptomyces* recorded high

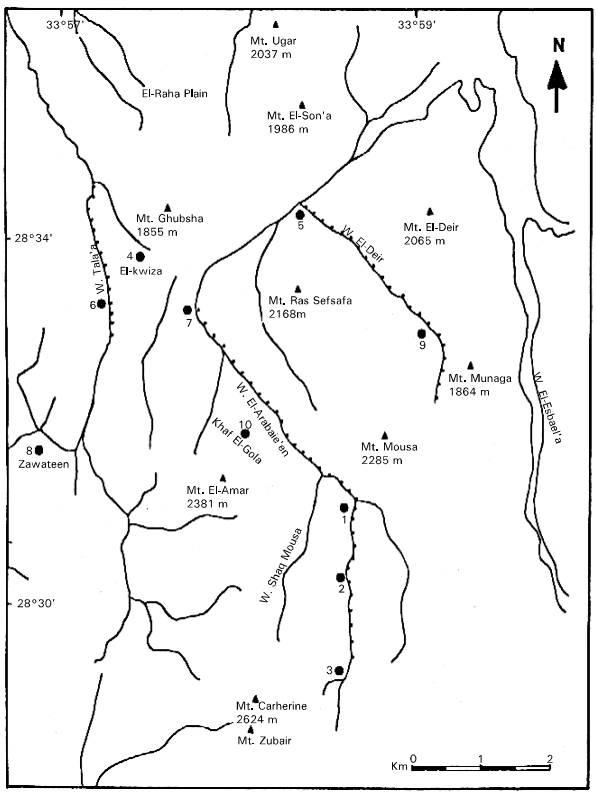

Fig. 1: Location map shows the position of ten min sites in the study area (Saint Catherine area)

Table 1: Soil characteristics

Location	pH	Nature of soil surface (%)				Moisture %	Organic matter %	EC (dSm^{-1})	Carbonate %	Salinity (ppm)
		Sand	Gravel	Cobbles	Boulders					
Wadi Garagnia	8.03	13.5	20.1	23.2	43.2	5.3	8.7	0.102	17.2	0.32
Wadi Garagnia	7.71	15.3	19.6	21.3	43.8	4.2	8.9	0.131	15.6	0.34
Wadi Garagnia	7.68	16.30	18.30	19.30	46.10	4.90	9.30	0.14	16.50	0.21
El-Kwiza	7.55	21.50	16.30	17.20	45.00	2.11	2.90	0.15	8.90	0.39
Wadi El-Deir	7.45	23.00	21.30	18.20	37.50	1.89	2.30	0.16	9.10	0.41
Wadi Tala'a	7.66	14.20	17.30	13.20	55.30	3.59	6.80	0.08	12.50	0.19
Mouth of W. El-Arabaeen	7.52	13.90	22.50	14.50	49.10	3.15	6.70	0.09	7.01	0.18
Zawateen	8.08	13.50	19.30	13.80	53.39	3.19	6.90	0.01	12.70	0.28
Wadi El-Deir	7.61	16.20	16.51	12.30	54.99	2.51	3.10	0.13	8.31	0.31
Kahf El-Gola	7.56	14.60	21.40	20.40	43.60	4.10	8.20	0.95	15.30	0.21

Table 2: Total count of actinomycetes and occurrence of genera isolated from soil samples collected from different sites

Location	Total Actinomycetes count gm^{-1} soil	Genera isolated							
		Streptomyces	*Actinomadura*	*Nocardia*	*Nocardiopsis*	*Pesudonocaria*	*Rhodococcus*	*Micromonospora*	*Streptosporangium*
Wadi Gargnia	48000	+	+	+	+	-	+	+	+
Wadi Gargnia	43600	+	+	-	-	-	-	+	-
Wadi Gargnia	59000	+	+	+	-	-	+	+	-
El-Kwiza	6840	+	+	+	-	-	-	-	-
Wadi El-Dier	5866	+	+	+	-	-	-	+	-
Wadi El-Talaa	26800	+	-	+	+	-	-	-	-
Mouth of W.El-Arabaeen	24000	+	+	-	-	-	-	-	-
Zawateen	27520	+	+	+	+	-	+	+	-
Wadi El-Dier	9520	+	+	+	+	+	-	-	-
Kahf El-Gola	35820	+	-	+	-	-	-	+	-

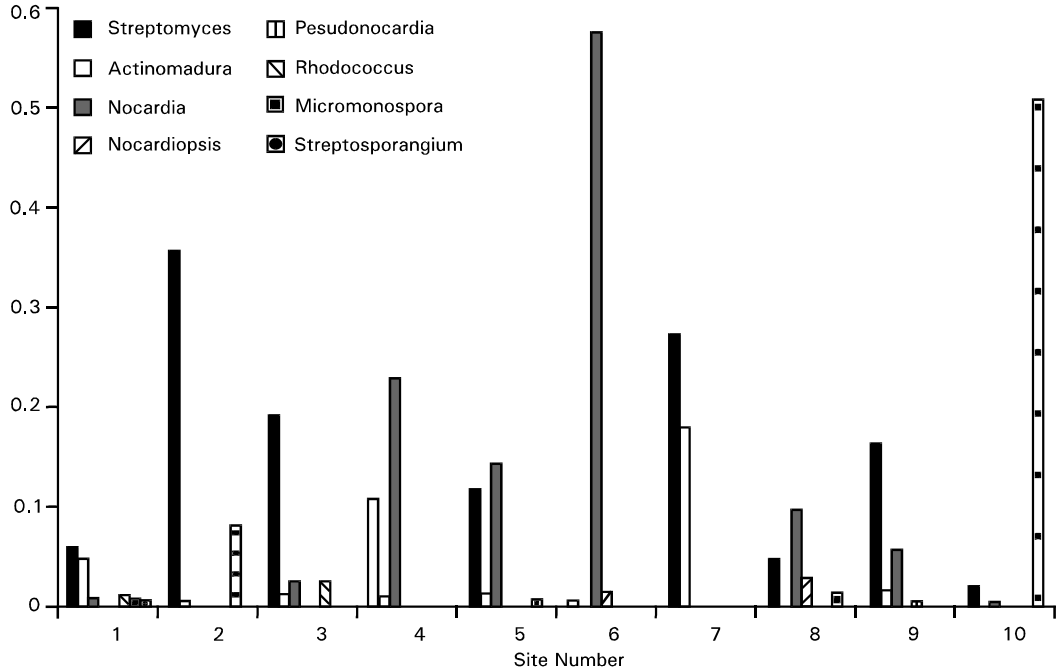

Fig. 2: The dominance of studied genera in the ten sites in Saint Catherine area, South Sinai

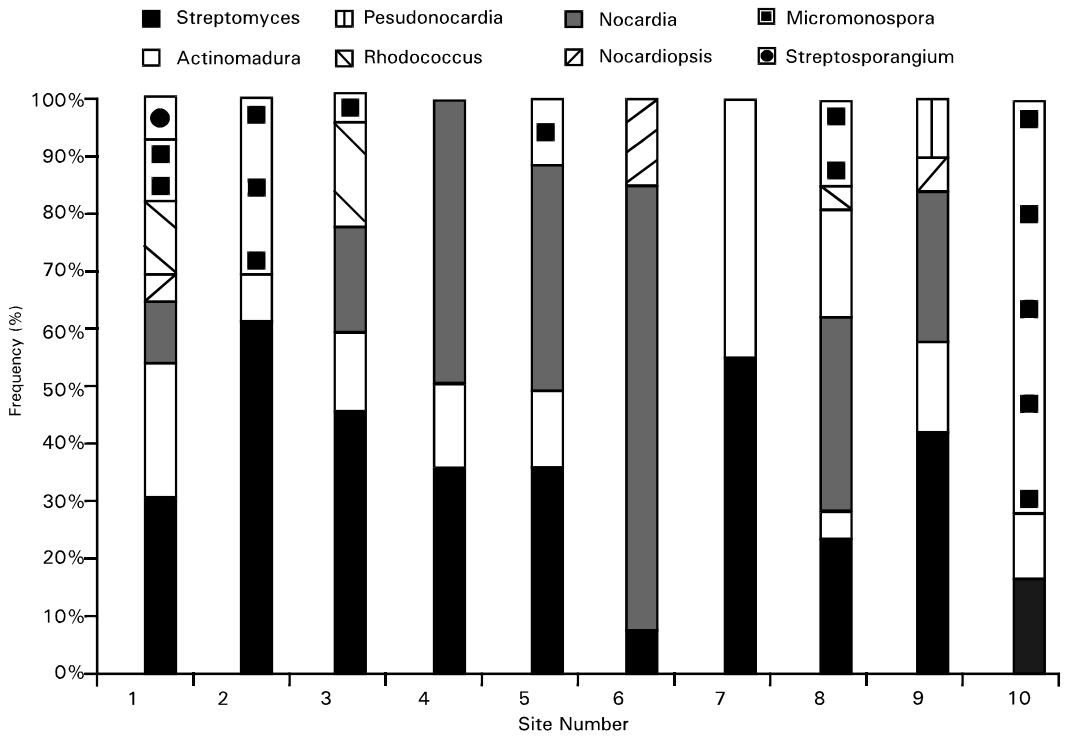

Fig. 3: The Frequecy of seven main genera throughout ten studied sties in Saint Catherine area, South Sinai

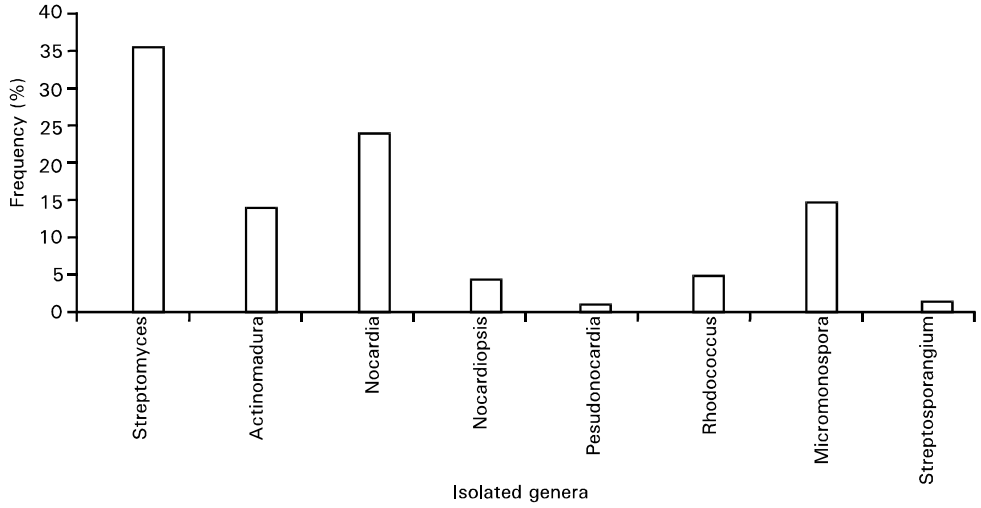

Fig. 4: The frequency of each genus throughout the whole study area

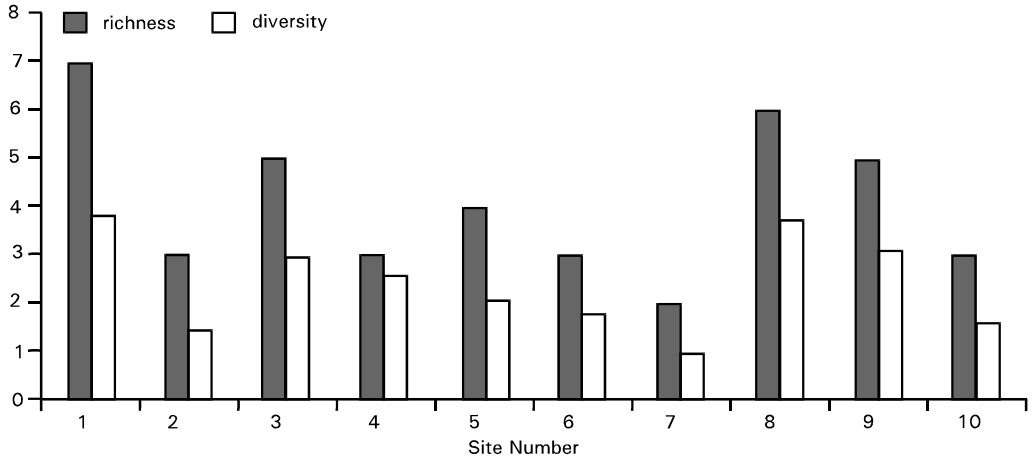

Fig. 5: The richness and diversity of common genera throughout the studies sites in Saint Saint Catherine, South Sinai

dominant values. However, genus *Nocardia* was the highest in its dominant value in sites 6 which recorded 0.15. Although, *Micromonospora* was the dominant genus in site 10 (Kahf El-Gola) where moisture % is high, it also recorded with less dominant value in site 5 (Wadi El-Deir) which characterized by low moisture % (Table 1, Fig. 2). No correlation between dominant value of any genus recorded and soil pH.

Frequency: Frequencies of identified genera of actinomycetes cultures, in different sites, were fluctuated. The frequency of genus *Streptomyces* was varied from 7.69 to 60.87% (Fig. 3). Genus *Actinomadura* recorded a range of frequency from 4.76 to 45.45%. Genus *Nocardia* recorded the highest value of frequency in site 6 (76.92%, Fig. 3). Genus *Micromonospora* was prevalent in many sites and site 10 recorded the highest frequency percentage (72.22%, Fig. 3). However, the other genera isolated recorded low frequency per cent, which ranged from 0 to 18.18%).

Frequencies of the different identified genera in all samples collected from the studied area indicate that *Streptomyces* was the most flexible genus which recorded the highest value (Fig. 4). It was represented by 74 isolates out of a total 208 actinomycetes cultures were grown from samples. However, genus *Nocardia* and genus *Micromonospore* were represented by 50 and 31 isolates respectively, for all sites and their frequencies were in sequence after *Strptomyces*.

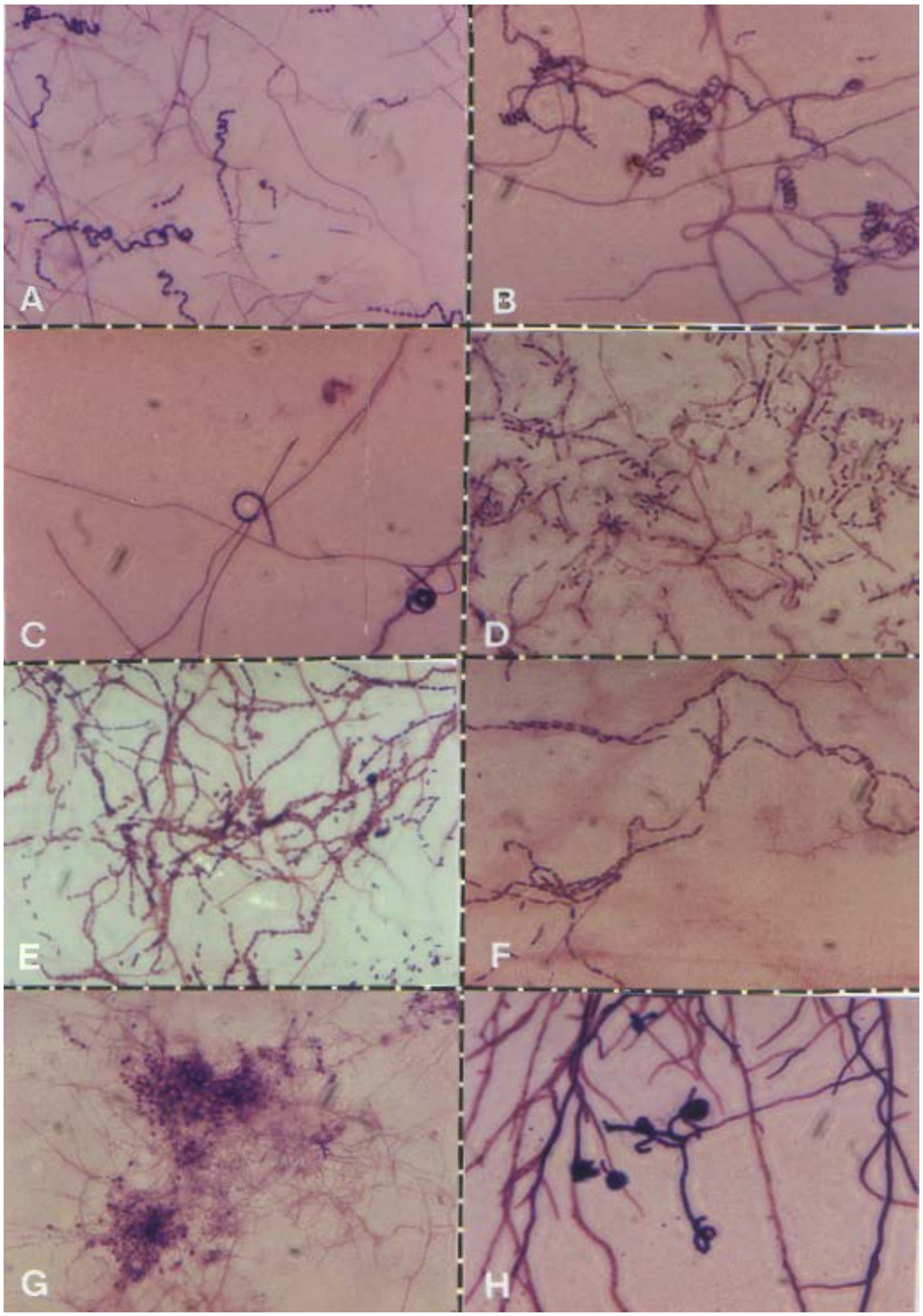

Fig. 6: Micromorphology of different genera isolated from soil samples of the study area, A and B illustrate the long chain of spores of genus *Streptomyces,* C is aerial hyphae bearing short chain of spores, in hook, of genus *Actinomadura*. D prepresents fragmented hyphae (substrate and aerial) of genus *Nocardia*. E represents the hyphae of genus *Nocardiopsis* which is showing areial mycelium totally sporulated. F shows budding and zig-zag-shaped hyphae of genus *Pesudonocardia.* G is substrate mycelium of genus *Micromonospora* bearing single spores in clusters. H illustrates aerial hyphae bearing globose sporangia of genus *Streptosporangium.* All have the same magnification (X 650)

Genera *Pesudonocardia* and *Streptosporangium* recorded the lowest frequency per cent (0.96, 1.44% respectively). They were represented by 3 and 2 isolates and their frequencies did not correlate with soil properties (Table 1).

Richness and diversity: Richness and diversity of genera, isolated from collected samples, gave an idea about the distribution of actinomycetes allover the study area. Site 1 recorded the highest value for genera richness, however, site 7 was the lowest in its value. Genera diversity was found to be proportionate with genera richness (Fig. 5). Sites 1 and 8 recorded the highest value for genera diversity followed by site 9. Site 7 was less diverse in genera occurred. Although all except site 9, values of richness and diversity were increased with the increase of carbonate % of soil samples, no significant correlation between them was detected. Other soil properties were not affecting the recorded values of both richness and diversity of genera in all studied samples.

Discussion

The results described in this paper are based on isolation of actinomycetes from soil collected from arid area located in Saint Catherine, South Sinai, Egypt. This is the first attempt to explore the different genera of actinomycetes that can inhabit such habitat of Egyptian origin.

The study revealed that actinomycetes are occurred in high population especially in sites with high percentage of moisture and organic matter. As also indicated by Tables 1 and 2, that slightly alkaline soil was favorable for actinomycetes to be survived and propagated. Two hundred and eight actinomycetes cultures were isolated and propagated from ten different sites which cover 21.5% of Saint Catherine area. The list of genera occurred and their distribution and frequency must be regarded as tentative, because any isolation procedure used is to some extent selective, especially those genera referred as rare genera. However, specific niches, where conditions for growth are favorable, are in need for certain genera of actinomycetes.

Among of the genera occurred, in this study, is genus *Streptomyces* which is the most prevalent in compared to others. This conclusion is in agreement with all studies that concerning actinomycetes isolation especially from soil, as a material for isolation (Lechevalier 1981, Balagurunathan *et al.*, 1996; Xu *et al.*, 1996; Zhang, 1997). Apart from *Streptomyces*, the genera most frequently appearing on medium used for isolation were *Actinomadura, Nocardia, Micromonospora and Rhodococus*. Occurrence of *Micromonospora,* especially in sites with high frequency values, was highly correlated with moisture content of soil from which it was isolated. This conclusion is in confirmation with the results reported by Williams and Wellington (1982) and Hatano (1997). Absence of occurrence of rare genera, that can inhabit such dry condition, among the identified genera in this study area, doesn't mean that they don't exist but they need more extensive study for using different specific and selective media suitable for their isolation.

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