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Spores of thermophilic actinomycetes in the atmosphere of Kuwait associated with allergic diseases

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ABSTRACT

The distribution of thermophilic actinomycetes in the atmosphere of Kuwait was studied during the year 1979. The concentration of these organisms per cubic metre was higher in the crowded commercial district than in the quieter residential one. Maximum counts were obtained during September, especially on nutrient agar–sea water medium. Higher counts were also recorded during April.

Five genera of thermophilic actinomycetes were recorded in the atmosphere of Kuwait: *Thermoactinomyces*, *Thermomonospora*, *Pseudonocardia*, *Streptomyces* and *Actinobifida*.

Thermoactinomyces vulgaris was dominant, being followed by *Thermomonospora viridis*. The *Streptomyces* isolates were characterised and classified into seven groups according to their morphology and carbon utilization pattern. Twelve *Streptomyces* isolates could be identified as *S. diastaticus* (7 strains), *S. galbus* (4 strains) and *S. griseoruber* (1 strain).

INTRODUCTION

Little is known about the distribution of bacteria, especially actinomycetes, in the outdoor atmosphere. Most studies on aerial dispersion of actinomycetes have been concerned with the investigation of respiratory diseases associated with the inhalation of spores, for example farmer's lung disease and bagassosis (Pepys *et al.* 1963; Sakula 1976; Lacey 1971).

Actinomycete spores ranging from 0·5 to 1·5 μm diameter are able to penetrate deeply into the lung. Hatch (1961) estimated that up to 50% of spores in this size range will be deposited in the alveolar spaces, and stated that a man doing light work will inhale about 10 l/min, so that about 750,000 spores will be deposited in the lung space per minute.

Gregory & Lacey (1963) found that when actinomycetes grow on hay and then sporulate, the spores are liberated into the air mainly as individuals. Lloyed (1969) explained that aerial dispersal of actinomycete spores from soil is different because actinomycetes within the soil grow vegetatively for a short period, sporulate and then the vegetative mycelium disappears, leaving behind localised regions of high spore concentrations. Any action which raises soil particles from the soil surface will therefore launch actinomycete spores adsorbed to dust particles into the air.

Thermophilic actinomycetes associated with allergic pneumonitis may be found in compost, soil or hay and when dispersed and inhaled may cause diseases.

The State of Kuwait occupies the northeastern part of the Arabian Peninsula. Kuwait is a tropical area, being hot in summer with a shade temperature above 40°C. In winter the weather is temperate with an average maximum temperature of about 19°C.

Hypersensitivity and allergic complaints have been repeatedly encountered in the State of Kuwait especially during March, April, September and October. Investigations of thermophilic actinomycetes in the atmosphere of Kuwait are lacking. The aim of this work is to study the numbers and groups of thermophilic actinomycetes in the atmosphere of Kuwait and to see whether a relationship exists between their occurrence and the outbreaks of allergic diseases.

MATERIALS AND METHODS

Air was sampled at weekly intervals during the year 1979 from January to December by the Casella Slit Sampler as described by Diab *et al.* (1976) at a height of 150 cm above ground between 9 and 10 a.m. from Mubarakiya and Sulaibikhat. The first locality is a crowded commercial area in the centre of Kuwait City while the second locality is a residential area at the west end of Kuwait City and mostly surrounded by desert.

After sampling, the plates were incubated at 50°C for 1–3 days and then counted. Nutrient agar (Oxoid), nutrient agar–sea water (nutrient agar dissolved in sea water) and Bunt and Rovira agar medium (Louw & Webley 1959) were used. Colonies of actinomycetes that appeared on plates of nutrient agar–sea water with maximum counts were isolated and purified on the same medium. Identification of the actinomycete isolates was carried out according to Bergey (1974) and Sykes & Skinner (1973).

Total cell hydrolysate analysis for the detection of diaminopimelic acid (M or L) and sugar patterns was carried out as described by Lechevalier & Lechevalier (1970). The morphology of the actinomycetes was studied using the cover slip culture technique and the methods of the ISP (Shirling & Gottlieb 1966). Photo- and electron-micrographs were taken as described by Diab *et al.* (1976).

For the classification within the genus *Streptomyces* the methods of the ISP (Shirling & Gottlieb 1966) were used. The keys of Küster (1972), Bergey (1974), Nonomura (1974) and Szabo *et al.* (1975), together with the descriptions of *Streptomyces* species of the ISP, were used.

RESULTS AND DISCUSSION

Results of the counts of total thermophilic bacteria and actinomycetes per cubic metre per hour are given in Table 1. From the table it can be seen that the counts of these organisms were much higher in the atmosphere of the crowded commercial district (Mubarakiya) than in the quieter residential one (Sulaibikhat). Working on mesophilic bacteria and actinomycetes, Diab *et al.* (1976, 1977) found similar results. They suggested that these may be due to various activities in the crowded area.

The nutrient agar supplemented by sea water gave the highest counts of thermophilic bacteria and actinomycetes in both localities. This medium was also the

Table 1. Total viable counts of thermophilic bacteria (TB) and thermophilic actinomycetes (A) × $10^3/m^3/hr$ in the atmosphere of two localities: Mubarakiya and Suliabikhat. Media used: NAY = nutrient agar yeast, SW = nutrient agar sea water and BR = Bunt and Rovira agar medium

		Mubarakiya			Suliabikhat		
Month		NAY	SW	BR	NAY	SW	BR
January	TB	424±24	608±29	354±31	71±12	90±6	73±10
	A		33±6				
February	TB	307±18	292±25	196±17	71±9	94±13	75±7
	A	33±6	59±6	17±6	13±4	9±3	
March	TB	408±30	708±43	387±28	48±7	71±5	25±4
	A	46±6	84±6	25±5	8±3	17±3	
April	TB	931±28	1183±68	566±56	111±14	123±15	50±4
	A	75±5	154±6	25±5	13±3	29±3	
May	TB	204±15	242±12	150±17	25±3	31±2	23±3
	A	29±4	63±4	17±6		2±2	2±2
June	TB	261±15	375±32	229±19	29±3	38±3	27±3
	A	17±6	67±7	17±6			
July	TB	225±10	466±23	217±12	27±3	31±2	23±3
	A	17±6	17±6				
August	TB	496±8	675±14	304±12	131±4	127±4	87±8
	A	58±5	79±6	8±5	15±2	25±3	6±3
September	TB	975±47	1016±64	471±10	231±17	344±12	84±8
	A	46±6	225±10	25±5	29±5	71±5	13±2
October	TB	241±14	346±15	254±27	73±8	108±6	27±3
	A	29±4	37±4	25±4	4±2	11±3	4±2
November	TB	534±77	600±61	362±64	75±7	94±5	56±6
	A	25±5	54±6	33±6	11±3	8±3	8±3
December	TB	442±45	512±35	400±57	58±5	81±6	42±7
	A	29±4	25±5	12±6			

best for counting thermophilic bacteria and actinomycetes in the soils of Kuwait (Diab 1978).

The month with the highest number of air spores was September in both localities, followed by April. High counts were also recorded during March and August. Diab *et al.* (1977) found that unlike the thermophilic actinomycetes the mesophilic actinomycetes of Kuwait had their highest air spore concentration during January and February.

The results in Table 1 indicate that thermophilic actinomycetes were distributed in the atmosphere of the crowded locality throughout the year of study. On the other hand, in Sulaibikhat, these organisms were not recorded during June–July (hot and dry) and December–January (wet and cold). During these months, lower counts were recorded in Mubarakiya. The lower counts in June–July may be due to the effect of desiccation. Similar results were obtained by Well & Zopposont (1942), Webb (1959) and Diab *et al.* (1976, 1977). On the other hand the depression observed during the cold

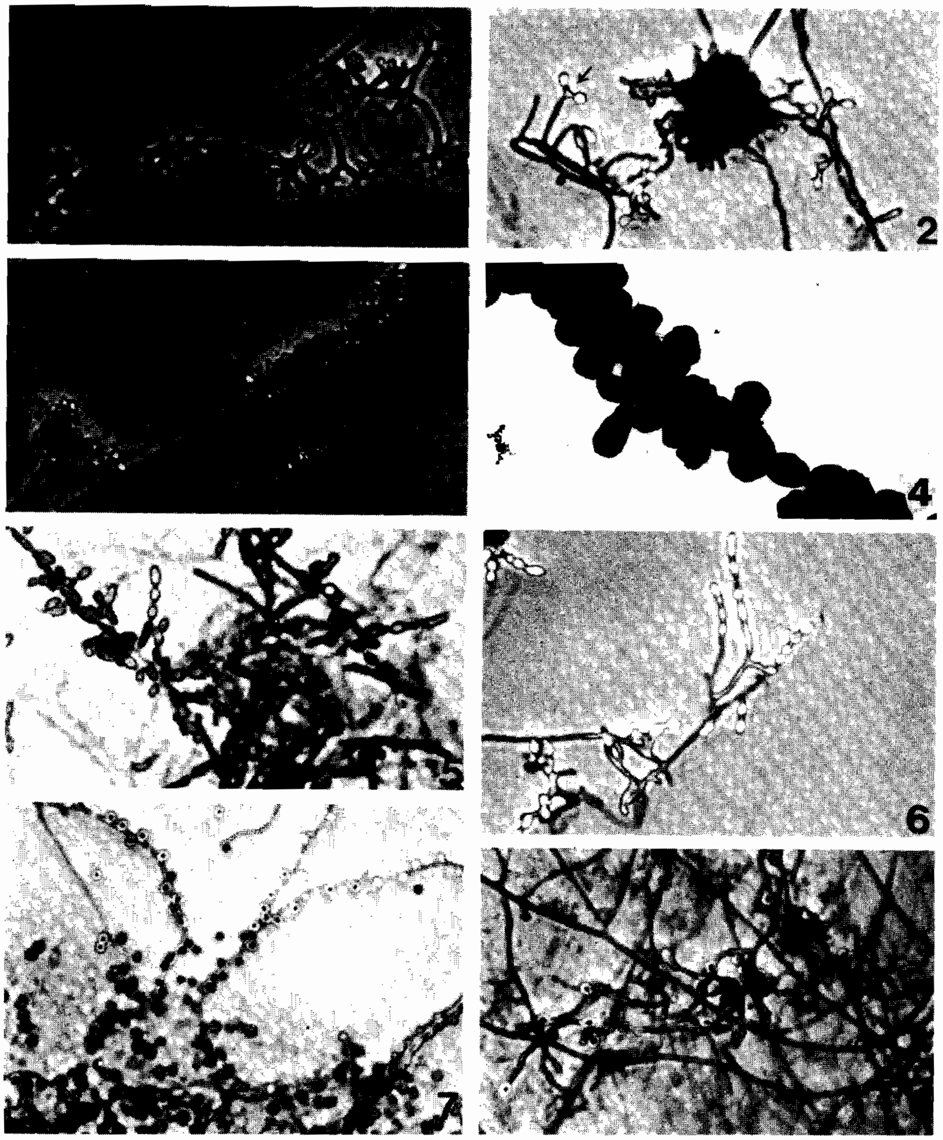

Plate I

Fig. 1. *Actinobifida*: photo-micrograph showing the characteristic dichotomous branching (× 1600). **Fig. 2.** *Actinobifida*: photo-micrograph showing heart-shaped terminal swellings. **Fig. 3.** *Thermomonospora*: photo-micrograph showing spike-like arrangement of spores (× 1600). **Fig. 4.** *Thermomonospora*: electron-micrograph showing spike-like arrangement of spores (× 6400). **Figs 5 and 6.** *Pseudonocardia*: photo-micrographs showing fragmentation of mycelia and acropetal succession of spores (× 1600). **Fig. 7.** *Thermoactinomyces vulgaris*: photo-micrograph showing endospores formed both on the aerial and substrate mycelia (× 1600). **Fig. 8.** *Thermoactinomyces* sp.: photo-micrograph showing terminal endospores and endospores formed on long stalks (× 1600).

season may be due to the slow propagation at low temperature. Allen (1953) reported that the spores of thermophilic actinomycetes could be killed by prolonged exposure to cold.

A total of 242 isolates of thermophilic actinomycetes were obtained during the year of study. Most of these (199) were from the crowded locality, Mubarakiya.

Results of the analysis of total cell hydrolysate for the presence of diaminopimelic acid (DAP) and sugar pattern indicated that 35 isolates out of the 242 contained L-DAP in their total cell hydrolysate. These strains produced long chains of spores which in most cases were arranged in spirals of different forms. According to Lechevalier & Lechevalier (1970) these organisms are grouped among the genus *Streptomyces*. This genus, as stated by Lechevalier, has no characteristic sugar pattern.

The other 207 isolates all contained the meso form of DAP in their total cell hydrolysate. According to their sugar pattern and the morphological characteristics, they can be put into two groups.

Group I. This group is represented by 98 isolates. They are characterised by the presence of M-DAP and the absence of arabinose, galactose, xylose or madurose in their hydrolysates (cell wall type III and C-sugar pattern). The morphological characteristics divide this group into two subgroups. One subgroup (94 isolates) is characterised by the formation of single endospores on both the aerial and substrate mycelium. Spores are sessile or stalked. Following Bergey (1974) and Lechevalier & Lechevalier (1970), these organisms were identified as members of the genus *Thermoactinomyces*. The other group was represented by only four isolates which were obtained during August from the atmosphere of the crowded locality. These four isolates produce single endospores on both substrate and aerial mycelium. The hyphae show characteristic dichotomous branching. The sporophores are dichotomously branched (Plate 1, Fig. 1). The young apical region of the sporophore exhibits heart-shaped terminal swellings (Plate 1, Fig. 2, arrow). This group can be ascribed to the genus *Actinobifida*.

Group II. This group is represented by 109 isolates. They have the A-sugar pattern (presence of arabinose and galactose, but no xylose and madurose). According to their morphology they can be divided into two subgroups: the first (71 isolates) is characterised by the formation of single spores only on the aerial mycelium. All of these organisms form greyish-green aerial mycelium; most of them produce dark green vegetative mycelium and dark green soluble pigments. The spores in most of them are produced as spike-like structures (Plate 1, Figs 3 and 4). Accordingly these organisms may be identified as strains of *Thermomonospora viridis*. The isolates of the second subgroup of A-sugar pattern (38 isolates) are probably strains of *Pseudonocardia thermophila*. They show evidence of acropetal succession of spores (Plate 1, Figs 5 and 6) and fragmentation of mycelia into bacillary elements. Their growth was orange-yellow and they did not hydrolyse starch.

The above results indicate the occurrence of five genera of thermophilic actinomycetes in the atmosphere of Kuwait: *Thermoactinomyces*, *Thermomonospora*, *Pseudonocardia*, *Streptomyces* and *Actinobifida*. The distribution of these genera during the period of study (Table 2) shows that the genus *Thermoactinomyces* was most numerous in the atmosphere of Kuwait, followed by the genus *Thermomonospora*. *Thermoactinomyces* was dominant during March and April, i.e. during the season in

Table 2. Distribution of the different genera of thermophilic actinomycetes in the atmosphere of Kuwait during the year 1979 in two localities: Mubarakiya (R) and Suliabikhat (K)

Genera	Locality	February	March	April	May	June	July	August	September	October	November	December	Total
Thermoactinomyces	R	5	18	16	3	0	3	6	6	7	9	4	77
	K	2	3	2	1	0	0	4	0	3	2	0	17
Thermomonospora	R	5	3	6	7	8	1	1	19	3	1	2	56
	K	2	5	0	0	0	0	1	2	4	1	0	15
Pseudonocardia	R	3	6	2	5	9	0	0	0	1	4	3	33
	K	0	1	0	0	0	0	0	0	0	4	0	5
Streptomyces	R	3	5	1	2	1	5	4	6	2	0	0	29
	K	0	1	0	0	0	0	5	0	0	0	0	6
Actinobifida	R	0	0	0	0	0	0	4	0	0	0	0	4
	K	0	0	0	0	0	0	0	0	0	0	0	—
Totals	R	16	32	25	17	18	9	15	31	13	14	9	199
	K	4	10	2	1	0	0	10	2	7	7	0	43

which some people in Kuwait suffer from allergy and other respiratory troubles. Diab *et al.* (1977) found that during March and April, the genus *Micropolyspora* was of higher occurrence in the atmosphere of the crowded locality, Mubarakiya. The above results show that thermophilic actinomycetes which cause respiratory troubles were obtained from the crowded commercial locality, i.e. this locality and similar other crowded commercial places may serve as reservoirs supporting these organisms which usually proliferate in decaying organic materials. Thus any action which raises soil particles from the surface will launch actinomycete spores adsorbed on dust particles (Lloyed 1969). Gregory & Lacey (1963) stated that the spores of actinomycetes growing on hay are liberated mainly as individuals. So, persons in the crowded locality are likely to be subjected to higher spore concentrations than in the quieter residential area of Sulaibikhat. Diab (1978) found no thermophilic actinomycetes in Kuwait desert soil, but the isolates of thermophilic actinomycetes he found in certain cultivated soils of Kuwait were all strains of *Thermoactinomyces vulgaris*. So, the absence or low concentrations of thermophilic actinomycetes in Sulaibikhat may be due to the fact that this locality is mostly surrounded by desert, and the allergic complaints observed in persons of this locality are probably due to factors other than thermophilic actinomycetes, for example pollen grains. Further investigations must be conducted on patients with exposure to thermophilic actinomycetes.

Gregory (1973) reported that *Streptomyces* species were abundant in mouldy hay, but only *Micropolyspora faeni* and *Thermoactinomyces vulgaris* were involved in farmer's lung disease and of these species the former is potent. Accordingly, the lower occurrence of *T. vulgaris* in August and September does not mean that these strains are not involved in allergic diseases.

All except three of the isolates of *Thermoactinomyces* were identified as *T. vulgaris*. They produce single spores, most of which are sessile and few are short stalked (Plate 1,

Table 3. Classification of the different *Streptomyces* spp. according to the carbon source which they failed to utilize (R = Mubarakiya, K = Suliabikhat)

Carbon source not utilized	Melanin	Spore surface	*Streptomyces* spp. No., place and month of isolation
I. Raffinose	+	Spiny	304 R9, 326 R9, 332 R9, 335 R9, 336 R9, 338 R9 and 340 R9
II. Sucrose, raffinose and rhamnose	+	Warty	106 R3
	—	Smooth to warty	53 R2, 58 R2, 60 R2, 83 K3, 104 R3, 118 R3, 88 R3 and 124 R3
III. Sucrose, raffinose and mannitol	+	Spiny	223 R7
	+	Smooth to warty	222 R7
		Warty	232 K8, 233 K8, 235 K8, 237 K8 and 238 K8
IV. Sucrose, raffinose and arabinose	+	Smooth	250 R8, 253 R8, 255 R8 and 256 R8
V. Sucrose, raffinose, rhamnose and mannitol	—	Warty	136 R4, 183 R5, 193 R5 and 295 R6
VI. Sucrose, raffinose, mannitol and inositol	+	Smooth to warty	322 R9
VII. Sucrose, raffinose fructose and mannitol	+	Smooth	227 R7, 228 R7 and 229 R7

Fig. 7) and their aerial mycelium is white to creamy. They showed good growth on nutrient agar. The reverse side of the growth was yellow-brown without soluble pigments. Their growth was good at 60°C. The physiological and biochemical characters were variable. The other three strains produced most of their spores on long stalks or apically on long branches (Plate 1, Fig. 8). Their aerial mycelium is white and well developed on nutrient agar. Morphologically, they differ from *T. vulgaris* and *T. sacchari*. They may be new species, possibly related to *T. candidus*. They require further study.

All 35 *Streptomyces* isolates were able to grow at 30–50°C. They belonged to the Gray series and section Spirales. According to their carbon substrate utilisation pattern these *Streptomyces* species can be divided into seven groups (Table 3). Out of the 35 isolated strains only 12 were identified as: *Streptomyces diastaticus* (7 strains of group I), *S. griseoruber* (1 strain from group II) and *S. galbus* (4 strains of group IV).

Further studies will be made on the unidentified *Streptomyces* species to confirm their taxonomic position.

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جراثيم الأكتينوميسيتات المحبة للحرارة في هواء الكويت وعلاقتها بأمراض الحساسية

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خلاصة

لقد درس توزيع بكتريا الأكتينوميسيت المحبة للحرارة في هواء الكويت وذلك في المدة من يناير إلى ديسمبر ١٩٧٩ في منطقتين مختلفتين في مدينة الكويت ، الأولى منطقة تجارية مزدحمة هي منطقة المباركية والثانية تمثل منطقة سكنية هادئة هي منطقة الصلبيخات .

وقد وجد ان تركيز هذه الكائنات في المتر المكعب من الهواء أعلى بكثير في المنطقة المزدحمة منه في المنطقة السكنية الهادئة ، وقد عزى سبب ذلك إلى عدة عوامل مختلفة ، وأظهرت النتائج أيضا ان هواء الكويت في الأماكن المزدحمة وخاصة في شهري مارس وأبريل تكثر فيه الأكتينوميسيتات المحبة للحرارة والتي عرف عنها في أماكن أخرى من العالم انها تسبب بعض أمراض الحساسية في الجهاز التنفسي للانسان ، ولذلك فقد أوصى هذا البحث بقيام بحوث أخرى خاصة بالمرضى الذين يصابون بأمراض الحساسية في هذه الشهور من السنة .

وفي أثناء هذه الدراسة تم عزل وتعريف ٢٤٢ عزلة من الأكتينوميسيتات المحبة للحرارة ووجد أنها تضم خمسة أجناس مختلفة .