Arab Gulf J. Scient. Res., **8** (3), pp. 121-135 (1990)

Existence of Soil Microflora Producing Amylases and Proteases in Eastern Region of Saudi Arabia

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ABSTRACT. Soil samples were collected from different location in Eastern region of Saudi Arabia in an attempt to isolate protease and amylase producing microorganisms. The soil dilution plate method was used for the isolation of microorganisms. Modified glucose yeast extract agar medium supplemented with gealtine (GYACe) and starch (GYAS) was used.

Thermophilic and mesophilic bacteria and mesophilic fungi producing protease enzymes were isolated in all location. No thermophilic actinomycetes and fungi producing the enzyme were recovered in any of the localities. The same pattern was observed in case of recoveries of amylase producing microorganisms. Species of thermophilic actinomycetes were recovered from soil of Dammam, Qatif and Safwa. Thermophilic fungi were also recovered from three localities (Dammam, Sayhat and Thoqbah). No thermophilic microflora producing protease and amylase enzymes were obtained from Abqaiq and Jubail soils.

Among the counts of protease producing organisms, *Bacillus* sp 2 was dominant in 9 localities. It was absent from Abquiq soil. *Streptomyces* sp 2 was found in Dhahran, Dammam and Naieria localities. *Penicillium* sp 1 was commonly found in Abquiq, Jubail, Hassa, Naieria, Qatif, Sayhat and Thoqbah localities.

Bacillus sp 2 as amylase producing was found in 8 localities. *Streptomyces* sp 1 and sp 2 were found from the same five localities (Abquiq, Dammam, Dhahran, Naieria, and Qatif). *Penicillium* sp 1, *Aspergillus* sp 2 and Mycelia Sterilia were recorded in four different localities for each.

Many investigations have been carried out of the existence and distribution of soil microflora in Saudi Arabia (Elwan and El-Naggar 1969 & 1972, Elwan *et al.* 1969, Elwan and Diab 1970a, 1970c and 1971, Abuzinada and El-Husseiny 1975, 1977, Abou-Heilah *et al.* 1982, Ali and Abou Heilah 1984 and Salam *et al.* 1975).

Little information is available about the microflora producing extracellular enzymes, in Eastern Region of K.S.A. especially thermophilic microflora. In the present study an attempt was made to isolate amylase and protease producing microorganisms at 30°C and 55°C from different soils collected from the Eastern Region.

Materials and Methods

Collection of soil samples: Soil samples were collected from ten different locations of Eastern Region (Fig. 1). The samples were collected from the upper layer at the depth of 5-10 cm at all localities by using sterile shovels, which were then put in sterile bags and labelled.

Analysis of soil: Soil samples were analysed for total soluble salts, organic matter content, and pH according to the methods adopted by Black (1965).

Microbial analysis: The soil dilution plate method of Johnson *et al.* (1959) was used. Modified glucose peptone yeast extract agar medium supplemented with gelatine (GPYA-Ge) or starch (GPYA-S) were used respectively for the recovery of protease and amylase producing microorganisms.

Recoveries of Bacteria and Actinomycetes:

i) Proteases producing microorganisms:

Three replicates containing GPYA-Ge medium were used for each sample. One ml portion of the proper dilution was plated. Dishes were incubated at 30 or 55°C for 48 hours; plates of developed growth were then flooded with mercuric chloride solution; the proteolytic activity of the recovered organisms was detected by observing the clear zone around bacterial growth due to the lysis of gelatine.

ii) Amylolytic producing organisms:

The previous procedure was followed using instead GPYA-S. Plates of developed growth were then flooded with iodine solution.

iii) Identification of bacteria and actinomyces:

Genera and species of microorganisms were identified according to Bergey's (1974).

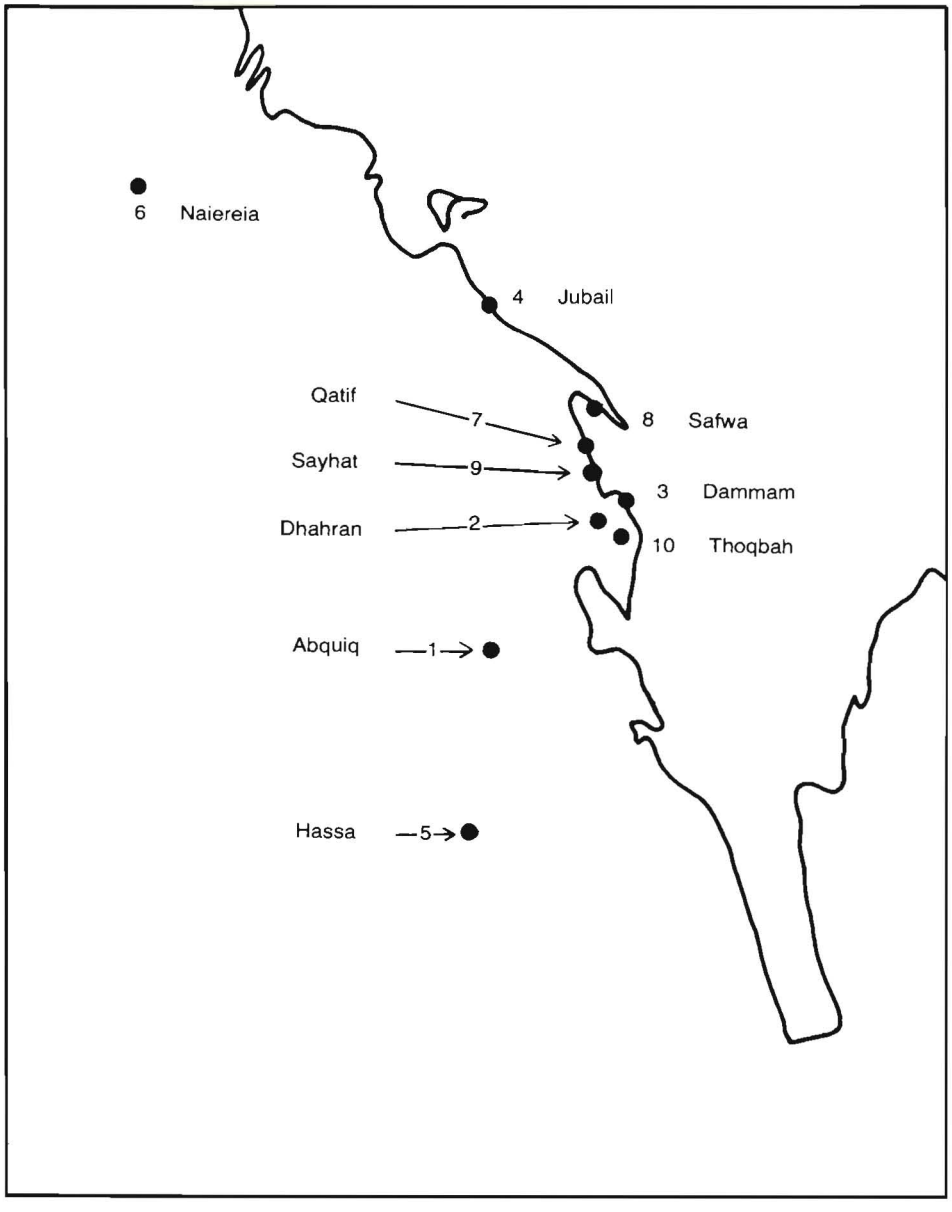

Fig. 1. Map of Eastern Region of Saudi Arabia showing the studied localities (1. Abquiq; 2. Dhahran; 3. Dammam; 4. Jubail; 5. Hassa; 6. Naiereia; 7. Qatif; 8. Safwa; 9. Sayhat and 10. Thoqbah).

Recoveries of protease and amylase producing fungi:

Three replicate plates of GPYA-Ge and GPYA-S, media containing rose bengal and streptomycine were used for each of soil sample for recovery respectively of protease and amylase producing fungi. One ml portion of the proper dilution was plated. Dishes were incubated at 30° and 55°C for 3-4 days. Plates (GPYA-Ge) of developed growth were flooded with mercuric chloride solution. The protease producing organisms were counted by observing the clear zone around the growth of fungal colonies. Plates (GPYA-S) of developed growth were then flooded with iodine solution. The amylase producing organisms were recovered by detecting the clear zone around the growth of fungal colonies.

The fungal genera and species were identified according to Gilman (1971) for soil fungi in general; Raper and Thom (1949) for *Penicilium* spp and Raper and Fennell (1965) for *Aspergilus* spp.

Results

The soil characteristics, pH values, total soluble salts, organic matter content and vegetation are summarized in Table 1. Data given in this table show that the studied soil samples were generally poor in organic matter content 3.0 - 3.8%. The total soluble salt contents varied slightly from 0.5 - 2.4%. The pH values of the soil samples revealed appreciable differences; all were slightly acidic (pH ranges from 5.25 to 5.85).

Table 2 gives the number of total viable microorganisms producing proteases per gm soil.

The results show that, the bacterial counts varied from one locality to another. The bacterial counts at 30°C ranged from 28×10^5 to 33×10^3 colonies per gram soil. The highest count was isolated from Dammam soil, while the lowest count was isolated from Abquiq soil. The highest bacterial counts at 55°C was 31×10^5 per gram soil from Thoqbah locality. No thermophilic proteases producers were isolated from Abquiq and Jubail. The lowest count at 55°C was 12×10^3 from Dhahran locality.

The data in Table 2 shows that the soil of the eastern region of Saudi Arabia was low in actinomycetes producing proteases. No thermophilic actinomycetes producing proteases were isolated at 55°C. But at 30°C it was isolated from 3 localities only, Dammam (41×10^3/gm soil), Naiereia (70×10^3/gm soil) and Dhahran (55×10^4/gm soil).

In respect of fungal content, the data in Table 2 shows, the fungal existence was less than the bacterial contents. The counts of fungi producing proteases at 30°C were variable. No fungi were isolated from Dhahran locality. The lowest count (24×10^3/gm soil) was isolated from both Abquiq and Hassa localities, while the highest count (53×10^4/gm soil) was detected in Thoqbah area. Thoqbah soil also contains the highest count of total viable bacteria at 55°C (31×10^5/gm soil).

Table 3 shows the numbers of total viable microorganisms producing amylases, isolated from the soil of different localities of easter region of Saudi Arabia at 30 and 55°C. This data reveals that, the thermophilic actinomycetes and fungi producing amylases were icolated from 3 different localities each, Dammam-Qatif-Safwa, and Dammam-Sayhat-Thoqba respectively.

No bacteria producing amylases were isolated at 55°C from Abquiq and Jubail localities. Fungi producing amylases were isolated at 30°C from 8 localities. The lowest count (12×10^3/gm soil) was isolated from Jubail, Hassa, Safwa and Sayhat localities. The highest count (25×10^4/gm soil) was recovered from Thoqbah locality.

Table 1. Chemical and physical characteristics of soil in the sampling sites of Eastern Region, Saudi Arabia (OMC, organic matter content; TSS, total soluble salt)

Localities	Soil texture	OMC %	TSS %	pH	Vegetation type
Abquiq	Sandy clay	0.4	0.5	5.60	Cultivated trees and fruits
Dhahran	Sandy clay	0.5	0.7	5.85	Cultivated trees and grasses
Dammam	Sandy clay	1.3	0.7	5.50	Residential area
Jubail	Sandy	0.3	0.87	5.25	Desert area with wide plant
Hassa	Clay loam	2.7	2.45	5.85	Agricultural area
Naiereia	Sandy	1.4	0.78	5.25	Desert area with wild plant
Qatif	Sandy clay	3.7	0.7	5.25	Cultivated trees and crops
Safwa	Sandy	0.3	1.74	5.60	Agricultural area
Sayhat	Clay loam	2.5	0.52	5.85	Agricultural area
Thoqbah	Sandy clay	3.8	1.65	5.50	Cultivated trees and residential (area)

Table 2. Numbers of total viable microorganisms producing proteases (No. × 10^4/gm soil) isolated from Eastern Region of Saudi arabia at 30 and 55°C.

Localities	Total viable microorganisms producing proteases (No. × 10^4/gm soil)					
	Bacteria		Actinomycetes		Fungi	
	30°C	55°C	30°C	55°C	30°C	55°C
Abquiq	3.34	0.00	0.00	0.00	2.04	0.00
Dhahran	71.66	1.25	55.00	0.00	0.00	0.00
Dammam	285.84	4.16	4.16	0.00	5.84	0.00
Jubail	7.91	0.00	0.00	0.00	3.34	0.00
Hassa	8.34	28.34	0.00	0.00	2.04	0.00
Naiereia	260.41	11.66	7.09	0.00	15.04	0.00
Qatif	15.84	7.91	0.00	0.00	7.09	0.00
Safwa	16.66	16.66	0.00	0.00	2.05	0.00
Sayhat	29.16	31.25	0.00	0.00	2.09	0.00
Thoqbah	198.34	312.5	0.00	0.00	53.75	0.00

Actinomycetes producing amylases were isolated at 30°C from 50% of the localities. The counts were 20×10^3, 79×10^3, 86×10^3, 12×10^4 and 22×10^4/gm soil from Dammam, Abquiq, Dhahran, Naiereia and Qatif localities respectively.

Bacteria producing amylases were isolated at 30°C from all localities with different values. The lowest count (79×10^3/gm soil) was isolated from Safwa, while the highest count (20×10^5/gm soil) was recorded from Qatif locality.

Microorganisms producing proteases enzymes are recorded in Table 4. The results show that four genera of bacteria, including 10 different species; one genera of actinomycetes including two species; four genera of fungi, including five species, were found in the soil of Eastern Region of Saudi Arabia.

Bacillus sp 2 was found in nine localities, but not isolated from Abquiq soil. Both species of *Streptomyces* were isolated from Dhahran and Dammam localities. In addition *Streptomyces* sp 2, was found in Naiereia locality.

Penicillium sp 1 was more common than the other fungal species. It was found in seven localities (Table 4). *Aspergillus* sp 1, was found in soil of six localities. In

Table 3. Number of total viable microorganisms producing amylase, isolated from soils of different localities from Eastern Region in Saudi Arabia at 30 and 55°C. (No. × 10^4/gm soil)

Localities	Total viable microorganisms producing amylases (No. × 10^4/gm soil)					
	Bacteria		Actinomycetes		Fungi	
	30°C	55°C	30°C	55°C	30°C	55°C
Abquiq	29.16	0.00	7.92	0.00	0.00	0.00
Dhahran	85.5	1.25	8.67	0.00	2.92	0.00
Dammam	83.34	65.84	2.09	13.34	0.00	2.09
Jubail	19.58	0.00	0.00	0.00	1.25	0.00
Hassa	20.83	25.83	0.00	0.00	1.25	0.00
Naiereia	11.67	1.25	12.50	0.00	3.75	0.00
Qatif	206.67	30.00	22.92	1.25	5.83	0.00
Safwa	7.92	13.33	0.00	1.25	1.25	0.00
Sayhat	25.83	11.25	0.00	0.00	1.25	3.75
Thoqbah	25.50	187.50	0.00	0.00	25.00	3.75

general Abquiq and Jubail localities are very low in microbial content. Abquiq contained only three species, *Micrococcus* sp, *Pseudomonas* sp and *Penicillium* sp 1. And also Jubail soil contained three species of microflora, *Bacillus* sp 2, *Penicillium* sp 1 and *Rhizoctonia* sp.

Table 5 shows that the presence of different amylase-producing-genera of microflora from different soil of Eastern Region of Saudi Arabia. The results show that three genera of bacteria, including nine species, one genera of Actinomycetes, including three species, and four genera of fungi, including six species, were found in soil samples.

Bacillus sp 2 was found in eight localities. It was not found in Abquiq and Jubail localities. *Pseudomonas* sp 2 which it was isolated from five localities (Dammam, Dhahran, Safwa, Sayhate and Thoqba). *Streptomyces* sp 1 and sp 2 were found in five localities (Abquiq, Dhahran, Dammam, Naiereia and Qatif).

Both *Penicillium* sp 1 and *Aspergillus* sp 1 were found in four localities. *Penicillium* sp 1 was recorded in Dhahran, Naiereia, Sayhat and Thoqbah; *Aspergillus* sp 1 was isolated from Naiereia, Qatif, Safwa and Thoqbah. The

Table. 4. General of microorganisms producing proteases enzymes; isolated from Eastern Region of Saudi Arabia at 30 and 55°C.

Genera of microorganisms producing proteases		Localities									
		Abquiq	Dhahran	Dammam	Jubail	Hassa	Naiereia	Qatif	Safwa	Sayhat	Thoqbah
Micrococcus	sp 1	+						+		+	
Micrococcus	sp 2								+	++	
Micrococcus	sp 3			+			+				
Pseudomonas	sp 1	+						+		+	
Pseudomonas	sp 2		+	+					+		+
Bacillus	sp 1		+				+				
Bacillus	sp 2		+	+	+	+	+	+	+	+	+
Bacillus	sp 3					+	+	+	+	+	+
Bacillus	sp 4						+	+			
Streptomyces	sp 1		+	+							
Streptomyces	sp 2		+	+			+				
Penicillium	sp 1	+			+	+	+	+		+	+
Aspergilus	sp 1					+	+	+	+	+	+
Aspergilus	sp 2							+			+
Rhizoctonia	sp				+			+		+	
Mycelia Sterilia	sp			+							+

results show that the *Penicillium* sp 1 and *Asperillus* sp 1 were more dominant in the studied localities than other fungal species.

Discussion

This work was planned to study the distribution of amylase and protease producing microorganisms from the soil of Eastern Region of Saudi Arabia.

Table. 5. Genera of microorganisms producing amylase enzymes; isolated from Eastern Region of Saudi Arabia at 30 and 55°C.

Genera of microorganisms producing proteases Amylase enzyme		Localities									
		Abquiq	Dhahran	Dammam	Jubail	Hassa	Naiereia	Qatif	Safwa	Sayhat	Thoqbah
Micrococcus	sp 1	+						+		+	
Micrococcus	sp 2	+					+				
Bacillus	sp 1		+								
Bacillus	sp 2		+	+		+	+	+	+	+	+
Bacillus	sp 3					+			+		+
Bacillus	sp 4							+			
Bacillus	sp 5							+			
Pseudomonads	sp 1							+			
Pseudomonads	sp 2		+	+					+	+	+
Streptomyces	sp 1	+	+	+			+	+			
Streptomyces	sp 2	+	+	+			+	+			
Streptomyces	sp 3			+				+	+		
Myceliasterila			+					+		+	+
Penicillium	sp 1		+				+			+	+
Penicillium	sp 2			+						+	+
Aspergilus	sp 1						+	+	+		+
Aspergilus	sp 2		+								+
Deuteromycetes							+				

In the present study amylase producing microorganisms were more common in the studied soil than protease producing microorganisms. This finding may be due to the abundance of plant residues in the soil which are considered as polysaccharide substrate. These substrates (residues of plant origin) act as a mediating attachment in the ecosystem (Marchall 1976). This attachment leads to aggregation of soil particles (Marchall 1976). Balkwill *et al.* (1977) showed that

many of the microbial cells in the natural soil are firmly attached to soil particles. In this study, soilpoor in organic matter lood to lower count of microorganisms, in agreements with Rosenzweig and Stotzky (1980) and Ali and Abou-Heilah (1984).

Table 1,2 and 3 show that clay soil contains higher bacterial count than fungal count. This may be due to clay minerals having a more antagonistic effect on fungi than bacteria. The most important constituents in clay composition are montomorillionate and kaolonate (in ratio 1:10). Monotmorillionate has been shown to enhance the growth of bacteria in soil (Filip 1973, Martin *et al.* 1976, and Rosenzweig and Stotzky 1979) and in pure culture (Stotzky 1966, Stotzky and Rem 1966, and Rosenzweig and Stotzky 1979). It maintains pH of the environment suitable for bacterial growth by basic cation exchange from their exchange complex for H^+ produced during metabolism. The stimulation of bacterial growth leading to depletion of nutrients in the presence of montomorillionate was apparently partially responsible for the inhibition of *A. niger* by bacteria (Rosenzweig and Stotzky 1979). In general, increasing montomorillionate concentration leads to the increase of inhibitory effect on fungal growth, but they found no correlation between the concentration of kaolinite and the level of inhibition of soil microflora.

With respect to the recorded fungal genera, *Penicillium* and *Aspergillus* were dominant in the soil of the studied localities. These results are in agreement with Elwan and Diab (1971) and Fathi *et al.* (1975), and Ali and Abou Heilah (1984). In this study *Penicillium* spp were dominant, but Ali and Abou-Heilah (1984) found that *Asperigillus* spp was commonly recorded. Five genera were of moderate count, these are *Penicillium* spp, *Aspergillus* spp, *Rhizoctonia* sp, *Sclerotrum* sp, *Colleotrichum* sp, *Mycelia sterilia* sp. and *Deuteuromycetes* sp. The results herein show that there is no correlation between the type of genera and the type of soil.

The studied localities are nearly acidic (pH 5.25 - 5.58). Streptomyces was isolated from 5 localities (Abquiq, Dhahran, Dammam), Naiereia and Qatif). *Streptomyces* are considered intolerant to acidic condition, but Alexander (1967) stated that from highly acidic environment the count of *Streptomyces* spp was less than total viable bacteria. Other investigators (Hagadorn 1976, Buckman and Bary 1969 and William *et al.* 1979) have mentioned that the growth of *Streptomyces* is inhibited in mineral soil having pH value of 5.0 or less. The results herein are closely related with Alexander (1967) and others. In this study, the isolated *Streptomyces* from nearlyacidic soil, were able to grow on neutral medium. This result is in agreement with Hagadoron and Halt (1975). In general, *Streptomyces* spp are widely spread in acidic soil (Kahn and Williams 1975 and Hagadorn 1976 and can be detected in neutral pH medium (Williams *et al.* 1979).

In this study *Streptomyces* counts were lower than the previously reported.

The presence of bacteria in different localities was recorded by many authors *e.g.*, desert soil (Reichenbach 1970), arid Mexican soil (Brockman 1976) and Arizona, New Mexico and Texas desert soils (Larkin and Dunigan 1973). In the present study bacterial cells have been detected in desert soils. This is agreement with Brockman (1976). Arid soil in Mexico contains different counts of bacterial cells, and in this work, the studied localities contain different counts of bacterial cells. This variability in counts may be due to plant debris in the soil.

It is well known, that the microbial flora of an arid soil is capable of producing varied and pronounced biological activity. The most frequently observed bacterial genera from the studied localities were *Micrococcous, Streptococcus, Pseudomonas, Bacillus. Bacillus* spp are quite abandant in the 9 localities. We must notice that the limitation of soil dilution plate methods might have masked the presence of other soil microorganisms.

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(Received 14/10/1989;
in revised form 12/08/1990)

وجود ميكروفلورا التربة المنتجة للاميليز والبروتييز في المنطقة الشرقية في المملكة العربية السعودية

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المملكة العربية السعودية

بذلت محاولة لالقاء الضوء على وجود وتعريف الكائنات الدقيقة المنتجة للبروتييز والاميليز. جمعت عينات التربة من أماكن مختلفة من المنطقة الشرقية في المملكة العربية السعودية وأجريت طريقة الاطباق المخففة في عزل الكائنات الدقيقة باستخدام بيئة آجار ـ جلوكوز ـ مستخلص الخميرة المعدل والمزود بالجيلاتين والنشا.

عزلت البكتريا المحبة للحرارة والمنتجة للبروتييز، وكذلك البكتريا المحبة للحرارة المتوسطة من جميع المناطق، ولم تعزل اكتينوميسيتات وفطريات محبة للحرارة في أي منطقة ووجدت الكائنات الدقيقة المنتجة للاميليز متطابقة مع الكائنات المنتجة للبروتييز. ظهرت أنواع الاكتينوميسيتات المحبة للحرارة والمنتجة للاميليز في تربة الدمام، القطيف وصفوى وكذلك الفطريات المحبة للحرارة وجدت في ثلاث مناطق (الدمام، سيهات، والثقبة). لم تظهر أي من الكائنات الدقيقة المحبة للحرارة والمنتجة للاميليز أو البروتييز في تربة الجبيل وأبقيق.

ومن ضمن الكائنات المنتجة للبروتييز، كانت الانواع التالية أكثر تمثيلاً: باسيلس رقم (٢) سائداً في تسعة من الأماكن، ولم يعزل من تربة أبقيق. كما وجد استربتوميسس رقم (٢) في الظهران، الدمام والنعيرية أما البنيسيليوم رقم (١)

فقد وجد في أبقيق، الجبيل، الاحساء، النعييرية، القطيف، سيهات والثقبة.

وبالنسبة للأنواع المنتجة للأميليز وجد الباسيلس رقم (٢) في ثمانية أماكن. ووجد النوعين (١)، (٢) من الاستربتوميسس معاً في خمسة أماكن (أبقيق، الدمام الظهران، النعييرية والقطيف). ووجد البنيسيليوم رقم (١) واسبرجلس رقم (٢) والميسيليا استريليا في أربعة أماكن مختلفة لكل منهم.