

CELLULOSE - DECOMPOSING FUNGI FROM SAUDI ARABIAN SOILS

By

ALI H. BAHKALI* AND MOHAMMAD A. KHIYAMI**

*Botany and Microbiology Department, College of Science, King Saud University, P. O. Box: 2455. Riyadh 11451, Saudi Arabia

**King Abdulaziz City for Science and Technology, General Directorate of Research, Grants Programs. Riyadh. Saudi Arabia

الفطريات المحللة للسليلوز في المملكة العربية السعودية

علي حسن عبد الرحمن بهكلي و محمد أحمد عبد الله خيمي

تم في هذه الدراسة جمع عينات تربة من ست مدن في المملكة العربية السعودية هي الدمام ، النماص ، مكة المكرمة ، الرياض ، تبوك وجيزان ، حيث تم عزل وتعريف ثلاثون نوعاً من الفطريات ينتمون إلى خمسة عشر جنساً ذات قدرة على تحليل السليلوز وأظهرت عينات الدمام أعلى معدل من الأنواع الفطرية (٢٠ نوع) تتبعها النماص (١٨ نوع)، مكة والرياض (١٧ نوع) ، تبوك (١٩ نوع) وجيزان (١١ نوع) . وتبين أن الأجناس السائدة المعزولة هي

Aspergillus, Penicillium, Alternaria, Ulocladium and Curvularia

كما تم خلال هذه الدراسة عزل وتعريف ستة أنواع فطرية ينتمون إلى أربعة أجناس لم يسجل وجودها من قبل في المملكة وهي .

Trichoderma pseudokoningii, T. harzianum, T. koningii, Ulocladium septosporum, Emericella nidulans and Cochliobolus lunatus.

مما تعتبر إضافة جديدة للفلورا الفطرية لتربة المملكة .

Key Words: Saudi Arabia. Cellulose, Decomposing., Mycoflora

ABSTRACT

Thirty fungal species belonging to fifteen genera were collected from 30 soil samples on cellulose Czapek agar. The highest number of fungal species was isolated from Dammam (20 species) followed by Niomas (18 species), Makkah and Riyadh (17 species each), Tabouk (16) species and Jizan (11 species). The most frequent genera isolated were *Aspergillus, Pencillium, Alternaria, Ulocladium* and *Curvularia*. Throughout this study, six fungal species belonging to four genera; *Ulocladiun septosporum, Emericella nidulans, Trichoderma harzianum, T. koningii, T. pseudokoningii* and *Cochliobolus lunatus* were found to be new records in Saudi Arabian soils.

INTRODUCTION

The fungal flora of the soil has attracted the attention of many investigators. In Saudi Arabia, with its huge desert areas, knowledge of the soil microflora would be of great interest. Reviewing the current literature showed many reports dealing with the existence and distribution of soil microbiota and a few of them traced those producing cellulase enzyme in Saudi Arabia (1-7). Abdel-Hafez (8) isolated seventy five species of cellulose-decomposing fungi belonging to twenty seven genera from soil samples collected from desert in Saudi Arabia, of which *Aspergillus*, *Alternaria*, *Stachybotrys* and *Penicillium* were the most frequent. The present work aims at making a preliminary survey to study the composition and frequency of cellulose-decomposing fungi in uncultivated soils collected from six different localities of Saudi Arabia, namely Riyadh, Makkah, Tabuk, Dammam, Nimas and Jizan.

MATERIALS & METHODS

Collection of soil samples:

Thirty soil samples (5 sample/place) were collected from six different locations of uncultivated areas in Saudi Arabia, during the period May-August 1993. (Fig. 1). The samples were collected from the upper layer at a depth of 5-10 cm by using sterile shovels. A composite sample from each location was prepared by thoroughly mixing the five collections of a total weight of 1000 g, then put in sterile plastic bags and labelled. From each composite sample 25 g were used for making fungal isolations (9).

Determination of cellulose-decomposing Fungi:

The soil dilution plate method was used (10). Cellulose Czapek's agar medium was selected to isolate cellulose-decomposing fungi (composition g/L: Cellulose powder, 20 g; NaNO₃, 3.0 g; K₂HPO₄, 1.0 g; MgSO₄·7H₂O, 0.5g; KCl, 0.5g; FeSO₄·7H₂O, 0.01 g; agar 15 g). Rose bengal (0.03 g/L) was added to reduce the spread of fast growing fungi, while streptomycin sulphate (0.033 g/L) was applied to eliminate bacterial growth (11). Rose bengal and streptomycin were aseptically and separately dissolved in sterile distilled water and saline solution respectively before adding to the medium. For each soil sample, five replicate plates were used and the plates were incubated at 28°C for 5-7 days. Fungal colonies were counted and isolated in pure culture and percent frequency was calculated as follows:

$$\% \text{ Frequency} = \frac{\text{No. of sites in which species found}}{\text{Total no. of sites}} \times 100$$

Identification of Fungi:

For the identification of fungal flora, slides were made from

pure cultures on potato-dextrose agar pH 5.6. which contains (per liter) 4 g potato, 20 g dextrose, 15 g agar and the taxonomic keys of (12-20) were used. Further confirmation of identification was provided by the International Mycological Institute, Egham, Surrey, U. K.

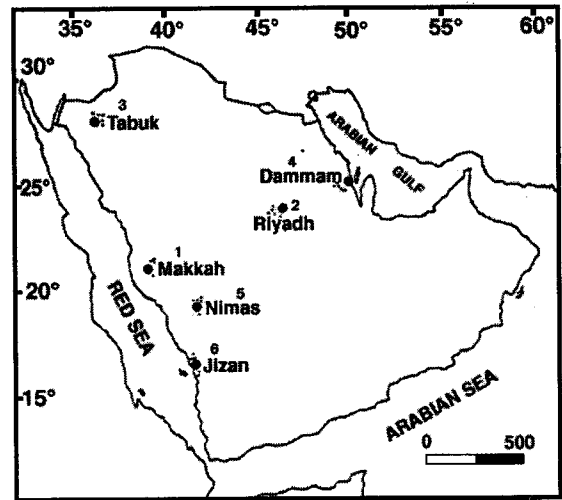


Fig. 1 A sketch map of Saudi Arabia showing the localities (1-6) from which the soil samples were collected

RESULTS

Thirty species belonging to fifteen genera were collected from different localities in the Kingdom of Saudi Arabia as shown in Table 1.

Although, the present study is the result of using one isolation technique and one isolation medium; *Ulocladium septosporum*, *Emericella nidulans*, *Trichoderma harzianum*, *T. koningii*, *T. pseudokoningii* and *Cochliobolus lunatus* were found to be new records to Saudi Arabian soils.

DISCUSSION

Despite the great role that fungi play in the soil, very little work has been done so far on cellulose-decomposing fungi in Saudi Arabian soil. The highest fungal species were isolated from Dammam soil samples (20 species) followed by Nimas (18 species), Riyadh (17 species), Tabouk (16 species) and Jizan (11 species).

The predominant genus was *Aspergillus*, with five species, followed by *Penicillium* and *Trichoderma* with four species each, then *Ulocladium* with three species, and *Alternaria*, *Cochliobolus* and *Fusarium* with two species each, and *Curvularia*, *Chaetomium*, *Drechslera*, *Mucor*, *Phoma*, *Rhizopus*, *Stachybotrys* and *Emericella* with one species each. Representatives of the *Mycelia sterilia* were also common and were found in soils from all the tested localities.

Table 1.

Occurrence and relative distribution of the fungal genera and species isolated from soil samples recovered on cellulose-Czapek's agar at 20°C, and collected from six different localities in Saudi Arabia.

FUNGAL SPECIES	IMI Number	M	R	T	D	N	J	T. N.	%	Frequency
<i>Aspergillus fumigatus</i> Fresen.	356162	06	02	04	02	03	04	21	4.2	100.0
<i>A. niger</i> van Tiegh.	356160	04	03	05	02	02	03	19	3.8	100.0
<i>A. flavus</i> Link	356149	04	04	03	01	02	03	17	3.4	100.0
<i>A. Terreus</i> Thom	-	---	03	02	---	01	02	08	1.6	66.70
<i>Aspergillus</i> sp	-	---	02	06	01	03	---	12	2.4	66.70
<i>Alternaria alternata</i> (:Fr.) Keissler	-	06	02	03	---	04	01	16	3.2	83.30
<i>Curvularia</i> sp	-	---	02	02	01	03	---	08	1.6	66.70
<i>Coffliobolus spicifer</i> R. R. Nelson.	356138	---	---	---	02	---	---	02	0.4	16.70
<i>C. lunatus</i> ** R. R. Nelson & F. A. Haasis	356145	01	---	---	---	---	---	01	0.2	16.70
<i>Chaetomium</i> sp	-	02	02	---	---	---	01	05	1.0	50.00
<i>Drechslera</i> sp	-	---	03	02	---	01	---	06	1.2	50.00
<i>Emericella nidulans</i> ** (Eidam) Vuill	356153	01	---	01	03	---	---	05	1.0	50.00
<i>Fusarium moniliforme</i> Sheldon	356146	03	02	---	04	---	---	09	1.8	50.00
<i>F. Solani</i> (Martius) Sacc.	356159	01	---	---	02	02	---	05	1.0	50.00
<i>Mucor</i> sp	-	01	---	---	01	---	---	02	0.4	33.30
<i>Mycelia sterilia</i>	-	03	06	06	04	07	02	28	5.6	100.00
<i>Penicillium chrysogenum</i> Thom.	356165	05	04	06	03	02	06	26	5.2	100.00
<i>P. griseofulcum</i> Dierckx	356166	---	06	05	04	03	---	18	3.6	66.70
<i>Penicillium</i> sp	-	02	04	03	01	02	01	13	2.6	100.00
<i>Phoma</i> sp	-	01	01	---	---	---	03	05	1.0	50.0
<i>Rhizopus</i> sp	-	---	---	01	---	02	02	05	1.0	50.0
<i>Stachybotrys</i> sp	-	---	---	02	---	---	---	02	0.4	16.70
<i>Trichoderma viride</i> Pers. : Fr.	356151	01	---	---	01	01	---	03	0.6	50.0
<i>T. harzianum</i> ** Rifai	356158	---	---	---	02	---	---	02	0.4	16.70
<i>T. koningii</i> ** Oudem	356164	---	---	---	---	01	---	01	0.2	16.70
<i>T. pseudokoningii</i> ** Rifai	350894	01	01	---	---	---	---	0.2	0.4	33.30
<i>Ulocladium atrum</i> ** Preuss	356143	02	03	---	04	03	---	12	1.4	66.70
<i>U. septosporum</i> * ** (Preuss) E. G. Simmons	356142	---	---	---	03	---	---	03	0.6	16.70
<i>Ulocladium</i> sp	-	---	---	---	02	---	---	02	0.4	16.70
Total number of genera	15	11	10	08	10	09	06			
Total number of Species	30	17	17	16	20	18	11			

IMI number = International Mycological Institute Number

T. N. : Total number of isolations 279

* Cultures deposited at International Mycological Institute, England.

** First record of the species from Saudi Arabia soils.

M: Makkah; R: Riyadh; T: Tabuk; D: Dammam; N: Nimas; J: Jizan

Most of the species recovered throughout the present study were reported to be cellulose-decomposing fungi (7, 21, 22).

Aspergillus fumigatus, *A. niger*, *A. flavus*, *Penicillium chrysogenum*, and *Penicillium* sp, were the most frequent and were represented in all tested samples. This is almost in agreement with the results obtained by Adel - Hafez (8)

Ulocladium was recovered in moderate frequency and this is in agreement with abdel - Hafez's (8) results.

The results also reveal that the fungi of Dammam and Jizan salt soils are basically similar to those of the Saudi Arabian desert and cultivated soils. This adds further support to the statement that there is no specific fungus flora of the different soil types.

However, the listing may differ in the order of frequency of some of the component fungi.

The occurrence of two *Trichoderma* spp. in Dammam soil (alkaline soil) is surprising since Rao (23) remarked that this genus is generally accepted as characteristic of acidic soils. It is possible that these species may have acquired an adaptability to local alkaline conditions (6).

In conclusion, the comparison of the cellulose-decomposing mycobiota of soil samples collected from different localities of the Kingdom with those of other areas in the world is often difficult; since there are differences in isolation techniques, isolation media, collecting seasons and depth of sampling or combinations of any of these factors (24).

More extensive study, with a greater variation in culture media, and isolation techniques, would reveal many additional fungal species.

ACKNOWLEDGEMENTS

The author wishes to thank Prof. Dr. M. A. El - Shanawany, King Abdulaziz City for Science and Technology, for reading the manuscript.

REFERENCES

- [1] **Moubasher, A. H. 1993.** Soil Fungi in Qatar and other Arab Countries. The Centre for Scientific and Applied Research, University of Qatar, Qatar.
- [2] **Abdel-Hafez, S. I. I. 1982.** Survey of the mycoflora of desert soils in Saudi Arabia. *Mycopath.* 80: 3-8.
- [3] **Hashem, A. R. 1990.** Studies on the fungal flora of Saudi Arabian soil. *Crypto. Bot.* 2 (3) : 179-182.
- [4] **Hashem, A. R. 1993.** Fungal flora of soils from Ashafa, Toroba, Wahat and Wehait. *J. King Saud Univ.* 5 (1): 47-53.
- [5] **Ali, M. L. and Abou-Heilah, A. N. 1984.** On the fungal of Saudi Arabia. III. Some fungi in soil from Eastern and Southern Regions. *J. Coll. Sci. King Saud Univ.*, 15: 309-320.
- [6] **Abou-Heilah, A. N.; Kassim, M. Y. and Basahy, A.Y. 1982.** Soil Mycoflora of Saudi Arabia. Isolation, Identification and Distribution in Riyadh Region. *Iraqi. J. Sci.* 23 (2): 197-216.
- [7] **Abdel-Monem, H. M. SA. A. and Aly, A. Om-Kolthom 1990.** Existence of soil micorflora producing amylases and proteases in Eastern Region of Saudi Arabia. *Arab Gulf J. Scient. Res.* 8 (3): 121-135.
- [8] **Abdel-Hafez, S. I. I. 1982.** Cellulose-decomposing fungi of desert soils in Saudi Arabia . *Mycopath.* 78: 73-78.
- [9] **Khiyami, M. A. 1994.** Physiological Studies on the Cellulolytic Enzymes of some Filamentous Fungi isolated from various soils of Saudi Arabia. M. Sc. Thesis, Fac. Sci. King Saud Univ. Dep. Bot. Mico.
- [10] **Johnson, L. F.; Curl, E. A. Bond, J. H. and Fribourg, H. A. 1959.** Methods for studying soil microflora-plant disease relationships. Burgess Publ. Co., Minneapolis, Minn.
- [11] **Abdel-Hafez, A. I. I. Mazen, M. B. and Galal, A. A. 1990.** Glycophiolic and cellulose-decomposing fungi from soils of Sinai Peninsula, Egypt. *Arab Gulf J. Scient. Res.* 8 (1): 153-168.
- [12] **Raper, K. B. and Thom, C. 1968.** "A manual of Penicillio, pp.135-138. Hafner publishing Company inc., New York.
- [13] **Raper, K. B. and Fennel, D. I. 1965.** The genus *Aspergillus*. Williams and Wilkins Co., Baltimore, U. S. A.
- [14] **Zycha, H.; Siepmann, R. and Linnemann, G. 1969.** "Mucorales", Verlag von J. Cramer. Lehre, 355 p.
- [15] **Gilman, J. C. 1971.** A manual of soil fungi. Iowa State College Press, Fourth edition.
- [16] **Ellis, M. B. 1976.** More dematiaceous hyphomycetes. Commonwealth Mycol. Instit., Kew. Surrey, England. 507 p.
- [17] **Barnett, H. L. and Hunter, B. B. 1987.** illustrated genera of imperfect fungi. MacMillan Publishing Company, New York. 218 p.
- [18] **Kalifa, O. 1976.** Detection of *Drechslera graminea* (Rab. ex. Sel.) Shoe and D. Teres (Sacc.) Shoe in barley seed. A report submitted to the Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark, 35 p.
- [19] **Carmichael, J. W., Kendrick, W. B.; Connoers. I. L. and Sigler, L. 1980.** Genera of hyphomycetes. The university of Alberta Press, Alberta. 386 p.
- [20] **Nelson, P. E.; Toussoun, T. A. and Marassas, W. F. O. 1983.** *Fusarium* species. An illustrated manual for identification; pp. 142-150. The Pennsylvania State University Press, USA.
- [21] **Mazen, M. B. 1973.** Ecological studies on cellulose-decomposing fungi in Egypt. Ph. D. Dissertation, Bot. Dept; Faculty of Sci., Assiut Univ.; Egypt. p. 285.
- [22] **Flannigan, B. 1970.** Degradation of arabinoxylan carboxymethyl cellulose by fungi isolated from barley kernels. *Trans. Brit. Mycol. Soc.* 55: 277-281.
- [23] **Rao, P. R. 1970.** Studies on soil gungi. III. Seasonal variation and distribution of microfungi in some soils of Andhra Pradesh (India). *Mycopath. Mycol. Appl.* 40: 277-298.
- [24] **Abou-Heilah, A. N. 1985.** Soil mycoflora of Saudi Arabia. II. Some microfungi in the forest of Asir region. *J. Bio. Sci.* 16(2): 1-16.

Received 20, March, 1996