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Extracellular Cellulase Enzyme Production by Soil Mycoflora in Saudi Arabia

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Abstract. Rhizosphere soil mycoflora of Alfalfa, Date-palm, Grape-vine, *Cucumis* sp. and *Zizyphus spina christi* were searched for cellulose degraders. A total number of 61 fungal species were isolated as cellulose degraders. *Aspergillus* was the most frequently isolated followed by *Penicillium, Ulocladium, Cheatomium, Alternaria* and *Drechslera.* The production of cellulase by isolated mycoflora was confirmed by cleared-zone technique. It was observed that the same fungal species isolated from different sources produced cellulase in different quantities.

Introduction

The disposal of waste or garbage has become the major problem for both developing and developed countries and needs serious attention for the sake of future generations [1]. Biodegration of cellulose is an important component of disposal, involving the degradation of waste materials containing plant cell wall components [2-5]. Fungi, along with other microorganisms, play a major role in the processes of cellulose degradation [6;7].

Numerous studies have been undertaken on the role of mycoflora as cellulose decomposers [7-10]. In Saudi Arabia, some records of cellulose decomposing fungi from soil are available but they are limited [11;12]. In contrast, soil mycoflora of Saudi Arabia are well documented [11;13-20]. This work was undertaken to study the cellulose decomposing fungi in the rhizosphere of agriculturally important plants, wild desert and sand-dune plants of Saudi Arbia, with the hope of isolating and using them to enhance degradation.

Materials and Methods

Soil samples were collected from rhizosphere soil of Alfalfa (*Medicago sativa*), Date-palm (*Phoenix dactylifera*), Grape-vine (*Vitis vinifera*), *Cuccumis* sp. (sand dune plant), and *Zizyphus spina christi*) Wild (desert plant).

Fifty samples of both rhizosphere and non-rhizosphere soils (approx. 100 g each) were collected in the month of March 1989. All the rhizosphere soil samples were mixed together. Five grams of soil were randomly taken from this mixture and placed into 45ml of sterilized water in flasks of 100 ml capacity. Five replicates were prepared for isolation of fungi by dilution plate method. Five replicates of non-rhizosphere soil samples were also prepared in the similar manner as for rhizosphere soil [16; 17; 20]. Bulk soil adhered to roots was separated from rhizosphere soil by gently shaking the roots of plants. Cellulose powder (BDH - Chemicals, London) was added (1% W/V) in the Dox medium as a sole source of carbon instead of sucrose. Rose bengal (0.03 g/L) was added in the medium to reduce the spread of fast growing fungi while streptomycin sulphate (0.033 g/L) was added to eliminate bacteria. Inoculated plates were incubated at room temperature (22-25°C). Isolated cultures were maintained on Dox - cellulose medium.

Detection of cellulase production by isolated fungi was carried out by the cleared-zone technique as described by Yoeh *et al.* [20]. Percent of cleared-zone was calculated according to formula given below. Literature for identification of fungi was the same as mentioned in our earlier reports [16-19].

$$\text{Percent of cleared-zone} = \frac{\text{Diameter of cleared–zone–Diameter of colony}}{\text{Diameter of colony}} \times 100$$

Results and Discussion

Rhizosphere soil of date-palm yielded the highest number of fungal colonies per gram while rhizosphere soil of sand dune plant, *Cuccumis* sp., yielded the lowest number (Fig. 1). The total number of colonies per gram of rhizosphere soil enumerated on cellulose agar medium was in the range of 880-1790 per gram including all the plants [16; 17; 20]. Earlier reports on the soil mycoflora of Saudi Arabia recorded a very wide range of number of fungal colonies per gram of soil (40-50,000 colonies/g) [11; 13; 14; 21].

Rhizosphere soils of alfalfa, date-palm, grape-vine, sand dune plant and desert plant yielded a total of 61 fungal species belonging to 20 genera as cellulose degraders (Table 1). *Aspergillus* was the predominant genus yielding 14 species. *Aspergillus flavus, A. fumigatus, A. niger* and *A. terreus* were found in the rhizosphere of all the plants. *Aspergillus* was also reported a predominant genus from different types of soil

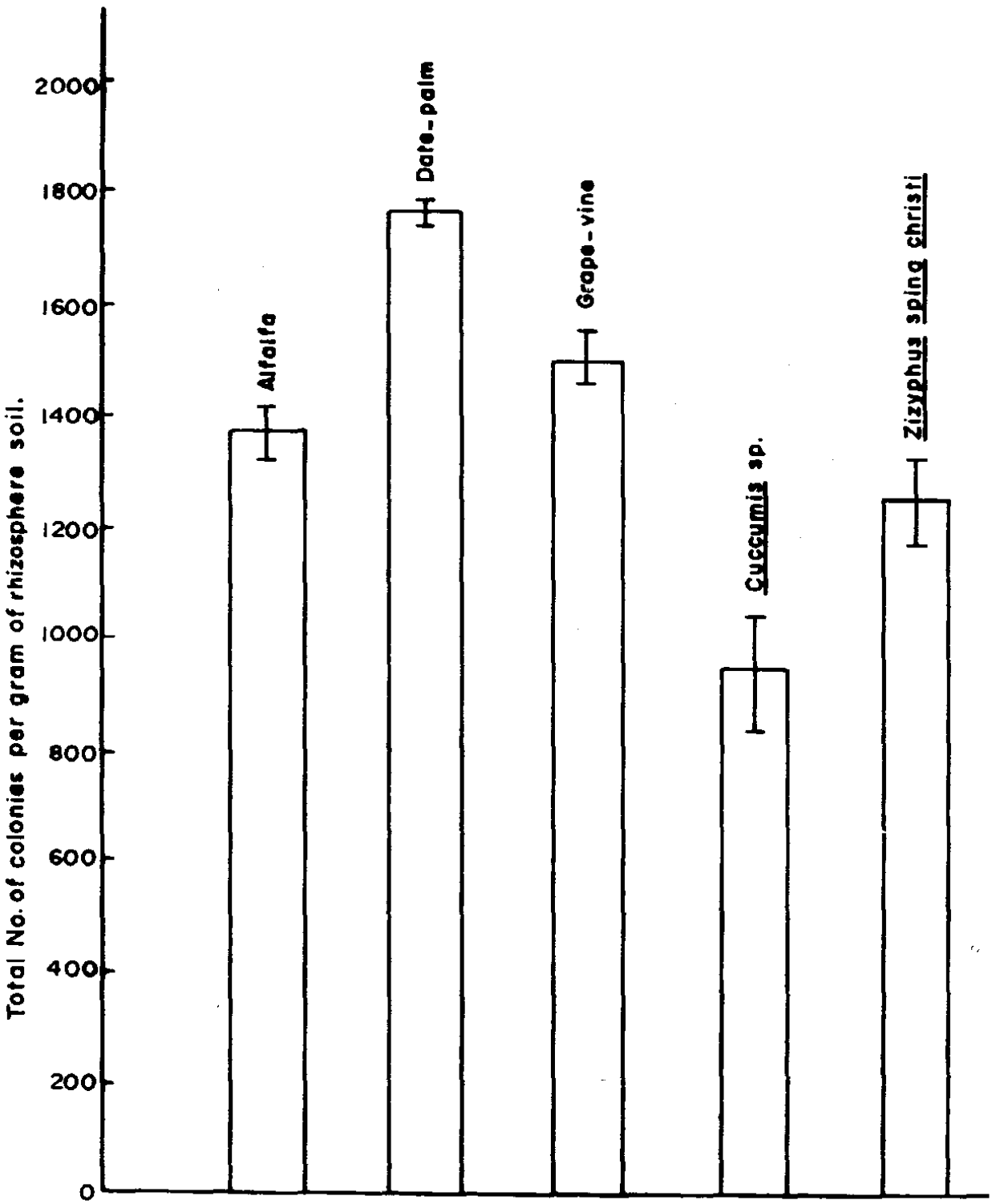

Fig. 1. **Total number of fungal colonies per gram of rhizosphere soil on cellulose agar medium at room temperature (22-25°C). Readings are the mean of 5 replicates.**
I = Standard Deviation.

Table 1. **Total no. of colonies per gram of soil yielded by each fungal species (Readings are the mean of 5 replicate plate count for each type of soil incubated at room temperature 22-25°C)**

Fungal species	Rhizosphere soil from				
	Alfalfa	Date-palm	Grape-vine	*Cuccumis*	*Z. spina christi*
Absidia corymbifera (Cohn.) & A. Trotter	18±6	29±9	—	—	—
Alternaria alternate (Fr.:Fr.) Keissler	39±7	46±16	59±11	24±9	16±5
A. chlamydospora Mouchacca	—	36±12	—	—	—
A. phragmospora van Emden	—	—	32±7	—	—
A. tenuissima (Kunze: Fr.) Wiltshire	26±5	42±12	—	—	—
Aspergillus candidus Link: Fr.	—	—	15±4	7±3	—
A. clavatus Desm.	29±11	49±16	32±11	—	—
A. flavipes (Bain. & Sartory) Thom.	—	—	—	15±3	19±2
A. flavus Link: Fr.	46±11	62±16	59±15	46±12	29±16
A. fumigatus Fres.	54±15	42±10	46±10	52±13	43±12
A. nidulans (Eidam) Winter	21±3	—	—	—	16±2
A. niger van Tieghem	63±14	69±15	44±9	33±10	29±8
A. ochraceus Wilhelm	—	16±6	12±6	29±5	13±7
A. parasiticus Speare	—	23±6	22±11	—	—
A. sydowii (Bain. & Sartory) Thom & Church	—	—	—	—	13±7
A. terreus Thom	29±9	15±5	23±7	16±4	7±3
A. terricola March.	—	—	26±7	—	—
A. ustus (Bain.) Thom & Church	—	19±6	31±9	—	19±4
A. versicolor (Vuill.) Tiraboschi	16±3	25±5	—	—	—
Botryotrichum atrogriseum van Beyma	—	—	—	13±4	—
Chaetomium bostrychodes Zopf.	15±5	8±3	29±5	9±3	18±6
C. globosum Kunze: Fr.	39±8	35±5	45±11	17±5	21±9
C. olivaceum Cooke & Ellis	—	—	9±4	—	—

Table 1. Continued

Fungal species	Rhizosphere soil from				
	Alfalfa	**Date-palm**	**Grape-vine**	***Cuccumis***	***Z. spina christi***
C. sphaerale Chivers	—	—	—	14±4	7±3
C. subglobossum Sergejeva	12±5	15±4	—	—	—
Cladosporium herbarum (Pers.: Fr.) Link	—	—	16±7	12±5	—
C. sphaerospermum Penzig	16±4	12±5	8±3	8±3	11±4
Drechslera hawaiiensis (Bugnicourt) Subram. & Jain ex M.B. Ellis	29±9	34±9	22±6	16±4	21±9
D. rostrata (Drechsler) Richardson & Fraser	14±9	6±2	—	—	—
D. spicifera (Bain) V. Arx	31±6	46±13	39±8	28±8	34±6
D. teres (Sacc.) Shoemaker	—	—	—	—	9±4
Humicola grisea Traaen	—	—	—	9±4	—
Macrophomina phaseoli (Maubl.) Ashby	—	13±3	—	—	—
Microascus cinereus (Emile-Weil & Gaudin) Curzi	—	—	—	8±6	—
Monodictys sp.	—	—	—	—	11±2
Mucor circinelloides van Tieghem	42±11	26±8	54±5	32±8	36±6
Paecilomyces varioti Bain	16±3	19±5	13±7	23±5	27±9
Penicillium asperosporum G. Smith	—	14±5	—	—	—
P. chrysogenum Thom	36±5	49±8	59±11	24±7	26±5
P. citrinum Thom	—	19±5	—	—	—
P. corylophilum Dierck x	—	—	—	13±4	—
P. decumbens Thom	—	13±5	—	—	—
P. frequentans Westling	16±2	—	—	—	—
P. funiculosum Thom	—	—	—	—	11±3
P. nigricans Bain. ex Thom	—	—	19±5	—	—
P. notatum Westling	23±5	16±6	31±8	—	—

Table 1. Continued

Fungal species	Rhizosphere soil from				
	Alfalfa	**Date-palm**	**Grape-vine**	***Cuccumis***	***Z. spina christi***
Phoma humicola Gilman & Abott	—	—	—	12±4	—
Stachybotrys atra Corda	—	16±5	33±9	—	—
S. bisbyi (Srinivasan) Barron	—	—	—	—	8±4
S. sp.	—	—	—	7±4	—
Torula ellissi	*16*±5	11±5	—	—	—
T. herbarum Link.: Fr.	—	—	11±5	17±3	—
Trichoderma harzianum Rifai	12±3	16±5	25±5	8±3	11±4
T. viride Pers.: Fr.	—	12±5	—	—	—
Ulocladium atrum Preuss	13±4	36±10	35±9	—	12±4
U. chartarum (Preuss) Simmons	—	19±5	29±4	7±4	—
U. botrytis Preuss	—	—	—	—	16±5
U. chlamydosporum Mouchacca	26±6	—	—	—	15±7
U. consortiele (Thum.) Simmons	—	16±5	—	—	—
U. tuberculatum Simmons	8±4	16±5	—	—	—
Zygorhinchus moelleri Vuill	—	—	15±6	8±3	—
Species 61	27	36	30	28	27
Genera 20	13	14	12	17	12

± Standard deviation

Table 2. Percent of cleared-zone by fungi (four days old culture) (readings are the mean of 5 replicates at room temperature 22-25°C)

Fungal species	Rhizosphere soil from				
	Alfalfa	**Date-palm**	**Grape-vine**	***Cuccumis***	***Z. spina christi***
Absidia corymbifere	86.6±5	46.7±11	—	—	—
Aleternaria alternata	20±5	46.6±7	26.6±4	53.3±7	26.6±8
A. chlamydospora	—	60.±10	—	—	—
A. phragmospora	—	—	13.3±3	—	—
A. tenuissima	33.3±6	35.4±4	—	—	—
Aspergillus candidus	—	—	23.3±6	22.9±5	—
A. clavatus	43.6±7	62.9±8	60±5	—	—
A. flavipes	—		—	22.9±6	23.4±8
A. flavus	34.6±6	47.7±5	73.6±9	40.5±7	20.5±5
A. fumigatus	39.3±8	43.4±7	35.8±7	45.5±6	42.4±7
A. nidulans	22.5±6	49.5±9	22.6±5	43.5±8	20.5±5
A. niger	32.6±6	49.5±9	22.6±5	43.5±8	20.5±5
A. ochraceus	—	22.6±6	26.6±6	18.6±5	25.5±7
A. parasiticus	—	24.7±5	35.5±6	—	—
A. sydowii	—	—	—	—	16.6±4
A. terreus	18.6±4	23.4±5	20.6±5	21.5±5	18±5
A. tericola	—	—	15.6±4	—	—
A. ustus	—	26.6±7	22.5±6	—	19.6±4
A. versicolor	29.6±6	24.8±6	—	—	—
Botryotrichum atrogriseum	—	—	—	13.7±5	—
Chaetomium bostrychodes	46.5±9	63.8±10	54.4±8	72.6±10	44.7±12
C. globosum	36.9±9	39.9±8	42.6±7	47.7±8	43.5±6
C. olivaceum	—	—	12.4±3	—	—
C. sphaerale	—	—	—	11.6±3	9.5±4

Table 2. Continued

Fungal species	Rhizosphere soil of				
	Alfalfa	Date-palm	Grape-vine	*Cuccumis*	*Z. spina christi*
C. subglobosum	26.5±5	36.5±8	—	—	—
Coladosporium herbarum	—	—	16.9±4	23.6±5	—
C. sphaerospermum	39±4	33±5	49±5	42.6±7	53.4±7
Drechslera hawaiiensis	22.4±3	29.5±5	28.5±5	28.7±4	26.6±7
D. rostrata	14.5±6	9.5±5	—	—	—
D. spicifera	34±4	36±4	36.5±5	41.3±7	35.5±5
D. teres	—	—	—	—	16.7±5
Humicola grisea	—	—	—	15.5±5	—
Macrophomina phaseoli	—	11.6±3	—	—	—
Microascus cinereus	—	—	—	11.5±4	—
Monodictys sp.	—	—	—	—	16.5±5
Mucor circinelloides	45±11	59±15	53.5±7	32.6±7	36.5±7
Paecilomyces varioti	23.6±7	26±5	24.5±6	21.6±6	25±5
Penicillium asperospermum	—	36.7±7	—	—	—
P. chrysogenum	62.6±9	55.9±7	69±11	62.5±9	49.5±8
P. citrinum	—	23.7±6	—	—	—
P. corylophilum	—	—	—	18.6±5	—
P. decumbens	—	42.5±13	—	—	—
P. frequentens	32.6±11	—	—	—	—
P. funiculatum	—	—	—	—	23±7
P. nigricans	—	—	39.8±9	—	—
P. notatum	46.5±7	26.6±7	59±11	—	—
Phoma humicola	—	—	—	16.6±6	—

Table 2. Continued

Fungal species	Rhizosphere soil from				
	Alfalfa	**Date-palm**	**Grape-vine**	***Cuccumis***	***Z. spina christi***
Stachybotrys atra	—	36.7±7	46±9		—
S. bisbyi	—	—	—		29.6±7
S. sp.	—	—	—	79±9	—
Torula ellissi	22.5±7	16.7±4	—		—
T. herbarum	—	—	16.5±3	18.5±3	—
Trichoderma harzianum	39.9±11	22.6±12	22.6±9	62.6±4	48.8±13
T. viride	—	36.6±8	—	—	—
Ulocladium atrum	42.9±6	59.6±7	46.6±11	—	33.3±6
U. botrytis	—	—	—	—	26.7±4
U. chartarum	—	36.5±5	56.7±7	42.2±9	—
U. chlamydosporum	36.9±8	—	—	—	23.5±6
U. consortiele	—	26±11	—	—	—
U. tuberculatum	18.8±8	16.7±4	—	—	—
Zygorhinchus moelleri	—	—	12.6±3	19.5±5	—

± Standard deviation

$$\text{Percent cleared-zone} = \frac{\text{Diameter of cleared-zone - Diameter of colony}}{\text{Diamter of colony}} \times 100$$

in Saudi Arabia [11; 12; 14; 22; 23 b]. Khaliel [24] reported *Penicillium* as the most dominant genus but *Aspergillus* was predominant in the present study, followed by *Penicillium* (9 species), *Ulocladium* (6 species), *Chaetomium* (5 species), *Alternaria* (4 species), and *Drechslera* (4 species). Almost the same sequence was also reported by Abdel - Hafez [11] for cellulase degrading fungi in soil. However, Abou - Heilah *et al.* [22] reported a different sequence of fungal genera. *Aspergillus parasiticus, Chaetomium sphaerale, Cladosporium sphaerospermum, Drechslera teres, Penicillium chrysogenum, Trichoderma harzianum, Torula ellissi, Ulocladium chartarum* and *U. tuberculatum* were not recorded by Abdel-Hafez [23] as cellulose degraders. On the other hand, many fungal species previously reported by Abdel - Hafez [23] as cellulose degraders, were not encountered in the present study.

The production of cellulase enzyme by a particular fungus varied with the source of isolation e.g. *Absidia corymbifera* isolated from alfalfa exhibits 87% cleared - zone, while the same fungus isolated from date-palm exhibit only 47% cleared-zone. Similarly, *Aspergillus flavus* isolate from grape-vine produced 64% cleared-zone while an isolate from *Zizyphus* produced 21% cleared - zone (Table 2). Lime *et al.*, (1985) reported similar results while working on the production of cellulase by *Aspergillus niger* isolated from different sources. *Absidia corymbifera* isolated from alfalfa produced a large cleared-zone (86.8%), indicating that it was an excellent producer of cellulase enzyme. Many fungi isolated from different sources produced a cleared-zone of over 60%. *Drechslera rostrata* was the only fungus that produced a cleared-zone of less than 10% when isolated from date-palm and a slightly higher quantity of cellulase (14.5%) by the isolate from alfalfa.

The present results indicated that production of cellulase enzyme by a particular fungus is highly dependant on the habitat from which the fungus was originated. These data may be useful for future researches on the type of materials needed to biodegrade because the production of enzyme is highly dependant on the origin of fungus. Furthermore, these local isolates may be of more useful for the biodegradation of waste materials (cellulosic) under indigenous environmental conditions than isolates of foreign origin.

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إنتاج إنزيم السيليولوز الخارجي بوساطة فطريات التربة بالمملكة العربية السعودية

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(سُلِّمَ في ٨/جماد الثانية/١٤١٣هـ، وقُبل للنشر في ٢٩/شعبان/١٤١٤هـ)

ملخص البحث. لقد تم فحص الفلورا الفطرية في المنطقة الجذرية للنباتات التالية: البرسيم، النخيل، العنب، شر الذئب (حيروق) والنبق بحثًا عن الفطريات التي لها القدرة على تحليل السيليولوز. كما تم عزل واحد وستين نوعًا فطريًا استطاع تحليل السيليولوز. ولقد كان فطر اسبرجيلس الفطر السائد يليه فطر بنسيليوم، بلوكلاديم، كيتوميم والترناريا ودريشيسليرا. كما تم التأكد من إنتاج إنزيم السيليولوز من الفلورا الفطريّة التي تم عزلها بوساطة طريقة المنطقة الشفّافة (عديمة اللون). وقد لوحظ من خلال الدراسة بأن النوع الفطري المعزول من مصادر مختلفة ينتج إنزيم السيليولوز بكميات مختلفة.