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Production of Extracellular Amylase by Soil Mycoflora

H.A. Bokhary and S. Parvez

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

ABSTRACT. Soils from the rhizosphere of cultivated plants (Alfalfa, Date-palm and Grape) and wild plants (*Cuccumis* sp. and *Zizyphus spina christi*) were screened for starch degrading fungi. A total number of 84 fungal species were isolated as starch-degraders. The predominant genera were *Aspergillus* (24 species), *Penicillium* (14 species), *Fusarium* (13 species), *Chaetomium* (10 species), *Mucor* (5 species) *Scytalidium* (4 species). The production of amylase by isolated fungi was confirmed by the cleared-zone technique.

The production of various enzymes by fungi has a great influence on human life, owing to its important role in the pharmaceutical, food, paper, textile and petroleum industries, (Pestana and Castillo, 1985, Kutzman 1983, Charney 1984). The biodegradation of waste materials is another example of the beneficial effects of these fungi (Hudson 1980), as the waste of garbage will become a great problem for our future generations, therefore preventive measure should be taken now to ease this future problem (Arab News Feb. 7, 1992).

Starch is a polysaccharide and a principal reserve food for seed plants. Hence, it should be degraded to smaller molecule sugars to be utilized by fungi (Kelley and Post, 1982), by the action of α-and β-amylase (Kelley and Post, 1982, Parry and Pawsey, 1984). Starch from various origins is used in the production of single-cell protein (SCP), provided it is first converted into maltose by amylolytic mycoflora (Casida 1991, Manilal *et al.* 1991).

Amylolytic fungi have hardly been studied in Saudi Arabia (Abdullah and El-Gindy 1987), in contrast to the biodegradation of other substrates like animal dung (Bokhary 1986a, Bokhary and Parvez 1986), truffles (Bokhary *et al.* 1990), cereal grains (Bokhary 1991), organic matters (Bokhary *et al.* 1984), cellulose

(Bokhary and Parvez 1992c), petroleum oil (Bokhary and Parvez 1992d) etc. Therefore, the present work was carried out to study the amylolytic mycoflora in the rhizosphere of cultivated and wild plants. The production of enzyme by fungi was confirmed by the cleared-zone technique (a semiquantitative method).

Materials and Methods

Soil samples were collected from rhizosphere soils of cultivated plants; Alfalfa, Date-palm, Grape-vine, and wild plants; *Cuccumis* sp. (sand-dune) and *Zizyphus spina christi* (desert plant).

Rhizospheric fungi were isolated by the dilution plate method as adopted earlier (Bokhary *et al.* 1984). Starch (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 also added in the medium to reduce the spread of fast growing fungi, while streptomycin sulphate (0.033 g/L) was added to eliminate bacterial growth. Inoculated plates were incubated at room temperature (22-25 °C). Isolated cultures were maintained on Dox-starch medium.

Detection of amylase production by isolated mycoflora was carried out by the cleared-zone technique (Lim *et al.* 1985). The percent cleared-zone with respect to colony size was taken as an indication of the level of enzyme activity. Identification of isolated fungi was carried out with the help of following leterature, Ellis (1971, 1976), Gilman (1971), Howard (1983), Nelson *et al.* (1983), Pitt (1979), Ramirez (1982), Raper and Fennell (1965), Schipper (1978), Zycha *et al.* (1969).

Results and Discussion

Rhizosphere soils of Alfalfa (*Medicago sativa* L.), Date-palm (*Phoenix dactylifera* L.), Grap-vine (*Vitis vinifera*), sand-dune plant (*Cuccumis* sp.) and desert plant (*Zizyphus spina christi* L. Wild) yielded between 1239 to 2836 colonies per gram of soil. The highest number of fungal colonies per gram of soil was found in Alfalfa and the lowest in *Cuccumis* sp. (Fig. 1).

A number of 84 fungal species belonging to 13 genera were isolated as starch degraders (Table 1). Rhizosphere soil of alfalfa yielded 26 species of 11 genera, Date-palm 42 species of 12 genera, grape-vine 50 species of 10 genera, *Cuccumis* sp. 26 species of 8 genera and there were 27 species of 9 genera from rhizosphere soil of *Z. spina christi*. *Aspergillus* was the predominant genus yielding 24 species, followed by *Penicillium* with 14 species, *Fusarium* with 13 species, *Chaetomium* with 10 species, *Mucor* with 5 species, and *Scytalidium* yielded 4 species.

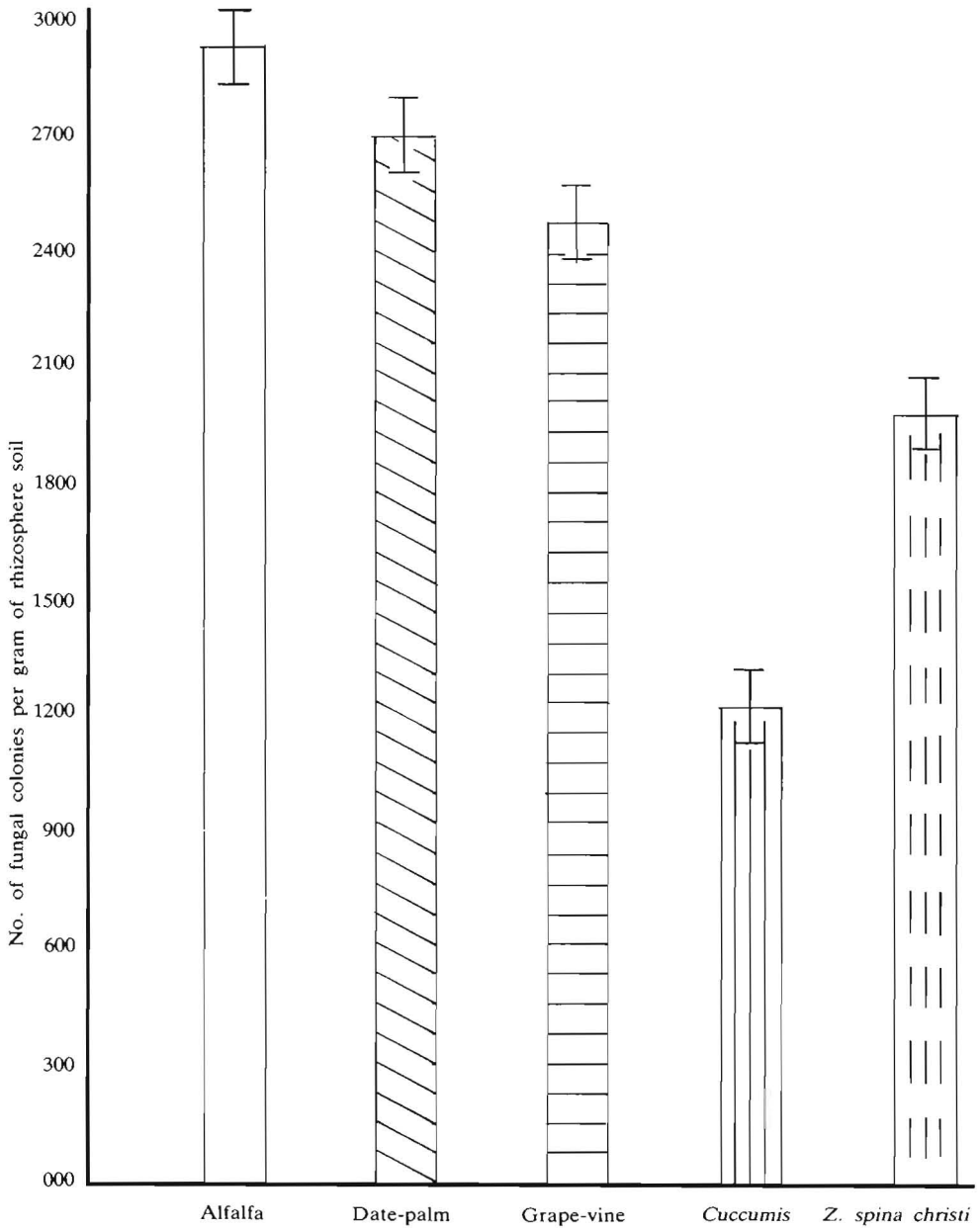

Fig. 1. Total number of fungal colonies per gram of rhizosphere soil isolated on Dox-starch medium at room temperature (22-22°C). Vertical bar represent standard deviation.

Table 1. Total number of colonies per gram of soil yielded by each fungal species* at room temperature (22-25°C)

Fungi	Rhizosphere soil of				
	A	B	C	D	E
Alternaria alternate (Fr.: Fr.) Keissler	39 ± 5	43 ± 5	28 ± 6	35 ± 7	49 ± 7
A. chlamydospora Mouchaca	–	46 ± 4	–	–	–
A. humicola Oudem	–	–	32 ± 6	–	–
Aspergillus amylovorus Panasenko ex Samson	49 ± 9	56 ± 8	62 ± 9	34 ± 5	46 ± 7
A. apica Mehrotra & Basu	–	–	36 ± 7	–	–
A. avenaceus G. Smith	–	–	26 ± 5	–	–
A. caespitosus Raper & Thom	–	–	35 ± 4	–	–
A. candidus Link : Fr.	29 ± 5	46 ± 6	52 ± 6	16 ± 4	26 ± 7
A. carbonarius (Bain.) Thom	43 ± 6	22 ± 5	57 ± 7	12 ± 3	22 ± 4
A. carneus (van Tiegh.) Blochwitz	18 ± 5	26 ± 5	39 ± 4	–	12 ± 3
A. clavatus Desm.	–	–	32 ± 4	–	–
A. ellipticus Raper & Fennell emend. Al-Musallam	36 ± 5	59 ± 11	66 ± 12	21 ± 5	32 ± 7
A. flavipes (Bain. & Sartory) Thom & Church	–	26 ± 3	–	–	–
A. flavus Link: Fr.	46 ± 5	49 ± 7	26 ± 5	16 ± 4	18 ± 4
A. fumigatus Fres.	26 ± 5	33 ± 5	39 ± 5	23 ± 5	29 ± 5
A. nidulans (Eidam) Winter	–	–	–	17 ± 4	23 ± 4
A. niger van Tieghem	43 ± 5	32 ± 4	46 ± 7	23 ± 5	29 ± 4
A. ochraceus Wilhelm	–	–	43 ± 4	18 ± 3	16 ± 4
A. oryzea (Ahlburg) Cohn	24 ± 5	39 ± 4	49 ± 6	–	–
A. parasiticus Speare	–	–	–	15 ± 2	19 ± 4
A. phoenicis (Corda) Thom	–	–	–	17 ± 4	29 ± 5
A. raperi Stolk & Meyer	–	–	–	16 ± 4	–
A. restrictus G. Smith	–	32 ± 4	–	–	23 ± 5
A. rugulosus Thom & Raper	–	–	–	17 ± 5	–
A. terreus Thom.	26 ± 4	29 ± 5	43 ± 7	16 ± 6	13 ± 5
A. ustus (Bain) Thom & Church	–	–	–	–	36 ± 5
A. versicolor (Vuill) Tiraboschi	–	–	–	17 ± 3	–
Blastomyces brasiliensis (Splendore) Conant	26 ± 5	23 ± 5	–	–	–
Chaetomium bostrychodes Zopf.	–	32 ± 5	–	–	–
C. carinthiacum Sorgel	–	–	18 ± 4	–	–
C. cochlioides Palliser	–	17 ± 5	–	–	–
C. globosum Kunze: Fr.	26 ± 4	32 ± 5	46 ± 6	–	–
C. indicum Corda	–	–	32 ± 5	–	–
C. murorum Corda	–	32 ± 5	–	–	–
C. nigricolor Ames.	–	–	25 ± 5	–	–
C. robustum Ames	–	–	–	16 ± 4	–
C. senegalens Ames	–	–	18 ± 4	–	12 ± 5
C. uniporum Aue & E. Muller	–	–	–	26 ± 6	39 ± 4
Drechslera australiensis Scharif ex Lam	26 ± 5	39 ± 4	46 ± 5	12 ± 2	18 ± 4
Fusarium chlamydosporum Wollenw. & Reinking	–	18 ± 4	29 ± 6	–	14 ± 5
F. ciliatum Link	23 ± 4	–	–	–	–

Table 1. Contd.

F. coeruleum (Lib.) Sacc.	–	–	36 ± 5	–	–
F. culmorum (W.G. Smith) Sacc.	–	–	26 ± 4	–	–
F. equisetum (Corda) Sacc.	–	46 ± 5	–	–	–
F. eumartii Carpenter	–	36 ± 6	46 ± 7	–	–
F. flocciferum Corda	26 ± 5	32 ± 5	43 ± 7	–	–
F. graminearum Schwabe	–	–	–	–	26 ± 4
F. longipes Wollenw. & Reinking	–	–	26 ± 4	16 ± 3	–
F. oxysporum Schlecht.	35 ± 6	43 ± 4	25 ± 5	–	–
F. proliferatum (Matsushima) Nirenberg	–	–	26 ± 4	–	–
F. solani (Mart.) Sacc.	49 ± 7	56 ± 8	63 ± 7	–	–
F. tricinctum (Corda) Sacc.	–	–	21 ± 5	–	–
Geotrichum candidum Link	32 ± 6	25 ± 6	39 ± 7	–	18 ± 5
G. capitatum (Diddens & Lodder) V. Arx	–	18 ± 4	–	–	–
Mucor circinellodides van Tighem.	52 ± 7	32 ± 6	24 ± 5	18 ± 7	26 ± 5
M. hiemalis Wehmer	16 ± 3	39 ± 7	26 ± 4	–	–
M. indicus Lender	–	–	16 ± 3	–	–
M. microsporus Namyslowski	–	16 ± 3	–	–	–
M. mucedo L. ex Fr.	16 ± 5	21 ± 5	–	–	–
M. pusillus Lindt	19 ± 3	26 ± 3	–	–	–
M. racemosus Fres.	–	18 ± 4	–	–	–
Penicillum brevicompactum Diercks	–	–	12 ± 3	–	–
P. brunneum Udagawa	–	–	16 ± 3	–	–
P. chrysogenum Thom	27 ± 4	24 ± 5	36 ± 5	18 ± 3	24 ± 4
P. citreonigrum Dierckx	–	–	12 ± 4	–	–
P. cyaneum (Bain. & Sartory) Biourge	–	13 ± 2	–	–	–
P. dierckxii Biourge	–	–	14 ± 2	–	–
P. expansum Link	–	17 ± 3	18 ± 3	–	–
P. funiculosum Thom	–	26 ± 4	–	–	–
P. islandicum Sopp	–	–	15 ± 4	–	–
P. phialosporum Udagawa	–	–	17 ± 3	–	–
P. rubrum Stoll	–	18 ± 5	–	–	–
P. sclerotiorum van Beyma	–	–	–	19 ± 4	–
P. thomii Maire	–	–	–	–	16 ± 4
P. verrucosum Dierecks	–	–	17 ± 3	–	–
Petriellidium sp.	21 ± 4	–	–	–	–
Phialophora sp.	–	24 ± 3	36 ± 4	–	–
Rhizopus microsporus van Tieghem	–	–	–	19 ± 3	25 ± 4
R. oryzea Went & Prinsen Geerligs	16 ± 3	26 ± 4	–	–	–
R. stolonifer (Ehrenb.: Fr.) Vuill.	–	–	–	19 ± 4	–
Scytalidium album Beyer & Klingstrom	–	–	23 ± 4	–	16 ± 4
S. aurantiacum Klingstrom & Beyer	–	39 ± 5	–	–	–
S. lignicola Pesante	–	–	41 ± 5	18 ± 4	–
S. terminale Rao & de Hoog	–	–	7 ± 2	–	–
Total Species/Genera 84/13	26/11	42/12	50/10	26/8	27/9

∗ Readings are the mean of 5 replicate for each type of soil
± Standard Deviation from the mean
A = Alfalfa B = Date-palm C = Grape-vine
D = *Cuccumis* sp. E = *Zizphus spina christi*

The production of amylase by a particular fungus appeared to be highly dependent on the origin of the fungus (Table 2). Considerable differences were thus observed in the percentage of cleared-zone exhibited by the same fungus isolated from different sources. For example, an *Aspergillus amylovorus* isolate from rhizosphere soil of grape-vine exhibited 89% cleared-zone, while an isolate of this fungus from the rhizosphere of *Z. spina christi* gave a percentage cleared-zone of 26% only. On the other hand, certain fungi like *P. islandicum, P. phialosporum* and *P. thomii* which grew poorly on starch agar medium, showed no cleared-zone, indicating they were not producers of amylase. *A phialosphora* sp. isolate of grape-vine did not produce amylase although its isolate from date-palm produce a cleared-zone of 39%. *Alternaria* sp. in general, were the poorest in the production of amylase, while *Aspergillus* sp. were the best. *Aspergillus amylovorus, A. carbonarius, A. ellipticus, A. fumigatus, A. niger* were the best producers of amylase among species of *Aspergillus*. *Fusarium oxysporum* was the best as compared to other *Fusarium* species. *Mucor circinelloides* produced more amylase than other *Mucor* species. *Penicillium chrysogenum* was not only the highest producer of amylase among Penicillium species but produced amylase more than *A. amylovorus*. *Mucor circinelloides* could be ranked third among the best producers of amlyase while *P. cyaneum, P. sclerotiorum* and *Petrillidium* sp. were isolated from one source only, they could be placed among good producers of amylase, exhibiting above 50% cleared-zone.

Table 2. Percentage of cleared-zone by fungi at room temperature (22-25°C)

Fungi	Percentage of cleared-zone*				
	Alfalfa	Date-palm	Grape-vine	*Cuccumis*	*Z. spina christi*
Alternaria alternate	9 ± 2	13 ± 3	26 ± 5	7 ± 2	11 ± 3
A. chalmydospora	–	3.2 ± 1	–	–	–
A. humicola	–	–	8.6 ± 1.5	–	–
Aspergillus amylovorus	36 ± 4	78 ± 9	89 ± 11	66 ± 9	26 ± 4
A. apica	–	–	11.5 ± 2	–	–
A. avenaceus	–	–	39 ± 5	–	–
A. caespitosus	–	–	24 ± 4	–	–
A. candidus	21 ± 4	24 ± 4	26 ± 5	22 ± 3	24 ± 6
A. carbonarius	42 ± 4	59 ± 6	82 ± 9	33 ± 5	46 ± 7
A. carneus	26 ± 5	36 ± 5	12 ± 3	–	59 ± 7
A. clavatus	–	–	26 ± 7	–	–
A. ellipticus	32 ± 6	39 ± 6	35 ± 7	63 ± 6	73 ± 7
A. flavipes	–	12 ± 2	–	–	–
A. flavus	23 ± 6	43 ± 5	16 ± 3	45 ± 5	69 ± 8
A. fumigatus	54 ± 4	78 ± 12	86 ± 11	69 ± 13	32 ± 6
A. nidulans	–	–	–	32 ± 5	9 ± 2

Table 2. *contd.*

Fungi	Percentage of cleared-zone*				
	Alfalfa	Date-palm	Grape-vine	*Cuccumis*	*Z. spina christi*
A. niger	33.4 ± 5	39.9 ± 5	42 ± 7	73.5 ± 9	67 ± 10
A. ochraceus	–	–	16 ± 3	39 ± 5	43 ± 7
A. oryzae	46.6 ± 11	53 ± 7	21.6 ± 3	–	–
A. parastiticus	–	–	–	43 ± 8	69 ± 10
A. phoenicis	–	–	–	*16 ± 6*	*41 ± 9*
A. raperi	–	–	–	17 ± 5	–
A. restrictus	–	43 ± 9	–	–	17 ± 5
A. rugulosus	–	–	–	29 ± 5	–
A. terreus	16 ± 3	20.5 ± 5	18 ± 4	52 ± 6	43 ± 5
A. ustus	–	–	–	–	33 ± 6
A. versicolor	–	–	–	31 ± 5	–
Blastomyces brasiliensis	15 ± 3	29 ± 5	–	–	–
Chaetomium bostrychodes	–	31 ± 5	–	–	–
C. carinthiacum	–	–	13 ± 2	–	–
C. cochlioides	–	25 ± 4	–	–	–
C. glabosum	21 ± 3	25 ± 4	23 ± 4	–	–
C. indicum	–	–	18 ± 4	–	–
C. murorum	–	29 ± 5	–	–	–
C. nigricolor	–	–	27 ± 4	23 ± 4	–
C. robustum	–	–	–	–	–
C. senegalens	–	–	31 ± 3	–	12 ± 2
C. uniporum	–	–	–	43 ± 7	71 ± 6
Drechslera australiensis	18 ± 3	29 ± 5	26 ± 5	22 ± 3	25 ± 4
Fusarium chlamydosporum	–	33 ± 3	45 ± 5	–	16 ± 3
F. ciliatum	12 ± 2	–	–	–	–
F. coeruleum	–	–	49 ± 9	–	–
F. culmorum	–	–	13 ± 2	–	–
F. equisetum	–	19 ± 3	–	–	–
F. eumartii	–	6 ± 2	9 ± 2	–	–
F. flocciferum	13 ± 2	19 ± 3	15 ± 4	–	–
F. graminearum	–	–	–	–	42 ± 5
F. longipes	–	–	19 ± 4	23 ± 4	–
F. oxysporum	41 ± 6	52 ± 5	16 ± 3	–	–
F. proliferatum	–	–	17 ± 5	–	–
F. solani	16 ± 3	27 ± 5	23 ± 5	–	–
F. tricinctum	–	–	6 ± 2	–	–
Geotrichum candidum	33 ± 4	47 ± 4	76 ± 4	–	39 ± 4
G. capitatum	–	12 ± 2	–	–	–
Mucor circinelloides	63 ± 6	79 ± 4	82 ± 9	76 ± 4	70 ± 7
M. hiemalis	20 ± 4	36 ± 6	41 ± 5	–	–
M. indicus	–	–	15 ± 4	–	–
M. microsporus	–	32 ± 5	–	–	–
M. mucedo	26 ± 7	32 ± 6	–	–	–
M. pusillus	32 ± 4	16 ± 5	–	–	–

Table 2. *contd.*

Fungi	Percentage of cleared-zone*				
	Alfalfa	Date-palm	Grape-vine	Cuccumis	*Z. spina christi*
M. racemosus	–	11 ± 3	–	–	–
Penicilium brevicompactum	–	–	17 ± 5	–	–
P. brunneum	–	–	12 ± 3	–	–
P. chrysogenum	63 ± 16	39 ± 4	82 ± 5	79 ± 9	89 ± 11
P. citreonigram	–	–	69 ± 11	–	–
P. cyaneum	–	53 ± 6	–	–	–
P. dierckxii	–	–	23 ± 4	–	–
P. expansum	–	–	29 ± 6	35 ± 5	–
P. funiculosum	–	46 ± 6	–	–	–
P. islandicum	–	–	**	–	–
P. phialosporum	–	–	**	–	–
P. rubrum	–	13 ± 5	–	–	–
P. sclerotiorum	–	–	–	52 ± 7	–
P. thomii	–	–	–	–	**
P. verrucosum	–	–	21 ± 4	–	–
Petriellidium sp.	69 ± 11	–	–	–	
Phialophora sp.	–	39 ± 5	**	–	–
Rhizopus microsporus	–	–	–	35 ± 6	39 ± 5
R. oryzde	14 ± 4	23 ± 6	–	–	–
R. stolonifer	–	–	–	22 ± 3	–
Scytalidium album	–	–	**	–	19 ± 4
S. aurantiacum	–	32 ± 5	–	–	–
S. lignicola	–	–	29 ± 4	**	–
S. terminale	–	–	21 ± 5	–	–

* Readings are the mean of 5 replicate for each sample
± Standard Deviation

** = Faint growth without production of amylase (No Cleared-Zone detected)

Aspergillus and *Penicillium* were the commonest genera in almost all types of habitat studied in Saudi Arabia (Abdel-Aziz and Kassim 1972, Abdel-Hafez 1982, Bokhary 1986b, Bokhary *et al.* 1984, Bokhary and Parvez 1986, 1992a, b, c). *Alternaria* species was reported earlier as poor producers of amylase (Abdullah and El-Gindy 1987) and *Aspergillus* species as good producers of extracellular enzymes capable of breaking down various types of complex organic compound (Barbesgaard 1977). The quantity and the production of extracellular enzymes were highly dependent on the source of the fungi (Hankin and Anagostakis 1975) and even the same fungus might not produce amylase from one substrate while producing much enzyme from other substrate. Lim *et al.* (1985) reported that

Aspergillus niger isolated from rice grains and soil, either did not produce amylase or exhibit partial hydrolysis only, while the same fungus from other sources produced a good quantity of amylase. This means that cultural conditions affect the production of amylase.

The different isolates of one fungus from different sources did not differ each other as far as morphology is concerned (Lim *et al.* 1985).

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انتاج أنزيم الاميلز خارج الخلية بواسطة فلورا التربة الفطرية

حسن عبدالحكيم بخاري و ثروت بارويز

قسم النبات والأحياء الدقيقة ـ كلية العلوم ـ جامعة الملك سعود
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تلعب الفطريات دوراً مهما في حياتنا لإنتاجها أنزيمات مختلفة الأنواع والتي بدورها تدخل أساساً في صناعات كثيرة مثل الصيدلة، والطعام، وصناعة الأوراق، والأنسجة وكذلك الصناعة البترولية، إضافة إلى استخدامها في التخلص من النفايات، ولقد تمت دراسة مادة النشا كمثل للمحللات البيولوجية التي تتم بواسطة الفطريات ومادة النشا هي احدى المواد الكربوهيدراتية المعقدة التي تخزن كمادة أساسية في بذور النباتات أو أعضاء التخزين أو الجذور، فقد وجد أن الفطريات لكي تستطيع استخدام النشا يجب أن تتحلل إلى جزيئات بسيطة حتى تستطيع النمو وتحليل هذه المادة.

ولهذا فلقد عني هذا البحث بدراسة الفطريات التي تقوم بتحليل مادة النشا، ولذلك فلقد تم أخذ عينات من التربة للمنطقة المحيطة بالجذر للنباتات التالية: البرسيم، النخيل، والسدر (النبق) ونبات الكيوميس وذلك لعزل الفطريات المحللة للنشا. ولقد كان العدد الكلي لهذه الأنواع ٨٤ أربعة وثمانون نوعاً. وكانت الأجناس السائدة هي *Aspergillus* (٢٤ نوعا)، *Penicillium* (١٤ نوعاً)، *Fusarium* (١٣ نوعاً) *Chaetomium* (١٠ أنواع) *Mucor* (٥ أنواع) و *Scytalidium* (٤ أنواع). كما تم التأكد من إنتاج أنزيم الأميليز من هذه الفطريات بواسطة طريقة المنطقة الشفافة (الرائقة) Cleared-zone technique.