Qatar Univ. Sci. Bull. (1984) 4: 85-93

SOME ENZYMATIC ACTIVITIES OF FUNGI ISOLATED FROM COTTON SEEDS AND COTTON SEED PRODUCTS

By

ISMAIL A. EL-KADY, MOHAMED B. MAZEN*
and SABAH M. SABER
Department of Botany, Faculty of Science,
Assiut University, Assiut,
Egypt.

Key words: Cotton seeds, Enzymes, Fungi, Lipase, Amylase and Protease.

ABSTRACT

Five hundred different cultures which belong to fifteen genera and forty-one species, isolated from cotton seeds and cotton seed products were studied for proteolytic, amylolytic and lipolytic activities. About 80% of the cultures tested proved to be protease-producers. High proportions of protease producing cultures were found in the genera *Aspergillus* (97.73%), *Fusarium* (95.83%) and *Penicillium* (46.34%). Most of the aspergilli which belong to the species *A. flavus, A. fumigatus, A. sydowii* and *A. terreus* displayed high proteolytic activities. About 88% of the cultures tested proved to be amylase-producers. High proportions of amylase producing cultures were found in the genera *Drechslera* (100%), *Fusarium* (95%), *Penicillium* (87%), *Alternaria* (81%) and *Aspergillus* (80%). About 88% of the cultures tested proved to be of lipolytic activity. High proportions of lipase producing cultures were found in the genera *Fusarium* (100%), *Aspergillus* (83.1%) and *Penicillium* (76.8%). Most of the lipase producers belonged to *F. moniliforme* (100%), *F. oxysporum* (98%), *P. chrysogenum* (95%), *A. flavus* (92%), *A. fumigatus* (91%), *A. niger* (85%) and *P. notatum* (81%).

INTRODUCTION

Stored grain and oil seeds and their products present a particularly fertile field for fungal colonization. These fungi have the potential of producing many changes associated with the reduction of seed quality. The extent of such changes depend upon storage conditions, length of storage, and predominating fungal species. The fungal-induced changes in stored grain can include loss of germination, heat damage, production of toxic metabolites, increase in nonprotein nitrogen, reduction in sugars, fatty acids, and loss of nutritive value (Christensen & Kaufmann, 1965 and Lynch, 1972).

Recently El-Kady *et al.*, (1984) studies were conducted in this laboratory to determine the mycoflora of cotton seeds and cotton seed products (cotton seed meal, cake, crude oil and refined oil). The material for this study was collected following the crop year 1981. The present study describes a survey of the enzymes activities (proteolytic, amylolytic and lipolytic) of the isolated cultures, which degrade some of the major constituents of cotton seed and cotton seed products.

* Present address: University of Qatar, P.O. Box 2713, Doha, Qatar.

MATERIALS AND METHODS

Production of enzymes by the different cultures were assayed using solid media, following the method previously used by Berkenkamp (1973) and Hankin & Anagnostakis (1975). The use of solid media permits the rapid screening of large populations of fungi for the presence or absence of specific enzyme.

Proteolytic activity

Detection of proteolytic enzymes was estimated as described by Society of American Bacteriologists (1957).

Two percent gelatin solution was prepared by dissolving the gelatin powder in water using a boiling water bath. The medium was adjusted to a pH of 6.5 - 7.0. Aliquots of 2 ml each, were poured in test tubes. The tubes were kept in a refrigerator until the solution setted. Standard disc (5 mm diameter) from a culture of each fungus, previously grown on Czapek's agar medium for 7 days at 28^{0}C, was placed upside down, on the surface of the solidified gelatin in the tube. The inoculated tubes were incubated at 28^{0}C for 24 hours. After the incubation period, the tubes were kept again in the refrigerator for 4 hours, to ensure that liquefaction is not due to heat effect. Fungi complete moderate, partial or no gelatin liquefaction denoting good, moderate, low or no proteolytic activity, respectively.

Amylolytic activity

The method of the Society of American Bacteriologists (1957) was used to determine the amylolytic activity of the fungi. Inoculum was grown on Czapek's agar medium for 7 days at 28^{0}C. Except for the fact that 1 gm of soluble starch was added per liter, the experimental medium was the same as the inoculum medium. One ml portions of the sterile medium were poured in each Petri dish (9 cm diameter). Each dish was inoculated with a 5 mm disc with the mycelium side on the agar. Following incubation in darkness for 3 days at 28^{0}C, the dishes were flooded with an iodine solution (KI, 15 gm; I_2, 3 gm per liter of distilled water). A zone void of blue indicated the production of amylase.

Lipolytic activity

Quantitative estimation of lipase(s) using agar diffusion assay, described by Lawrence *et al.*, (1967) was used. A 0.2% tributyrin was emulsified then supplemented with 1.5% agar Difco) for solidification. 10 ml portions of the sterile medium were poured in each Petri dish of 9 cm diameter. Each dish was inoculated with a 5 mm. disc (with the mycelium side on the agar) from each fungus, previously grown on Czapek's agar medium for 7 days at 28^{0}C. The dishes were incubated for 24 hours at 28^{0}C after which diameters of clear zones were measured. Diameter of clear zones were compared with those obtained with standard curve of pencreatic lipase. Readings were taken in triplicates, means were calculated and values were approximated for the nearest 0.5 mm diameter.

RESULTS AND DISCUSSION

The results indicated that about 61%, 11% and 6% of the cultures tested proved to be good, moderate or low protease producers, respectively. About 20% of the tested isolates did not show any proteolytic activities. The proteolytic cultures are representative of different groups of fungi. Members of the same genus and even species, varial from nonproteolytic types to high protease producers. This is in harmony with the results of Dion (1950) and Das *et al.* (1979),

who found that within the same species, different isolates showed great variation in the production of proteases. Actually gross differences in physiological activities between different strains of the same species have been reported for almost every species of micro-organisms used in an industrial process (Gray, 1959).

High proportion of protease producing cultures were found in the genera *Aspergillus* (97.73%), *Fusarium* (95.83%) and *Penicillium* (46.34%). This is contrary to the finding of Dion (1950) who reported the complete absence of any detectable proteases in the culture medium of 29 isolates of *Penicillium* and only two moderate proteases producers among 25 isolates of *Aspergillus*. However, those cultures were grown in submerged conditions in contrast to the present investigation in which solid medium was used. On the other hand, the present results are in complete agreement with those of Koch & Dedic (1957), who reported that aspergilli, followed by fusaria and penicillia are the most active producers of proteolytic enzymes.

A. flavus, A. fumigatus, A. sydowii and *A. terreus* proved to be of highly proteolytic activities. Mayne (1956) listed *A. flavus* as common on deteriorated cotton seeds and showed it to be the most active of several moulds isolated from this source. Members of the *Aspergillus flavus* group are commonly cultivated for the production of proteolytic enzymes.

Traditionally, they have been used in the preparation of soy source and other protein-rich fermented foods in the orient (Underkofler *et al.* (1959).

Although *A. terreus* is not known to be particularly, enzymatically active, the results clearly indicated that all the isolated tested of this species to have high to moderate proteolytic activities.

Most of the isolates belonging to *A. niger* headly gave any detectable proteases. The weakness to absence of proteolytic activity by some of the tested isolates could be explained by the test that our trials for proteolytic activity were done at the neutral pH (6.5 - 7.0). According to Zielinska and Naczkowski (1972), *A. niger* produced acid protease at pH (2.5 - 3.0).

Amylolytic activity

Most of the tested fungi (about 88%) showed amylolytic activity. This result agrees with the study of Trigiano and Fergus (1979), who reported that about 80% of different fungi tested showed amylolytic activity. However, the results obtained from this study do not agree with Cochrane's (1958) view which states that amylolytic ability is virtually universal among the fungi.

A high proportion of moderate to good amylase producing cultures was found in the genera, *Drechslera, Fusarium, Penicillium, Alternaria* and *Aspergillus*. Amylase-producers constituted about 100%, 95%, 87%, 81% and 80% of the tested fungi of these genera, respectively. These moulds are very commonly found on decaying materials and may play an important part in the spoilage of agricultural products such as cotton seeds and seed products.

Of the different aspergilli tested, *A. clavatus, A. quadrilineatus, A. flavus* and *A. nidulans* were the most active species tested. All to 88% of them proved to be α-amylase producers (Table 1). Aspergilli are well-known producers of extracellular amylase (Lulla *et al.* 1955, Jonson *et al.* 1968, Jayaraman & Prasad, 1971, Kundu *et al.* 1973) and thus they have a wide application in the fermentation industry.

Lipolytic activity

It is evident that the isolates tested were able to produce lipase in varying degrees. Many of

Table 1
Total number of isolates and percentage of proteolytic, amylolytic and lipolytic activities of fungi isolated from cotton seeds and cotton seed products.

Organism	*TNI	Isolates activity (%)									
		Proteoplytic				Amylolytic			Lipolytic		
		G	M	L	N	G	M	N	G	M	N
Aspergillus	308										
A. candidus Link	1	100	0	0	0	100	0	0	100	0	0
A. clavatus Desmazieres	4	75	25	0	0	75	25	0	0	0	100
A. flavipes (Bain. & Sart.) Thom & Church	1	0	100	0	0	100	0	0	100	0	0
A. flavus Link	84	94	4	2	0	61	27	12	66	27	7
A. funigatus Fresenius	90	80	18	1	1	41	11	48	69	22	9
A. nidulans (Eidam) Winter	42	79	12	7	2	76	12	12	60	38	2
A. niger Van Teighem	7	14	0	14	72	29	29	43	57	14	29
A. ochraceus Wilbelm	1	100	0	0	0	100	0	0	100	0	0
A. quadrilineatus Thom & Raper	5	60	40	0	0	80	20	0	80	10	10
A. sydawii (Bain. & Sart.) Thom & Church	24	63	25	8	4	63	17	21	8	38	54
A. terrus Thom	46	96	4	0	0	63	15	22	43	20	37
A. terricola Marchal	2	100	0	0	0	100	0	0	0	50	50
A. ustus (Bain.) Thom & Church	1	0	100	0	0	100	0	0	100	0	0
Penicillium	82										
P. citrinum Thom	6	50	0	0	50	50	50	0	33	33	33
P. chrysogerum Thom	18	22	22	17	39	78	11	11	78	17	5
P. corylophilum Dierchx	16	12	0	19	69	50	25	25	37	36	37
P. cyclopium Westling	2	0	0	50	50	100	0	0	50	0	50
P. duclauxi Delacroix	3	0	0	0	100	100	0	0	33	67	0
P. funicluosum Thom	2	50	0	0	50	50	50	0	0	50	50
P. jensenii Zaleski	6	0	17	33	50	50	17	33	33	17	50
P. lanosum Westling	1	0	0	0	100	0	100	0	100	0	0
P. n.. .cans (Bainier) Thom	1	0	0	0	100	0	100	0	0	100	0
P. notatum Westling	21	14	9	29	48	67	33	0	52	29	19
P. purpurogenum Stoll	2	0	0	0	100	0	50	50	0	100	0
P. rubrum Stoll	2	0	0	0	100	50	50	0	100	0	0
P. verruculosum Peyronel	2	50	50	0	0	50	50	0	0	50	50
Other genera:	110										

Table (1 cont.)

Organism	*TNI	Isolates activity (%)									
		Proteoplytic				Amylolytic			Lipolytic		
		G	M	L	N	G	M	N	G	M	N
Alternaria alternata (Fr.) Keissler	27	15	7	22	56	37	44	19	22	33	45
Cladosporium cladosporioides (Fresen) de Vries	2	0	0	100	0	0	100	0	0	50	50
Curuvularia lunata (Walker) Boedijn	4	50	25	25	0	25	50	25	25	25	50
Drechslera spicifera (Bainier) Von Arx.	2	0	100	0	0	100	0	0	100	0	0
Drechslera halodes (Drechsler) Subram & Jain	3	0	67	33	0	100	0	0	33	33	33
Fusarium moniliforme Sheldon	16	100	0	0	0	81	13	6	88	12	0
Fusarium oxysporum Schlechtendahl	8	87	0	0	13	87	13	0	100	0	0
Humicola grisea Traaen	1	100	0	0	0	0	100	0	0	0	100
Mucor hiemalis Wehmer	6	50	0	50	0	0	0	100	0	17	83
Myrothecium roridum Tode ex Fr.	3	0	0	0	100	100	0	0	100	0	0
Rhizopus stolonifer (Ehrenb. ex Fr.) Lindt	27	7	4	7	82	45	22	33	26	18	56
Sepedonium chrysospermum (Bulliard) Fries	3	33	0	67	0	33	33	33	0	0	100
Stachybotrys chartarum (Ehrenb. ex Link) Hughes	2	0	0	50	50	100	0	0	0	50	50
Trichoderma viride Pers. ex. S.F. Gray	1	0	0	0	100	100	0	0	0	100	0
Ulocladium botrytis Preuss	5	60	0	0	40	0	40	60	80	20	0

* TNI = Total number of isolates tested.

G = Good activity.

M = Moderate activity.

L = Low activity.

N = No activity.

them (about 78%) showed lipolytic activity. This agrees with Cochrane's statement (1958), that most fungi have the capability to produce lipase. High proportions of moderate to good lipase producing cultures were found in the genera *Fusarium,* (24 out of 24), *Aspergillus* (256 out of 308) and *Penicillium* (63 out of 82). Most of the lipase producers belonging to *F. moniliforme* (100%). *F. oxysporum* (100%). *A. nidulans* (98%), *P. chrysogenum* (95%), *A. flavus* (92%), *A. fumigatus* (91%), *A. niger* (85%) and *P. notatum* (81%).

The present results are in concordance with those of Raper and Fennell (1965). They reported that members of *A. flavus, A. fumigatus, A. nidulans* and *A. niger* to produce lipolytic enzymes. These fungi occur quite common on oil seeds, and on vegetable oils in storage and may cause substantial deterioration of these commodities. Vishwanthan *et al.* (1957) reported *A. flavus* to hydrolyze peanut oil rapidly. Gonzales (1957) found that *A. flavus* was the most lipolytic of several species of *Aspergillus* isolated from olive stored prior to pressing. Mayne (1956) listed *A. flavus* as common on deteriorated cotton seeds and showed it to be among the most active of several moulds isolated from this source. Coursey (1960) and Coursey & Eggins (1961a,b) found *A. niger* to be one of the most common and most active fungi implicated in the lipolysis of palm oil during storage. The lipolytic activity of *A. fumigatus* in peanut oil and saf-flower oil was reported by Vishwanathan *et al.* (1957) and Ramakrishnan & Banerjee (1951 a,b & 1952). The extracellular production of lipase by most of the fungi tested in this investigation is an indication of their probable roles in the spoilage of oil seeds and its products. Hiscocks (1965) reported that tropical oil seeds deteriorate rapidly with the development of acidity during storage. Hanson *et al.* (1973) reported that the free fatty acid contents increased rapidly when cocao beans were inoculated with storage fungi (*A. niger, A. flavus, A. amstelodami, A. ruber* and *A. repens*). Lipolytic fungi have been implicated in the spoilage of Nigerian palm fruits (Eggins, 1964; Eggins & Coursey, 1964). Hutchinson (1961) reported increases in fat acidity in wheat due to fungal growth. Dorworth & Christensen (1968) reported that increases in fat acidity in stored grains or seeds depend upon the fungus involved and the moisture content of the grain or seeds.

The results clearly displayed that all the isolates of *F. moniliforme* and *F. oxysporum* to be or high to moderate lipolytic activities. This is in complete concordance with those of Svabova *et al.* (1980) who detected the presence of esterase, ∝-amylase and protease in all the isolates tested of *F. moniliforme* and *F. oxysporum.*

The three enzymes surveyed in this investigation are those which degrade some of major constituents of cotton seeds and cotton seed products. The results indicated that 88%, 80% and 78% of fungi studied have the capability to utilize starch, proteins and lipids of the cotton seeds and cotton seed products. There is also evidence that many of these fungi may be able to degrade cellulose and lignin.

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بعض الأنشطة الأنزيمية للفطر المعزولة من بذور القطن ومنتوجاتها

اسماعيل القاضي ، محمد بهى الدين مازن ، صباح محمد صابر

درست ٥٠٠ عزلة مختلفة تنتمى الى خمسة عشر جنسا وواحد واربعون نوعا من الفطر معزولة من بذور القطن ومنتوجاتها . شملت الدراسة الأنشطة الأنزيمية المحللة للمواد البروتينية والنشوية والدهنية والتى قد تساعد على سرعة اتلاف هذه المنتوجات اثناء فترة التخزين . ثبت من الدراسة ان ما يقرب من ٨٠٪ من الفطر المختبرة لها نشاط انزيمي محلل للبروتينات ، واوضحت الدراسة ان نسبة مرتفعة من الفطر ذات الأنشطة الأنزيمية المحللة للبروتينات قد وجدت في جنس اسبرجيلس فيوميجاتس ، اسبرجيلس سيدوي ، اسبرجيلس تيريس لها نشاط انزيمي قوي محلل للبروتينات .

ثبت كذلك ان ما يقرب من ٨٨٪ من الفطر المختبرة يمكنها تحليل النشا .

ووجد ان نسبة مرتفعة من مزارع الفطر المنتجة للأنزيم المحلل للنشا تتبع اجناس درشسليرا (١٠٠٪) . فيوزاريوم (٩٥٪) ، بنسيليام (٨٧٪) ، الترناريا (٨١٪) واسبرجلس (٨٠) . اثبتت الدراسة كذلك ان حوالى ٨٨٪ من الفطر المختبرة منتجة لانزيم الليبيز المحلل للدهون . وقد وجد ان نسبة مرتفعة من مزارع الفطر المنتجة لهذا الأنزيم تنتمى الى اجناس فيوزاريوم (١٠٠٪) ، اسبرجيلس (٨٣,١٪) ، بنسيليام (٧٦,٨٪) . ومن الأنواع التى تميزت بارتفاع نسبة الفطر المنتجة لأنزيم الليبيز كل من : فيوزاريوم مونيليفورمى (١٠٠٪) ، فيوزاريوم اوكسيسبوريم (١٠٠٪) ، اسبرجيلس نيديولانس (٩٨٪) ، بنسيليام كريزوجينم (٩٥٪) ، اسبرجيلس فلافس (٩٢٪) ، اسبرجيلس فيوميجاتس (٩١٪) ، اسبرجيلس نيجر (٨٥٪) وبنسيليام نوتاتم (٨١٪) .