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Short Communication

Amylolytic, Cellulolytic and Acid Proteolytic Activities of Seed-borne Fungi of Foxtail Millet and Sorghum

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Deterioration of seed is usually attributed to the action of extracellular enzymes secreted by seed-borne microorganisms [1]. Fungi, in particular play a prominent role in seed deterioration during storage. As the carbohydrates and proteins of seed are the major constituents, the ability of fungi to secrete amylolytic, cellulolytic and proteolytic enzymes becomes important for their invasion and colonization in the seed tissue, which ultimately renders them unfit for human consumption. Though there have been a number of studies dealing with deteriorative changes in food reserves under the influence of fungi [2, 3 & 4], comparatively few reports exist to elucidate the role of hydrolytic enzymes that facilitate the infection of the seed and hydrolysis of its complex reserve food. In the present investigation, amylolytic, cellulolytic and acid proteolytic activities of seed-borne fungi of foxtail millet [*Setaria italica* (L.) Beauv.] and sorghum [*Sorghum bicolor* (L.) Moench] were assayed under *in vitro* conditions.

The isolates of *Aspergillus flavus, A. niger, A. terreus, Penicillium citrinum, Curvularia lunata, C. maculans, Alternaria alternata, Mucor* sp. and *Rhizopus* sp. isolated from foxtail millet and *Fusarium oxysporum* obtained from sorghum were initially screened for their ability to secrete hydrolytic enzymes such as amylase (starch hydrolysis), cellulase (cellulolysis) and acid protease (caseinolysis and gelatin liquefaction). All the fungi tested were found to be positive and attempts were made to quantify the enzyme activity, which may differ with the efficiency of different isolates at different incubation periods.

For determining amylolytic activity, the fungi were cultivated on Czapek-Dox broth containing 1 per cent starch as sole 'C' source. Observations for amylase and glucoamylase production were made at 4 days interval up to 16th day of incubation at 37°C. Culture filtrates were then centrifuged and the supernatant was used as the crude enzyme extract. Enzyme activity was determined on the basis of reducing sugars [5] calculated with reference to a standard curve of maltose (amylase) and glucose (glucoamylase) and expressed in Units/ml of the broth filtrate.

For quantitative assessment of the cellulolytic activities of fungi, similar protocol described for amylases was followed except that the fungi were grown on Czapek-Dox broth containing 1 per cent pure cellulose powder. Enzyme extracts obtained after centrifugation at 3 days interval up to 15 days served as enzyme source and cellulolytic activity was analyzed by filter paper activity [6]. The amount of reducing sugars released by cellulase enzyme was expressed in terms of Units/ml of the broth filtrate.

Acid protease production was studied by growing the test fungi on glucose-casein broth for 16 days and the culture filtrates were analyzed at 4 days interval, centrifuged and the supernatant was used for assay [7]. Enzyme activity was expressed in Units/ml of the broth filtrate.

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Most of the fungi tested *viz.*, *A. terreus*, *P. citrinum*, *F. oxysporum*, *A. alternata*, *C. maculans*, *C. lunata*, *Rhizopus* sp. and *Mucor* sp. exhibited high amylase and glucoamylase activity on 8 days of incubation that declined gradually. Other fungi such as *A. niger* and *A. flavus* secreted maximum amylase on 4th day and glucoamylase on 4 and 8 days of incubation respectively (Table 1). Among the fungi tested, a high degree of amylase production was observed in *Rhizopus* sp. followed by *Mucor* sp. while the production of glucoamylase was vice versa. Of the three *Aspergillus* species tested, *A. terreus* recorded high amylase as well as glucoamylase production in 8 days old culture and are in conformity with the observations made earlier [8, 9].

Data presented in Table 2 revealed that *P. citrinum* followed by *A. niger*, *A. terreus*, *A. flavus* and *F. oxysporum* showed peak value of filter paper activity on 6 days of incubation and a sudden fall was observed thereafter. However, the cellulase activity for *C. lunata*, *A. alternata*, *Rhizopus* sp. and *Mucor* sp. was almost negligible during the initial stages of growth and increased gradually, achieving the peak value on 9th day. In case of *C. maculans*, maximum activity was found with 12 days old culture. Trivedi and Rao [11] recorded that all the activities of cellulase reached maximum on 12 days of growth for some fungi. Rajendran *et al.* [2] also observed maximum filter paper activity of *Humicola fuscoatra* after 6-8 days of incubation.

Of all the fungi tested, *Mucor* sp. followed by *Rhizopus* sp. stood first in secreting maximum amount of cellulase whereas *P. citrinum*, *A. niger*, *A. terreus*, *A. flavus* and *F. oxysporum* were moderate producers and rest of the fungi were proved to be less efficient.

It appeared from the data (Table 3) that all the fungi could produce acid proteases. Among the fungi tested, *A. alternata* recorded higher proteolytic activity followed by *A. terreus*, *P. citrinum*, *A. flavus* and *F. oxysporum*. In general, maximum enzyme activity was observed with

Table 1. Estimation of amylase and glucoamylase produced by certain seed-borne fungi

Fungi	Days of incubation							
	4		8		12		16	
	A*	G**	A	G	A	G	A	G
Aspergillus flavus	0.12	3.26	0.07	3.56	0.05	2.10	0.01	1.10
A. niger	0.16	4.86	0.06	3.88	0.04	2.88	1.00	-
A. terreus	0.09	1.92	0.40	9.16	0.06	2.32	1.06	-
Penicillium citrinum	0.15	3.20	0.22	5.12	0.10	2.56	0.10	0.16
Fusarium oxysporum	0.09	2.56	0.16	3.40	0.01	1.08	0.64	-
Alternaria alternata	0.07	2.36	0.15	4.38	0.02	1.82	0.16	-
Curvularia maculans	0.06	1.88	0.16	5.14	0.01	1.08	0.01	0.22
C. lunata	0.05	1.60	0.15	4.10	0.02	1.50	0.13	-
Rhizopus sp.	0.07	2.20	1.54	35.00	0.35	4.23	0.18	3.12
Mucor sp.	0.06	2.00	1.10	35.68	0.24	8.58	0.06	1.48

*Units of amylase activity (hydrolyzed starch in 15 min of incubation)

**Expressed as the amount of glucose liberated in Units/ml '- -' Represents no activity

Table 2. Cellulolytic activities of seed-borne fungi *in vitro*

Fungi	Cellulase activity (Units/ml)				
	Days of incubation				
	3	6	9	12	15
Aspergillus flavus	0.22	0.32	0.01	—	—
A. niger	0.24	0.39	0.03	0.02	—
A. terreus	0.08	0.34	0.04	0.03	0.01
Penicillium citrinum	0.20	0.42	0.04	0.03	—
Fusarium oxysporum	0.28	0.29	0.02	0.02	—
Curvularia lunata	0.01	0.02	0.08	0.05	0.001
C. maculans	0.02	0.02	0.03	0.06	0.02
Alternaria alternata	-	0.02	0.08	0.07	—
Rhizopus sp.	0.01	0.03	0.80	0.12	0.01
Mucor sp.	0.02	0.04	0.90	0.03	0.02

'—' Represents no activity

Table 3. Acid Protease production by predominant seed-borne fungi

Fungi	Acid protease (Units/ml)			
	Days of incubation			
	4	8	12	16
Aspergillus flavus	0.13	0.20	0.16	0.03
A. niger	0.12	0.18	0.08	0.02
A. terreus	0.22	0.23	0.12	0.03
Fusarium oxysporum	0.09	0.20	0.01	0.03
Penicillium citrinum	0.16	0.20	0.14	0.01
*Alternaria alternata**	0.14	0.29	0.11	0.04
Curvularia lunata	0.15	0.17	0.18	0.085
C. maculans	0.14	0.15	0.11	0.08
Rhizopus sp.	0.11	0.14	0.11	0.02
Mucor sp.	0.14	0.15	0.12	0.03

8 days old cultures, but in *C. lunata*, 12 days old culture secreted highest quantity. These results are similar to those found on 8 days of incubation for *Phoma exigua* and 12 days for *Graphium penicillioides* [10].

The present study clearly indicates the production of hydrolytic enzymes by seed-borne fungi which may play a vital role in pathogenesis.

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