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EFFECT OF CARBOHYDRATES ON PROTEASE AND LIPASE PRODUCTION IN SEED-BORNE FUNGI OF SOYBEAN

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ABSTRACT

During the process of biodeterioration, seed mycoflora produce enzymes to degrade protein, carbohydrate and oil. These enzymes are called as hydrolytic enzymes. The enzymes which degrade proteins are called protease and enzymes, which degrade oil are lipase. Among the protease activity, most of the monosaccharides and disaccharides stimulate protease production; however, among monosaccharides are xylose-inhibited protease activity of Alternaria alternata and Aspergillus flavus. Fructose-inhibited protease activity of Aspergillus glaucus. Among disaccharides, only maltose-inhibited protease production of Aspergillus flavus and A. niger. It is interesting to note that CMC (Carboxymethyl Cellulose) inhibited considerably the protease production in maximum fungi. Lipase production of fungi also inhibited with different carbon sources. Aspergillus sps. reveals maximum inhibition in monosaccharides and diasaccharides, whereas xylose-inhibited lipase production in almost all the fungi, whereas disaccharides and polysaccharides do not affect on lipase activity.

KEYWORDS: *Carbohydrates, Protease, Lipase, Fungi*

INTRODUCTION

Seed plays a vital role in the production of healthy crop. These seeds are also responsible for disease transmission. This takes place either in the field or in ill storage condition. Neergard (1977) reported that in the presence of seed-borne pathogens, several types of abnormalities like reduction in seed size, seed rotting, discoloration of seeds, seed necrosis, loss in germ inability, toxification and other physiological disorders. According to Sandikar (1990), the species of *Fusarium* are found to be significantly destructive and responsible to cause harmful effect on seed health, resulting in seed deterioration and poisoning of seeds. During the process of biodeterioration, fungi produce enzymes to degrade proteins, carbohydrates and oil. Sharma and Satyanarayana (1980) studied production of protease by some fungi, such as *Helminthosporium, Glomerella cingulata, Curvularia geniculata and Alternaria pelandui.*

Umatale (1995), Charya and Reddy (1982) also studied lipase production in certain oil seeds. Umatale found *Aspergillus flavus, A. helianthi,* Macrophomina phaseolina and *Rhizopus nigricans* are more active to produce lipase.

MATERIALS AND METHODS

Collection of samples and detection of seed mycoflora. For the collection of seed samples, the method described by Neergaard (1973) has been adopted. Accordingly, from fields, store houses market places and seed companies. A composite sample of each variety was prepared by mixing the individual samples together. The seed mycoflora was isolated by using Standard Moist Blotter paper method (SMB) and Agar Plate Method (APM), as recommended by International seed testing

association (ISTA 1966), De Tempe (1970), Neergaard (1973) and Agarwal (1976).

Identification of Seed Borne Fungi

The fungi occurring on each and every seed in the plates were identified primarily on the basis of sporulation characters, like sexual or asexual spores with the help of stereoscopic binocular microscope. The identification and further confirmation of seed-borne fungi was made by preparing slides of the fungal growth and observing them under compound microscope. The identification was made with the help of manuals as per Nelson *et al*. (1983), Singh *et al.* (1991), Mukadam, D. S. (1997) and Mukadam *et al*. (2006).

Production of Protease

Production of protease(s) was made by growing the fungi on a liquid medium containing glucose 10 g, gelatin 10 g, dipotassium hydrogen phosphate 1.0 g, $MgSO_4.7H_2O$ -500 mg and distilled water -1000 ml. pH of the medium was adjusted at 5.5. 25 ml of medium was poured in 100 ml Erlenmeyer conical flasks and autoclaved as 151 bs pressure for 20 minutes. The flasks on cooling were inoculated separately with 10 ml standard spore/mycelial suspension of test fungi prepared from 7 days' old cultures grown on PDA slants. The flasks were incubated for 6 days at 25±1°C with diurnal periodicity of light. On the 7th day, the flasks were harvested by filtering the contents through Whatman's filter No. 1. The filtrates were collected in the pre-sterilized bottles and termed as crude enzyme preparation.

Assay Method (Cup–Plate Method)

Determination of protease(s) activity was done with the help of cup–plate method, adopted by Hislop *et al.* (1982) and Rajamani (1990). A basal medium was prepared by adding 2% (w/v) agar and 1% (w/v) gelatin. pH of the medium was adjusted at 5.6 with Mcilvaine's buffer. Then, it was sterilized at 15 lbs pressure for 15 minutes. About 15 ml of the medium was poured in pre-sterilized petri-plates under aseptic condition. On solidification 6-mm diameter cups/cavities were made in the centre of each of the agar plate with a sterilized cork borer (No. 4). The cups/cavities were filled carefully with about 0.5 ml of culture filtrate (crude enzyme preparation). The plates were incubated at 25°C for 24 hours. Then, the plates were flooded with 15 % mercuric chloride in 7N HCl. After 10 minutes of standing, a clear transparent zone indicated the hydrolysis of gelating by extracellular proteolytic enzymes, whereas the rest of the region of the petri-plates become opaque due to the coagulating (protein) by mercuric chloride. The diameter of the clear zone was used as a measure (mm) of protease activity, while non-appearance of clear zone considered absence of protease (s) in the culture filtrates.

Production of Lipase

Lipase activity was studied by growing the fungi on liquid medium at pH 5.6 containing oil-10 g, KNO_3 – 2.5 g, KH_2PO_{4-} – 1.0 g, $MgSO_2$ – 0.5 g and distilled water 1000 ml, 25 ml of the medium was poured in 100 ml conical flasks and autoclaved at 151 bs pressure for 30 minutes, then on cooling the flasks were inoculated separately with 1.0 ml spore suspension of the fungi, which were incubated for 6 days at 25± 1°C with diurnal periodicity of light. On the 7th day, the flasks were harvested by filtering the contents through Whatman's filter paper no. 1. The filtrates were collected in pre-sterilized culture filtrate bottles and termed as crude lipase.

Assay Method (Cup–Plate Method)

Determination of lipase activity was done with the help of cup–plate method. The medium contains Difco peptone – 10 g,

NaCl – 5 g, $Cac1_2.2H_2O$ – 1.0 g, agar 2% and 10 ml lipid substrate sorbitan monolaurate (Tween 20) (pre-sterilized) was added to it. The pH of the medium was adjusted to 6.00. The medium was poured in each petri-plate. On solidifying the medium with the help of a cork borer (No. 4) was made in the centre and was filled with 0.1 ml culture filtrate. The plates were incubated at 28^oC. After 24 hours, a clear circular zone was measured (mm) as lipase activity. Similar procedure was followed for the culture filtrate in the central cavity instead of the active enzymes.

RESULT AND DISCUSSIONS

Effect of six sources of carbohydrates was studied against the protease and lipase activity of fungi, and the results are given in table 1.Among the protease activity, most of the monosaccharides and disaccharides stimulate protease production; however, among monosaccharides, xylose inhibited protease activity of *Alternaria alternata* and *Aspergillus flavus.* Fructose inhibited protease activity of *Aspergillus glaucus.* Among disaccharides, only maltose inhibited protease production of *Aspergillus flavus* and *A. niger.* It is interesting to note that CMC (carboxymethyl cellulose) inhibited considerably the protease production in maximum fungi. Lipase production of fungi also inhibits with different carbon sources. *Aspergillus* sps. reveals maximum inhibition in monosaccharides and diasaccharides, whereas xylose-inhibit lipase production in almost all the fungi, whereas disaccharides and polysaccharides do not affect lipase activity.

Table 1: Effect of Carbohydrates on Protease and Lipase Production in Seed-Borne Fungi

Carbohydrates (0.5 % Conc.)	Fungi									
	Aal	Asf	Asn	Asg	Asu	Cul	Fur	Fuo	Spv	Triv
	Activity Zone (mm)									
Protease Production										
Monosaccharides										
Glucose (C)	18	20	18	16	12	13	18	12	11	11
Fructose	20	22	20	13	18	22	21	19	14	20
Xylose	17	19	22	20	28	16	18	14	18	12
Disaccharides										
Sucrose	20	22	20	25	20	18	18	13	18	22
Maltose	16	16	14	16	17	14	25	20	16	25
Polysaccharides										
CMC	3	6	5	6	10	1	1	1	4	6
Starch	36	18	20	17	30	18	24	20	17	15
Lipase Production										
Monosaccharides										
Glucose (C)	38	14	23	24	28	20	21	23	30	32
Fructose	50	12	25	25	26	22	20	25	28	50
Xylose	50	10	11	11	11	14	13	12	12	10
Disaccharides										
Sucrose	34	19	10	18	10	20	40	48	31	18
Maltose	31	12	30	15	18	30	20	22	26	45
Polysaccharides										
CMC	4	15	20	16	16	26	13	25	18	18
Starch	26	23	32	24	28	40	22	38	30	25
Aal - *Alternaria alternata*						Cul -	*Curvularia lunata*			
Asf - *Aspergillus flavus*						Fur -	*Fusarium roseum*			
Asn - *Aspergillus niger*						Fuo -	*Fusarium oxysporum*			
Asg - *Aspergillus glaucus*						Spv -	*Spicaria violecia*			
Asu - *Aspergillus ustus*						Triv -	*Trichoderma viride*			

CONCLUSIONS

Among the protease activity, most of the monosaccharides and disaccharides stimulate protease production; however, among monosaccharides, xylose inhibited protease activity of *Alternaria alternata and Aspergillus flavus.* Fructose inhibited protease activity of *Aspergillus glaucus.* Among disaccharides, only maltose inhibited protease production of *Aspergillus flavus* and *A. niger.* Lipase production of fungi also inhibited with different carbon sources.

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