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

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ABSTRACT

During the process of bio-deterioration, 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.

It is observed that total of thirty (30) species of fungi were isolated from ten varieties of Soybean. It is also observed that different amino acids such as Alanine stimulate almost all fungi except Alternaria alternata, Aspergillus flavus, A. niger and A.ustus.

KEYWORDS: *Amino Acids, 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 in causing harmful effect on seed health, resulting into seed deterioration and poisoning of seeds. During the process of bio-deterioration, fungi produce enzymes to degrade proteins, carbohydrates and oil. Sharma and Satyanarayana (1980) studied production of protease due to some fungi, which includes *Helminthosporium, Glomerella cingulata, Curvularia geniculata, Alternaria pelandui.*

Umatale (1995), Charya and Reddy (1982) also studied on lipase production in certain oil seeds. Umatale found *Aspergillusflavus,A. helianthi,* Macrophominaphasiolina and *Rhzopus nigricans* are more active in producing 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 (19760).

Identification of Seed Borne Fungi

The fungi occurring on each and every seed in the plates were identified preliminary 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 liquid medium containing glucose 10g, gelatin 10g, dipotassium hydrogen phosphate 1.0g, $MgSO_4.7H_2O$ -500mg and distilled water-1000ml. pH of the medium was adjusted at 5.5. Twenty five ml of medium was poured in 100ml Erlenmeyer conical flasks and autoclaved as 151bs pressure for 20 minutes. The flasks on cooling were inoculated separately with 10ml 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^oC with diurnal periodicity of light. On 7th day, 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 one percent (w/v) gelatin. pH of the medium was adjusted at 5.6 with Mcllavaine's buffer. Then, it was sterilized at 15 lbs pressure for 15 minutes. About 15 ml of the medium was poured in pre-sterilized petriplates under aseptic condition. On solidification, 6mm 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 0.5ml of culture filtrate (crude enzyme preparation). The plates were incubated at 25^oC for 24 hours. Then, the plates were flooded with 15 percent 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 regions of the petriplates become opaque due to the coagulatin (protein) by mercuric chloride. Diameter of the clear zone was used as measure (mm) of protease activity, while non appearance of clear zone is considered absence of protease (s) in the culture filtrates.

Production of Lipase

Lipase activity was studied by growing the fungi on liquid medium at pH5.6 containing oil-10g, KNO_3 -2.5g, KH_2PO_{4-} -1.0g, $MgSO_2$ – 0.5g and distilled water 1000ml. 25ml of the medium was poured in 100ml conical flasks and autoclaved at 15 lbs pressure for 30 minutes, and then, on cooling, the flasks were inoculated separately with 1.0ml spore suspension of the fungi, which were incubated for 6 days at 25± 1^oC with diurnal periodicity of light. On 7th day, the flasks were harvested by filtering the contents through Whatman 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-10g, NaCl-5g, $Cac1_2.2H_2O$-1.0g, agar 2 percent and 10ml lipid substrate serbitan mono laurate (Tween-20) (Pre-sterilized) were 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 0.1ml culture filtrate. The plates were incubated at 28°C. 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.

RESULTS AND DISCUSSIONS

Six amino acids at concentration of 100ppm were tested against ten seed borne fungi. The results are shown in Table 1.

It is observed from the results that Alanine stimulates almost all fungi except *Alternaria alternata, Aspergillus flavus, A. niger* and *A. ustus.* Agrinine also stimulates all the fungi for protease production except *A. flavus* and *A. niger* while it produces no protease by *A. glaucus* and *A.ustus.* Mithionine also stimulates protease activity to all fungi except *A flavus, A.niger* and *A.ustus.* Aspartic acid stimulates protease production except *A. alternata, A. flavus, A. niger* and *Fusarium roseum.*

In case of lipase production, Alanine completely inhibited lipase activity except *Trichoderma viride* as it produces no lipase by *A. niger, Fusarium roseum, F. oxysporum* and *Spicaria violecia.* Arginine also completely inhibited lipase activity by all fungi and it produced no activity of lipase by *A. alternata, F. roseum and Spicaria violecia.* Methionine also acts as inhibitory to all fungi except *A. alternata* and it produces no lipase by *Fusarium. roseum.* Threonine also inhibited almost all fungi except *A. alternata, Aspergillus ustus* and *Trichoderma viride* while it produces no lipase by *Fusarium roseum.* Aspartic acid also shows same results except *A. alternata, Aspergillus flavus, A. ustus* and *Trichoderma. viride* whereas it produced no lipase by *Fusarium roseum.*

Table 1: Effect of Amino Acids on Protease and Lipase Production in Seed-Borne Fungi of Soybean

Oils (1% Conc)	Fungi									
	Activity Zone (mm)									
	Aal	Asf	Asn	Asg	Asu	Cul	Fur	Fuo	Spv	Triv
	Protease Production									
Alanine	14	12	11	20	11	35	25	25	23	20
Arginine	18	11	12	-	-	35	25	18	20	25
Monohydrochloride	-	20	21	12	22	30	14	40	23	18
Methionine	14	15	16	18	15	13	18	16	12	-
Threonine	22	12	13	16	32	40	42	11	40	14
Aspartic acid	14	16	14	20	16	17	13	25	13	16
Control	18	20	18	16	12	13	18	12	11	11
	Lipase Production									
Alanine	19	12	-	22	16	18	-	-	-	22
Arginine	-	11	11	16	14	16	-	16	-	11
Monohydrochloride	30	14	18	16	16	15	-	12	11	11
Methionine	28	14	18	20	16	14	-	18	13	12
Threonine	30	22	24	18	18	20	-	13	12	11
Aspartic acid	27	28	24	20	22	20	-	26	20	22
Control	27	23	24	25	20	24	35	17	18	12

Aal – Alternaria alternata

Asf – Aspergillus flavus

Asn – Aspergillus niger

Asg – Aspergillus glaucus

Asu – Aspergillus ustus

Cul – Curvularia lunata

Fur – Fusarium roseum

Fuo – Fusarium oxysporum

Spv – Spicaria violecia

Triv – Trichoderma viride

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