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EFFECT OF ANTIBIOTICS 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 thirty species of fungi were isolated from ten varieties of soybean. It is also observed that Ampicillin completely inhibits the activity of protease production except A. alternate, as same results were found with streptomycin except A. glaucus.

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 to cause harmful effect on seed health, resulting into seed deterioration and poisoning of seeds. During the process of bio-deterioration, fungi produces 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, Alternaria pelandui.*

Umatale (1995), Charya and Reddy (1982) also studied 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 a septic 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 region of the petriplates becomes 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.

RESULT AND DISCUSSIONS

Five different antibiotics at 100 pm concentration were employed against ten seed borne fungi to study their effect on protease and lipase production. The results are given in Table 1.

Ampicillin completely inhibits the activity of protease production except *A. alternate*. Thesame results were found with streptomycin except *A. glaucus* and it produces no protease by *Fusarium roseum* and *F. oxysporum.* Terramycin only stimulates *Alternaria alternata, while Griseofulvin stimulates A. alternata, Aspergillus glaucus, A. ustus Curvularia lunata* and *Spicaria violecia* while no enzyme production by *F. oxysporum.*

Doxycyclin only stimulates to fungi like *A. alternata, S. violecia* and *Trichoderma viride* while it produces no protease activity by *C. lunata* and *F. roseum.*

Lipase production in the presence of ampicillin shows inhibitory effect on almost all fungi except *Trichoderma viride* and it shows no lipase activity by *Fusarium roseum.* Streptomycin also inhibits all the fungi except *A. alternata.* Terramycin completely inhibits all fungi and it produces in lipase by *F. roseum.* Griseofulvin also inhibits all fungi except T. *viride* and it produces no lipase zone by *F. roseum* and *Spicaria violecia.* Doxycyclin completely inhibits all the fungi and it produces no lipase zone by *F. roseum.*

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

Antibiotics(100ppm conc.)	Fungi									
	Aal	Asf	Asn	Asg	Asu	Cul	Fur	Fuo	Spv	Triv
	Activity Zone (mm)									
Protease Production										
Ampicillin	22	18	19	18	-	16	16	15	18	14
Streptomycin	14	13	18	17	16	16	-	-	18	18
Terramycin	20	14	19	16	16	16	18	18	16	15
Griseofulvin	16	17	19	22	20	20	11	-	30	12
Doxycyclin	17	14	15	17	17	-	-	11	21	30
Control	16	19	21	16	18	17	21	18	20	22
Lipase Production										
Ampicillin	20	14	12	16	11	18	-	14	12	13
Streptomycin	30	14	22	17	23	20	11	14	13	11
Terramycin	26	16	14	16	20	19	-	20	14	11
Griseofulvin	23	12	11	14	12	16	-	11	-	13
Doxycyclin	26	20	11	14	22	18	-	12	11	11
Control	27	23	24	20	25	24	35	17	18	12

Aal - Alternaria alternata	Cul -	Curvularia lunata	
Asf - Aspergillu flavus	Fur -	Fusarium roseum	
Asn - Aspergillus niger	Fuo -	Fusarium oxysporum	
Asg - Aspergillus glaucus	Spv -	Spicaria violecia	
Asu - Aspergillus ustus	Triv -	Trichoderma viride	

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