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

Kesare Umesh

Associate Professor, Department of Botany, Adarsh Senior College, Osmanabad, Maharashtra, India

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ABSTRACT

During the process of biodeterioration, seed mycoflora produces enzymes to degrade protein, carbohydrate and oil. These enzymes are called as hydrolytic enzymes. The enzymes which degrade proteins are called protease and enzymes and which degrade oil are lipase. Five different salts at 0.05% concentration were added separately in to the basal medium, and it is observed that Barium chloride shows inhibitory effect on protease production by the fungal species, such as Aspergillus niger, A. glaucus, A. ustus and Trichoderma viride, while there is no activity by the fungi like Curvularia lunata, Fusarium roseum, F. oxysporum and Spicaria violecia. Sodium chloride also reveals similar effect except some fungi like A. niger, A. glaucus, C. lunata and F. oxysporum. Potassium chloride also is an inhibitory factor for protease production by almost all the fungi except Alternaria alternata, A. flavus and A. niger. Barium chloride stimulates the lipase activity by A. alternata and inhibited by A. glaucus, while it was in total inhibition in the other fungi.

KEYWORDS: *Salts, Protease, Lipase, Fungi*

INTRODUCTION

Seed plays a vital role for the production of a 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. Acording to Sandikar (1990) the species of *fusarium* are found to be significantly destructive and responsible to cause harmful effect on seed health resulting in to seed deterioration and poisoning of seeds. During the process of biodeterioration, fungi prduce 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 on lipase production in certain oil seeds. Umatale found *Aspergillus flavus, A.helianthi,* Macrophominaphasiolina and *Rhzopus 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 standered 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-sterlised 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 presterilized 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 region 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 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 1bs pressure for 30 minutes, 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 of the flasks were harvested by filtering the contents through Whatman filter paper no.1. The filtrates were collected in presterilized 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) 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 0.1ml 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

Five different salts at 0.05% concentration were added separately in to the basal medium and their effect on protease production was studied and the results are shown in Table 1.

Barium chloride reveals inhibitory effect on protease production by the fungal species, such as *Aspergillus niger, A. glaucus, A. ustus* and *Trichoderma viride,* while there is no activity by the fungi like *Curvularia lunata, Fusarium roseum, F. oxysporum* and *Spicaria violecia.* Sodium chloride also reveals similar effect except some fungi like *A. niger, A. glaucus, C. lunata and F. oxysporum*. Potassium chloride is also an inhibitory factor for protease production by almost all the fungi except *Alternaria alternata, A. flavus and A. niger*. No production of protease was seen by *S. violecia*. While calcium chloride completely inhibits the production of protease in all the ten fungi, Magnesium chloride also inhibits protease production except *A. alternata* and *F. oxysporum.*

Barium chloride stimulates the lipase activity by *A. alternata* and inhibited by *A. glaucus,* while it was total inhibition in the other fungi. Sodium chloride reveals stimulatory effect in lipase production in most of the fungi except A. *flavus, A. ustus, Curvularia lunata* and *Trichoderma viride*. Potassium chloride also reveals stimulatory effect in most of the fungi, except *C. lunata,* while it was totally inhibited in *A. flavus, A. ustus, Spicaria violecia* and *Trichoderma viride.* Calcium chloride reveals inhibitory effect on lipase production by the *Aspergillus niger* and *A. glaucus,* and it is totally inhibited in most of the fungi except it is stimulatory by *Alternaria alternata*. Magnesium chloride reveals stimulatory effect on lipase production by most of the fungi except *A. niger,* which is inhibited with this salt and other produce totally inhibited lipase, such as *A. glaucus, Curvularia lunata, Fusarium roseum, F. oxysporum* and *S. violecia*.

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

Salts (0.05% conc.)	Fungi									
	AAL	ASF	ASN	ASG	ASU	CUL	FUR	FUO	SPV	TRIV
	Activity Zone (mm)									
Protease Production										
Barium chloride	18	21	16	16	20	-	-	-	-	13
Sodium chloride	14	18	18	21	16	21	18	20	16	14
Potassium chloride	20	20	19	14	20	14	16	14	-	11
Calcium chloride	13	16	17	13	15	14	18	16	14	12
Magnesium chloride	18	17	16	16	18	14	20	21	16	17
Control	18	20	18	19	21	20	22	20	19	20
Lipase Production										
Barium chloride	12	-	-	12	-	-	-	-	-	-
Sodium chloride	15	12	18	17	11	15	17	18	15	16
Potassium chloride	14	-	14	14	-	12	15	12	-	-
Calcium chloride	13	-	13	12	-	-	-	-	-	-
Magnesium chloride	11	16	11	-	15	-	-	-	-	18
Control	11	16	14	14	15	17	11	11	11	18

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*

CONCLUSIONS

Five different salts at 0.05% concentration were added separately in to the basal medium and their effect on protease production was studied. Barium chloride reveals inhibitory effect on protease production by the fungal species, such as *Aspergillus niger, A. glaucus, A. ustus* and *Trichoderma viride*, while there is no activity by the fungi like *Curvularia lunata, Fusarium roseum, F. oxysporum* and *Spicaria violecia*. Barium chloride stimulates the lipase activity by *A. alternata* and inhibited by *A. glaucus*, while it was in total inhibition in the other fungi.

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