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Effect of physical factors on hydrolytic enzyme action of seed bone *Alternaria* Species

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Abstract
Impact of physical factors on hydrolytic enzyme production of *Alternaria* species was studied. Continuous light favored enzyme production in *Alternaria* species. Maximum enzyme activity occurred in 15 – 20 days in all the *Alternaria* species. *Alternaria* species produced hydrolytic enzymes maximum at 20 – 30°C temperature.All the species of *Alternaria* at 5.5 to 6.5 pH value produced maximum enzyme, however pH 3.5 and 8.5 inhibited enzyme activity.

Keywords: Light, pH, enzyme, fungi, seed

INTRODUCTION

The seed borne fungi are known to deteriorate the seeds and its contents [1]. Degradation of these seeds chemical content has been due to production of hydrolytic enzyme like lipase, protease, cellulose etc. and production of hydrolytic enzyme related with physical factors. However the less information is available about the impact of physical factors on hydrolytic enzyme produced by *Alternaria*. Considering the fact attempt were made to study the impact of physical factors on amylase, protease and lipase produced by *Alternaria*.

MATERIALS AND METHODS

Production of hydrolytic enzyme

Production of hydrolytic enzyme was studied by growing the fungi in liquid medium containing Starch (in case of amylase)/ gelatin (In case of protease)/ oil (In case of lipase), 1%, KNO_3, 0.25% KH_2PO_4 0.1% and $MgSo_4$. $7H_2O$ 0.05%, pH of the medium was adjusted at 5.5. Twenty five ml of medium was poured in 100 ml conical flask autoclaved and inoculated separately with 01ml spore suspension of the fungi which were grown for 7 days on POA slants. The flasks were incubated for 6 days at 25 ± 1°c with diurnal periodicity of light. On 7th day of the flasks were harvested filter no.1 the filtrates were collected in presterilized bottle and teemed as crude enzyme preparation.

Assay of hydrolytic enzyme

Assay of hydrolytic enzyme was done by cup-plate method.

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RESULTS AND DISCUSSION

Impact of light, incubation period, temperature and pH on amylase, lipase and protease of *Alternaria* species were studied and results are summarized in table 1, 2, 3 and 4.

Amylase and lipase production was more in continuous light in all the species of *Alternaria*. Whereas continuous dark light inhibited amylase and lipase production. Protease production of *A.crassa* and *A. Alternata* inhibited by dark light and continuous light (Table 1).

Alternaria alternata, *A. dianthicola* and *A. tenuissima* do not show lipase activity on 5th days of incubation period. *A. crassa* and *A. dianthicola* do not produce protease at 5th day incubation however as the incubation period increase upto 20th day protease production increased. Effect of temperature on the enzyme activity of Alternaria produced enzyme. At 10°c temperature *A. citri, A. crassa, A. dianthicola and A. macrospora* do not show any amylase production. However 40°c temperature for *A. citri and A. crassa* also inhibited amylase production (Table 2).

Lipase production of *A.citri and A. crassa* initiated at 10°c whereas *A. citri, A. crassa,* and *A. macrospora* inhibited lipase production at 40°c similarly *A. citri* initiated its protease production at 60°c whereas *A. dianthicola and A. macrospora* inhibited protease production at higher temperature (Table 3).

At pH 3.5 none of the species of *Alternaria* produced hydrolytic enzyme Lipase production of *A. citri and A. dianthicola* was also inhibited at pH 4.0 similarly *A. crassa and A. macrospora* inhibited protease enzyme production at pH 4.0 however the maximum hydrolytic enzyme production was reported at pH 5.5 to 6.5 in all species of *Alternaria* (Table 4).

Growth of the microorganisms is directly related to their metabolic activity, therefore, physical factor which are related to enzyme production were also studied in detailed. It is observed from the results that *Alternaria species* produced maximum amylase, lipase and protease in between 15 to 20 days at 20 to 30°c temperature. It is interesting to note that in case of *Alternaria species* continuous light favored the enzyme production whereas 5.5 to 6.5 pH favored maximum enzyme production, however temperature up to 5 to 10°c and pH below 4.5 inhibited the enzyme activity of *Alternaria* species similar types of work have been reported by

earlier workers Adam (1981) [2] reported optimum period for amylase production is more 7 days in *Humicola lanunginora*. Similarly Charya and Reddy (1982) [3] reported optimum period for protease production is more than week. Tarrat et al (1973) [4] reported majority of microorganisms showed their protease activity between 25 to 35^{0}c temperature. Sonwane (2002) [5] observed *A. alternata* favored 20 to 30^{0}c for protease production under *Alternata* light and dark condition similar type of observation in seed mould of Jowar for amylase production have been reported by Panchal (1984) [6].

The range of pH for enzyme production in fungi was found to be variable. Bhosale (1989) [7] reported pH 5.0 to 6.0 is ideal for amylase production in *Aspergillus flarus*. Jonson (1968) [8] reported that alkaline pH was favored in protease production of *A. tenuisima*.

Table 1. Effect of light on enzyme production in *Alternaria* species

Species of *Alternaria*	Illumination of light		
	Continuous light	Continuous dark	*Alternata* dark and light
	Amylase Production		
A.alternata	29	18	19
A.citri	28	18	20
A.crassa	30	20	20
A.dianthicola	32	08	24
A.macrospora	33	20	20
A.tenuissima	27	20	26
	Lipase Production		
A.alternata	30	25	27
A.citri	27	26	30
A.crassa	20	21	25
A.dianthicola	29	18	26
A.macrospora	25	17	20
A.tenuissima	28	15	20
	Protease Production		
A.alternata	21	26	25
A.citri	23	23	20
A.crassa	16	20	12
A.dianthicola	18	15	20
A.macrospora	15	15	13
A.tenuissima	21	15	20

Activity zone in mm

Table 2. Effect of incubation period on enzyme species in *Alternaria* species

Species of *Alternaria*	Incubation period				
	5 days	10 days	15 days	20 days	25 days
	Amylase Production				
A. alternata	08	11	14	18	18
A. citri	11	18	13	14	14
A. crassa	05	14	15	14	10
A. dianthicola	12	15	15	16	15
A. macrospora	07	14	17	15	17
A. tenuissima	10	17	14	14	15
	Lipase Production				
A. alternata	--	12	30	14	18
A. citri	06	06	25	15	20
A. crassa	08	10	26	18	21
A. dianthicola	--	10	18	20	19
A. macrospora	05	11	25	23	20
A .tenuissima	--	08	30	19	19
	Protease Production				
A. alternata	07	18	22	21	20
A. citri	08	16	20	19	20
A. crassa	--	12	17	20	21
A. dianthicola	--	15	16	19	20
A. macrospora	07	12	18	20	17
A. tenuissima	10	17	21	18	18

Activity zone in mm

Table 3. Effect of temperature on enzyme production in *Alternaria* species

Species of *Alternaria*	Temperature 0^{o}C					
	5	10	15	20	30	40
	Amylase Production					
A.alternata	--	13	12	12	18	17
A.citri	--	--	08	10	20	--
A.crassa	--	--	09	10	15	--
A.dianthicola	--	--	10	12	17	21
A.macrospora	--	--	10	11	14	18
A.tenuissima	--	12	13	09	20	14

			Lipase Production			
A.alternata	--	12	17	16	20	13
A.citri	--	--	15	19	21	--
A.crassa	--	--	10	15	18	--
A.dianthicola	--	10	20	20	18	12
A.macrospora	--	14	16	18	19	--
A.tenuissima	--	15	15	14	17	15
			Protease Production			
A.alternata	--	14	17	20	20	12
A.citri	--	--	15	18	21	15
A.crassa	--	18	19	20	23	18
A.dianthicola	--	15	17	19	20	--
A.macrospora	--	10	12	20	18	--
A.tenuissima	--	13	18	15	17	15

Activity zone in mm

Table 4. Effect of pH on enzyme production in *Alternaria* species

Species of *Alternaria*	**pH**					
	3.5	4.5	5.5	6.5	7.5	8.5
			Amylase Production			
A.alternata	--	15	19	20	21	--
A.citri	--	17	18	21	18	--
A.crassa	--	16	18	20	21	--
A.dianthicola	--	19	20	19	19	--
A.macrospora	--	15	16	18	20	--
A.tenuissima	--	13	15	20	19	--
			Lipase Production			
A.alternata	--	20	25	30	30	--
A.citri	--	18	23	28	20	--
A.crassa	--	20	19	18	21	--
A.dianthicola	--	18	20	21	22	--
A.macrospora	--	19	23	27	20	--
A.tenuissima	--	13	18	26	19	--
			Protease Production			
A.alternata	--	17	18	18	17	--
A.citri	--	16	16	20	20	--
A.crassa	--	16	18	17	16	--
A.dianthicola	--	18	16	18	21	--
A.macrospora	--	15	17	18	21	--
A.tenuissima	--	16	18	19	19	--

Activity zone in mm

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