

Effect of physical factors on hydrolytic enzyme action of seed borne *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, cellulase 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%, KNO₃, 0.25% KH₂PO₄ 0.1% and MgSO₄. 7H₂O 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 0.1ml 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.

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 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

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earlier workers Adam (1981) [2] reported optimum period for amylase production is more 7 days in *Humicola lanuginosa*. 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°C temperature. Sonwane (2002) [5] observed *A. alternata* favored 20 to 30°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 flavus*. Jonson (1968) [8] reported that alkaline pH was favored in protease production of *A. tenuissima*.

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

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

Activity zone in mm

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

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

Activity zone in mm

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

Species of <i>Alternaria</i>	Temperature 0°C					
	5	10	15	20	30	40
Amylase Production						
<i>A. alternata</i>	--	13	12	12	18	17
<i>A. citri</i>	--	--	08	10	20	--
<i>A. crassa</i>	--	--	09	10	15	--
<i>A. dianthicola</i>	--	--	10	12	17	21
<i>A. macrospora</i>	--	--	10	11	14	18
<i>A. tenuissima</i>	--	12	13	09	20	14

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

Activity zone in mm

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

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

Activity zone in mm

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