



Extra-cellular cellulase enzyme production by post-harvest fungi under the influence of physical factors

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ABSTRACT

The present study deals with impact of physical factors on extracellular cellulase activity of some dominant post-harvest fungi. Post-harvest fungi were isolated from different varieties of papaya fruits by agar plate method. Out of 20 species 10 species of fungi were selected to study their cellulase enzyme activity. Different physical parameters such as light, incubation period, temperature and pH were studied in order to determine the optimum conditions for cellulase production of ten dominant fungi. The cellulase present in the broth was assayed by cup-plate method. It is observed that cellulase activity of post-harvest fungi was found to be optimum at temperature 20°C, pH 6.0-6.5, incubation period of 20 days and continuous light

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Introduction

Papaya (*Carica papaya* L) is an economically important fruit crop cultivated in tropical and subtropical regions of the world. During post-harvest condition papaya fruits gets infected by several fungi, during their infection, these fungi secrete their biological weapons, that is, enzyme like cellulase and pectinase which causes spoilage of fruits. Since very little information is available on the effect of carbon sources, nitrogen sources, phosphorous sources, sulphur sources, antibiotics, vitamins, fungicides and trace elements on cellulase activity of post-harvest fungi of papaya fruits, attempts were made to determine the impact of these physical factors on cellulase activity of post-harvest fungi.

Material and Methods

Fresh, healthy and mature papaya fruits of Taiwan variety were collected from Aurangabad fruit market. Papaya fruits were surface sterilized with 0.1 % HgCl₂, pricked to a depth of 2 mm and washed with sterile distilled water. The injured fruits were dipped in spore suspension (100spore/ml) of selected dominant fungi for 2 min. Then the fruits were placed in sterilized polythene bags as on fruit per bags. These polythene bags containing papaya fruits were incubated to different level of temperature and relative humidity.

of temperature, incubation periods and pH on activity of cellulase enzyme of post harvest fungi was investigated by incubating inoculated fruits at different temperature and different pH. On 8th day of inoculation 5g of rotted tissue was macerated with distilled water and 0.5N NaCl. The extract was filtered and filtrate was centrifuged at 4000 rpm for 25min. the supernatant was used as enzyme sample. Cellulase was assayed gives in 2ml of enzyme sample, 5ml of 1% pectin dissolved in buffer solution (pH- 4.5), 1.8ml of phosphate citrate buffer solution (pH-4.0) and 1.5ml of distilled water. The cellulolytic were assayed using 2ml of enzyme sample, 5ml of 1% CMC (Carboxy Methyl Cellulose), dissolved in buffer solution (pH-

4.5), 1.8ml of sodium citrate buffer (pH-4.8) and 1.8ml of distilled water.

The enzyme activity was assayed by determining loss in viscosity of the reaction mixture after 120 min at 30°C following the method of Bell et al (1955). The data were statistically analyzed for C.D. following Panse V.G. and Sukhatme P.V. (1978).

Results And Discussion

Studies on production of ten post-harvest fungi were made in relation to the different types of light illuminations and results are given in table 1 (Graph 1).

It is evident from the results that *Fusarium oxysporum*, *Alternaria alternata*, *Aspergillus flavus*, *Colletotrichum gloeosporioides* and *Penicillium digitatum* were active in continuous light for cellulase production while *Alternaria alternata*, *Fusarium moniliforme* and *Rhizopus stolonifer* were very less efficient in continuous dark for cellulase production where as alternate light and dark illumination was proved to be favourable for cellulase activity of all fungi.

In order to find that the optimum period for cellulase production, the culture filtrates of test fungi from 5 to 25 days of incubation were assayed for cellulase activity and results are summarized in table 2 (Graph 2).

It was observed from the results that out of ten fungi studied *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Fusarium moniliforme* and *Penicillium digitatum* could not produce cellulase at 5th day incubation period whereas, other test fungi showed production of cellulase on 5th days of incubation. The production of cellulase increased gradually up to 10th days. It is interesting to note that the post-harvest fungi increase the cellulase production up to 15 –20 days, however on 25th days there was no any considerable difference of cellulase production. On tenth days *Aspergillus flavus*, *Aspergillus niger*, *Alternaria alternata*, *Curvularia lunata*, *Colletotrichum gloeosporioides*, *Fusarium equiseti* and *Penicillium digitatum* were showed maximum cellulase activity whereas, *Fusarium oxysporum*,

Fusarium moniliforme and *Rhizopus stolonifer* showed minimum cellulase activity.

Cellulase production of post-harvest fungi was studied at six different temperatures and the results are summarized in table 3, (Graph 3 and plate 1).

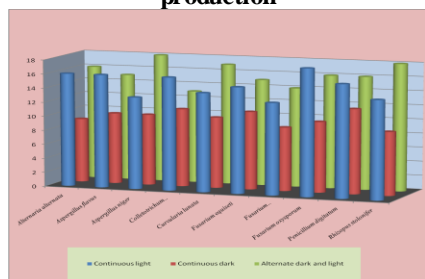
It was observed from the results that at low temperature (10°C) except *Curvularia lunata* and *Penicillium digitatum* other tested fungi were unable to produce cellulase as well as at temperature (20°C) except *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata* and *Fusarium oxysporum* other tested fungi were unable to produce cellulase. The temperature range 20 to 35°C was found to be stimulatory for cellulase production in all the fungi whereas, as temperature goes on increasing it was also reduced the cellulase enzyme activity.

The fungi were cultured on glucose nitrate medium at twelve different pH values from 3.0 to 8.5 and incubated for seven days at room temperature and cellulase production was studied and results are summarized in table 4, graph 4.

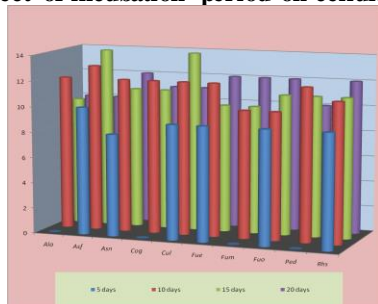
It was observed that at pH 3.5, except *Curvularia lunata* none of the test fungi produced cellulase enzyme. At pH 5.0 to 7.0 cellulase activity of all tested fungi was optimum. At pH 4.0 *Colletotrichum gloeosporioides* and *Fusarium equiseti* as well as at pH 4.5 *Alternaria alternata* and *Fusarium moniliforme* were found completely inhibitory for cellulase production. *Penicillium digitatum*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Rhizopus stolonifer* were produced maximum cellulase at 6.0 pH, whereas *Curvularia lunata*, *Aspergillus flavus*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Penicillium digitatum* were produced maximum cellulase activity at 6.5 pH.

Impact of physical factors like illumination of light, incubation period, temperature and pH on cellulase production was studied by several scientists. Alternate light and dark stimulated cellulase activity in all tested fungi. Maximum cellulase activity of all post-harvest fungi was found in between 15-20th days of incubation period. Temperature range between 20-35°C is more suitable for cellulase production. Rathod (2007), Kesare (2008) and Kulkarni (2009) reported similar findings about the effect of incubation period, temperature, pH and light on hydrolytic enzyme of fungi.

Graph 1: Effect of illumination of light on cellulase production



Graph 2: Effect of incubation period on cellulase production



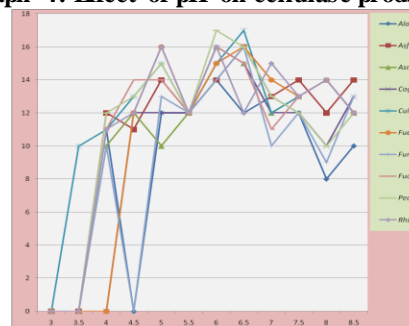
Ala = *Alternaria alternata*
Asf = *Aspergillus flavus*
Asn = *Aspergillus niger*
Cog = *Colletotrichum gloeosporioides*
Cul = *Curvularia lunata*
Fue = *Fusarium equiseti*
Fum = *Fusarium moniliforme*
Fuo = *Fusarium oxysporum*
Ped = *Penicillium digitatum*
Rhs = *Rhizopus stolonifer*

Graph 3: Effect of temperature on cellulase production



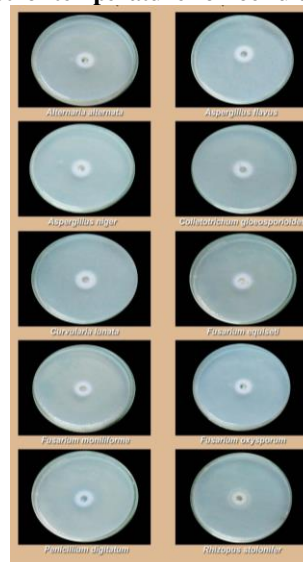
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Rhs = *Rhizopus stolonifer*

Graph 4: Effect of pH on cellulase production



Ala = *Alternaria alternata*
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Fig. 1: Effect of temperature on cellulase production



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Table 1: Effect of light illumination on cellulase production

Fungi	Light		
	Continuous light	Continuous dark	Alternate dark and light
Alternaria alternata	16	09	16
Aspergillus flavus	16	10	15
Aspergillus niger	13	10	18
Colletotrichum gloeosporioides	16	11	13
Curvularia lunata	14	10	17
Fusarium equiseti	15	11	15
Fusarium moniliforme	13	09	14
Fusarium oxysporum	18	10	16
Penicillium digitatum	16	12	16
Rhizopus stolonifer	14	09	18

Zone of enzyme activity expressed in mm

Table 2: Effect of incubation period on cellulase production

Incubation period	Fungi									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
5 days	--	10	08	--	09	09	--	09	--	09
10 days	12	13	12	12	12	12	10	10	12	11
15 days	10	14	11	11	14	10	10	11	11	11
20 days	10	10	12	11	11	12	12	12	10	12

Zone of enzyme activity expressed in mm

Ala = Alternaria alternata

Asf = Aspergillus flavus

Asn = Aspergillus niger

Cog = Colletotrichum gloeosporioides

Cul = Curvularia lunata

Fue = Fusarium equiseti

Fum = Fusarium moniliforme

Fuo = Fusarium oxysporum

Ped = Penicillium digitatum

Rhs = Rhizopus stolonifer

Table 3: Effect of temperature on cellulase production

Temperature (°C)	Fungi									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
10°C	--	--	--	--	10	--	--	--	10	--
20°C	--	12	13	--	10	--	--	10	--	--
25°C	13	14	17	13	12	12	11	13	14	12
30°C	14	15	14	16	15	13	14	12	13	12
35°C	17	18	16	14	12	12	12	16	14	14
40°C	15	16	13	12	16	15	14	14	16	13

Zone of enzyme activity expressed in mm

Ala = Alternaria alternata

Asf = Aspergillus flavus

Asn = Aspergillus niger

Cog = Colletotrichum gloeosporioides

Cul = Curvularia lunata

Fue = Fusarium equiseti

Fum = Fusarium moniliforme

Fuo = Fusarium oxysporum

Ped = Penicillium digitatum

Rhs = Rhizopus stolonifer

Table 4: Effect of pH on cellulase production

pH	Fungi									
	Ala	Asf	Asn	Cog	Cul	Fue	Fum	Fuo	Ped	Rhs
3.0	--	--	--	--	--	--	--	--	--	--
3.5	--	--	--	--	10	--	--	--	--	--
4.0	11	12	10	--	11	--	10	11	12	11
4.5	--	11	12	12	13	12	--	14	13	12
5.0	12	14	10	12	15	16	13	14	15	16
5.5	12	12	12	12	12	12	12	12	12	12
6.0	14	14	16	16	15	15	14	16	17	16
6.5	12	16	15	15	17	16	16	15	16	12
7.0	13	13	12	12	12	14	10	11	13	15
7.5	12	14	13	12	13	13	12	13	12	13
8.0	08	12	14	10	14	14	09	14	10	14
8.5	10	14	12	13	12	12	13	12	12	12

Zone of enzyme activity expressed in mm

Ala = Alternaria alternata

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