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Cellulase enzyme production of post-harvest fungi under the influence of carbon and nitrogen sources

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Abstract
Present research work embodied the effect of nutritional sources like carbon and nitrogen on cellulase enzyme production of post-harvest fungi. It was found that, among carbon sources fructose and sucrose stimulated cellulase activity, while lactose, CMC and starch inhibited the cellulase activity of test fungi. Nitrate source like sodium nitrate, sodium nitrite and calcium nitrate stimulated the cellulase activity while, ammonium sources in the form of nitrate, phosphate and sulphate were proved inhibitory for cellulase production of all post-harvest fungi.

Keywords: Cellulase enzyme activity, post-harvest fungi, carbon sources and nitrogen sources.

INTRODUCTION

In developing countries, postharvest losses are often more severe due to ill storage conditions and transportation facilities. Fungal fruits infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Nutritionally, fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decayed [1]. Cellulase is a complex enzyme, act synergistically to convert complex carbohydrates present in lignocellulosic biomass into glucose efficiently [2]. Post-harvest fungi of fruits are specific in their nutritional requirements. Therefore, they attack their respective susceptible host and causes changes in the stored product by absorbing or by hydrolysing complex forms into simple substances through the hydrolytic enzyme activity. Pectic and cellulolytic enzymes are the two main biological weapons by which fungi can break down the pectic and cellulosic substances of the host cell wall. Several workers have recorded the elaboration of such enzymes e.g. pectinases, hydrolases, oxidases and other enzymes by pathogens and storage fungi causing post-harvest diseases [3]. Several factors like physical and nutritional factors affect on cellulase enzyme production. Hence, in present investigation an attempt was made to study the effect of carbon and nitrogen sources on cellulase enzyme production.

MATERIALS AND METHODS

Production of cellulase

Production of cellulase was made by growing the fungi on liquid medium containing CMC – l0gm, KNO_3 – 0.25%, KH_2PO_4 – 0.1% and $MgSO_4.7H_2O$ – 0.05%, pH – 5.0. Out of which 25ml of medium was poured in 100 ml Erlenmeyer conical flasks and autoclaved at 151bs pressure for 15 minutes. The flasks on cooling were inoculated separately with 1ml spore suspension of test fungi prepared from the 7 days old cultures grown on PDA slants. The flasks were inoculated for 6 days at 25 ± 1°C with diurnal periodicity of light. On the 7th day the flasks were harvested by filter the contents through Whatman filter paper no.l. The filtrates were collected in the pre-sterilized bottles and termed as crude enzyme.

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Assay for cellulase

Cellulase activity was assayed by viscometric method as viscosity loss % after 60 minutes.

RESULTS AND DISCUSSION

Effect of carbon sources on cellulase activity of post harvest fungi isolated from mango fruits

In order to study the effect of carbon sources on cellulase activity of test fungi, carbon sources other than glucose were supplemented individually in the basal medium of which two sources belongs to monosaccharide, three belongs to disaccharides and three belongs to polysaccharides. Effect of these carbon sources on cellulase production was studied and results are given in table 1. Among carbon sources fructose and galactose induced cellulase activity, while monosaccharide like sucrose inhibited cellulsae activity of test fungi. CMC and starch inhibited the cellulase activity of same enzymes. On the other hand, lactose stimulated pectinase enzyme production in case of *Botryodiplodia theobromae, Penicillium chrysogenum* and *Rhizopus stolonifer.*

Effect of carbon sources on cellulase activity of post harvest fungi isolated from papaya fruits

Monosaccharides like fructose and galactose induced cellulase activity, while sucrose inhibited cellulsae activity of

Aspergillus niger and *Colletotrichum gloeosporioides*. CMC and starch inhibited the cellulase activity of same enzymes. On the other hand, polysaccharides like pectin only inhibited cellulase activity of *Aspergillus niger, Colletotrichum gloeosporioides* and *Phoma caricae* (Table 2).

Effect of nitrogen sources on cellulase activity of post harvest fungi isolated from mango fruits

Nitrogen sources in the form of nitrate, nitrite and ammonium forms at 0.25% concentration were incorporated separately in the basal medium. Basal medium containing potassium nitrate served as the control. Effect of these different nitrogen sources on cellulase activity of post harvest was studied and results are given in table 3. Sodium nitrate inhibited cellulase activity of all post-harvest fungi while, calcium nitrate, sodium nitrate and urea were responsible to induce the cellulase activity of *Alternaria alternata, Fusarium oxysporum* and *Rhizopus stolonifer.* Ammonium oxalate stimulated cellulase activity of *Fusarium oxysporum, Phoma caricae* and *Rhizopus stolonifer.* On the contrary of thisa mmonium nitrate, ammonium phosphate and ammonium sulphate hampered the cellulase enzyme production in tested fungi.

Effect of nitrogen sources on cellulase activity of post harvest fungi isolated from papaya fruits

Sodium nitrate and calcium nitrate inhibited cellulase activity of all post-harvest fungi except *Aspergillus niger* while, sodium nitrate and urea hampered cellulase activity of all post-harvest fungi except *Aspergillus flavus* and *Aspergillus niger.* Ammonium oxalate in *Aspergillus niger* and *Penicillium chrysogenum*; ammonium phosphate in *Botryodiplodia theobromae, Penicillium chrysogenum* and *Phytophthora nicotiana*; ammonium sulphate in *Aspergillus niger, Penicillium chrysogenum* and *Phytophthora nicotiana* were found to be stimulatory for cellulase activity (Table 4).

CMC, starch, ammonium phosphate, diammonium hydrogen phosphate, ferrous sulphate, zinc sulphate and copper sulphate inhibited the cellulase activity produced by post-harvest fungi isolated from mango fruits [4]. Among carbon sources fructose and sucrose significantly induced cellulase and pectinase activity, while lactose, CMC and starch inhibited the cellulase and pectinase activity of *Colletotrichum gloesporioides* and *Rhizopus stolonifer* [5]. Nitrogen sources in the form of urea and peptone significantly increased pectinase activity of *Colletotrichum gloesporioides* and *Rhizopus stolonifer* but ammonium phosphate significantly reduced it [5].

Cellulase production of all tested fungi except *Aspergillus niger* and *Colletotrichum gloeosporioides* was maximum in presence of xylose as compared to glucose and minimum as compared to fructose, while among disaccharides, sucrose was proved to be stimulatory for cellulase production in all ten fungi. CMC and starch inhibited cellulase action in all the tested fungi [6]. Nitrogen sources like nitrites, nitrates, urea and ammonium forms were found to be inhibitory for the cellulase production of post-harvest fungi [6].

Table 1. Effect of carbon sources on cellulase activity of post harvest fungi isolated from mango fruits

Carbon sources	*Aspergillus flavus*	*Aspergillus niger*	*Colletotrichum gloeosporioides*	*Botryodiplodia theobromae*	*Penicillium chrysogenum*	*Rhizopus stolonifer*
Fructose	84	84	86	85	89	84
Galactose	81	85	84	84	84	82
Sucrose	81	81	83	83	85	81
Maltose	79	82	82	84	84	80
Lactose	77	82	78	86	85	80
CMC	71	80	78	76	77	72
Pectin	77	81	80	81	84	74
Starch	69	78	70	77	76	78
Control	77	82	80	82	84	78

Values are expressed in viscosity loss (%) after 60 minutes

Table 2. Effect of carbon sources on cellulase activity of post harvest fungi isolated from papaya fruits

Carbon sources	*Alternaria alternata*	*Aspergillus niger*	*Colletotrichum gloeosporioides*	*Fusarium oxysporum*	*Phoma caricae*	*Phytophthora nicotiana*
Fructose	85	79	80	90	86	88
Galactose	83	80	78	86	80	90
Sucrose	82	75	76	85	78	84
Maltose	79	76	76	86	78	84
Lactose	77	76	74	84	79	83
CMC	70	72	72	80	71	71
Pectin	79	74	74	83	72	81
Starch	67	71	64	78	74	74
Control	78	76	76	84	78	78

Values are expressed in viscosity loss (%) after 60 minutes

Table 3. Effect of nitrogen sources on cellulase activity of post harvest fungi isolated from mango fruits

Nitrogen sources	*Alternaria alternata*	*Aspergillus niger*	*Colletotrichum gloeosporioides*	*Fusarium oxysporum*	*Phoma caricae*	*Rhizopus stolonifer*
Sodium nitrate	83	90	74	90	87	75
Calcium nitrate	80	86	60	87	81	76
Sodium nitrite	79	83	70	85	81	72
Urea	77	88	68	86	81	71

Ammonium oxalate	75	88	66	90	83	74
Ammonium nitrate	69	82	64	82	74	66
Ammonium phosphate	75	85	66	83	75	66
Ammonium sulphate	67	84	56	79	73	64
Control	75	88	68	84	81	70

Values are expressed in viscosity loss (%) after 60 minutes

Table 4. Effect of nitrogen sources on cellulase activity of post harvest fungi isolated from papaya fruits

Nitrogen sources	*Aspergillus flavus*	*Aspergillus niger*	*Colletotrichum gloeosporioides*	*Botryodiplodia theobromae*	*Penicillium chrysogenum*	*Phytophthora nicotiana*
Sodium nitrate	82	68	43	68	51	60
Calcium nitrate	84	69	42	70	54	66
Sodium nitrite	88	72	46	76	66	65
Urea	86	73	42	60	58	60
Ammonium oxalate	76	72	44	72	70	58
Ammonium nitrate	80	66	58	64	66	70
Ammonium phosphate	82	62	64	80	70	74
Ammonium sulphate	81	70	65	69	71	76
Control	84	68	68	76	68	72

Values are expressed in viscosity loss (%) after 60 minutes

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