Current Botany 2012, 3(3): 01-03
ISSN: 2220-4822
Available Online: http://currentbotany.org/

# Impact of carbon and nitrogen sources on pectinase production of post-harvest fungi

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**Abstract**
In the present investigation, emphasis has been given on to study the pectinase enzyme production of post-harvest fungi isolated from mango and papaya fruits, under the influence of carbon and nitrogen sources. It was found that, among carbon sources fructose and sucrose induced pectinase activity, while lactose, CMC and starch inhibited the pectinase activity of test fungi. Nitrate source like sodium nitrate, sodium nitrite and calcium nitrate were found to be stimulated the pectinase activity while, ammonium sources in the form of nitrate, phosphate and sulphate were proved inhibitory for pectinase production of all post-harvest fungi.

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

## INTRODUCTION

In India, fruits have been found to be infected with several diseases in the field as well as very significantly in the transport and storage. Most of the diseases have been studied in detail in relation to epidemiology and management strategies. A post-harvest fruit and food loss constitutes a vast complex of physical and biological changes due to microorganisms like fungi and bacteria. Biochemical estimation of infection process showed that the microbial plant pathogens must produce a set of enzymes which degrades carbohydrate polymers and protein composition of the infected plant's cell wall [1 and 2]. These enzymes are either intracellular or extracellular [3 and 4]. The intracellularly produced enzymes are insoluble pectic polymers which cement the plant cells together, they include: Cellulase, amylase and pectinolytic enzymes. Cellulase, which is a cell wall degrading enzyme found within the tissue of plants and it is capable of tissue maceration especially when secreted by pathogens [5, 6 and 7]. Pectinolytic enzymes are also involved in host-pathogen interactions by degrading the middle lamella and cell walls leading to tissue maceration. This degradation and breakdown lead to loss of tissue coherence, separation of individual cells and finally, reduced food production. Several factors like physical and nutritional factors affect on pectinase enzyme production. Hence, this study reveals the effect of carbon and nitrogen sources on pectinase enzyme production.

## MATERIALS AND METHODS
### Pectinase
### Production of pectinase

Received: June 14, 2012; Revised: July 11, 2012; Accepted: Aug 25, 2012.

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Production of pectinase was made by growing the fungi in liquid medium containing pectin – 10gm, $KNO_3$ – 0.25%, $KH_2PO_4$ – 0.1%, $MgSO_4.7H_2O$ – 0.05%, pH – 5.0. Out of which 25 ml of medium was poured in 100 ml Erlenmeyer conical flasks and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with 1 ml standard spores / mycelial suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 days at $25^0$C with diurnal periodicity of light. On $7^{th}$ day, the flasks were harvested by filtering the contents through Whatman filter paper no.1. The filtrates were collected in the presterilized bottles and termed as crude enzyme.

### Assay for pectinase

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

The Ostwald's viscometer was thoroughly cleaned with distilled water and dried before use. 6ml of pectin in 2ml of 0.2 M acetate buffer (pH 5.2) and 4ml of enzyme source were taken in viscometer and were thoroughly mixed and incubated at $25^0$C temperature. The efflux time of the mixture at 0, 5, 10, 20, 30, 40, 50 and 60 minutes was recorded with the help of stop watch. The percent loss of viscosity was calculated by using the formula

$$\text{Percent loss of viscosity} = \frac{T_0 - Tx}{T_0 - Tw} \times 100$$

**Where, $T_0$ = Flow time in seconds at zero time**
Tx = Flow time of the reaction mixture at time T
Tw = Flow time of distilled water

## RESULTS AND DISCUSSION
### Effect of carbon sources on pectinase activity of post harvest fungi isolated from mango fruits

In order to study the effect of carbon sources on pectinase activity of post harvest 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 pectinase production was studied and results are given in table 1.

Among carbon sources fructose and sucrose induced pectinase activity, while, CMC and starch inhibited the activity of same enzymes. On the contrary of this pectinase activity of *Aspergillus niger, Botryodiplodia theobromae, Penicillium chrysogenum* and *Rhizopus stolonifer* was stimulated due to pectin.

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

In order to study the effect of carbon sources on pectinase activity of post harvest fungi isolated from papaya fruit, 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 pectinase production was studied and results are given in table 2.

Among carbon sources fructose and sucrose induced pectinase activity of post-harvest fungi, while CMC and starch inhibited pectinase activity. Maltose and lactose stimulated the pectinase activity of *Fusarium oxysporum, Phoma caricae* and *Phytophthora nicotiana.* On the contrary of this pectinase activity of *Aspergillus niger* and *Phytophthora nicotiana* was stimulated due to polysaccharides like pectin.

**Effect of nitrogen sources on pectinase 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 was studied and results are given in table 3.

Nitrate source like sodium nitrate, Sodium nitrite and calcium nitrate were found to be stimulated the pectinase activity of all test fungi except *Colletotrichum gloeosporioides*. Nitrogen sources in the form of urea hampered pectinase activity *Aspergillus niger, Colletotrichum gloeosporioides* and *Phoma caricae.* Ammonium oxalate stimulated the pectinase of *Fusarium oxysporum, Phoma caricae* and *Rhizopus stolonifer*. Ammonium sources in the form of nitrate, phosphate and sulphate were proved inhibitory for pectinase production of all post-harvest fungi.

**Effect of nitrogen sources on pectinase activity of post harvest fungi isolated from papaya 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 was studied and results are given in table 4.

Nitrate source like sodium nitrate in all post-harvest fungi except *Penicillium chrysogenum* and sodium nitrite and calcium nitrate in all post-harvest fungi except *Aspergillus flavus* were proved to be inhibitory for pectinase activity. Urea was found to be stimulatory for *Aspergillus niger* and *Aspergillus flavus* to produce pectinase enzyme. Ammonium phosphate and ammonium sulphate hampered the pectinase activity of post-harvest fungi except *Penicillium chrysogenum* and *Phytophthora nicotiana.*

Similar results were reported [8]. It was reported that, glucose was stimulatory for *Fusarium oxysporum, Fusarium equiseti* and *Penicillium digitatum* whereas *Alternaria alternata, Curvularia lunata* and *Colletotrichum gloeosporioides* inhibited production of pectinase. Fructose was proved stimulatory for pectinase production in *Fusarium oxysporum, Fusarium equiseti* and *Penicillium digitatum*. CMC proved poor source of carbon for pectinase production all the tested fungi. Sucrose was proved to be stimulatory for pectinase production to *Aspergillus niger* and *Alternaria alternata*. On the other hand, nitrogen sources like sodium nitrate favoured maximum production of pectinase in case of *Aspergillus niger, Penicillium digitatum* and *Aspergillus flavus* whereas, sodium nitrate was unfavourable for pectinase production in case of *Colletotrichum gloeosporioides, Fusarium moniliforme, Alternaria alternata, Curvularia lunata, Fusarium equiseti* and *Rhizopus stolonifer*. *Alternaria alternata, Curvularia lunata, Aspergillus niger, Fusarium moniliforme* were inhibited the production of the pectinase enzyme in presence of sodium nitrite. Urea proved to be inhibitory to *Penicillium digitatum, Fusarium moniliforme* and *Curvularia lunata* for pectinase enzyme production. Carbon sources like fructose and sucrose significantly induced cellulase and pectinase activity, while lactose, CMC and starch inhibited the cellulase and pectinase activity of *Aspergillus flavus, A. fumigatus, A. niger, Colletotrichum gloesporioides* and *Rhizopus stolonifer*. On the other hand, nitrogen sources in the form of urea and peptone increased pectinase activity but ammonium phosphate reduced it [9].

Urea supported the maximum production of cellulases when compared with $(NH_4)_2SO_4$ [10]. Fructose and sucrose stimulated lipase activity while lactose, carboxyl methyl cellulose and starch inhibited lipase activity of pathogenic fungi [11].

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

| Carbon sources | *Aspergillus flavus* | *Aspergillus niger* | *Colletotrichum gloeosporioides* | *Botryodiplodia theobromae* | *Penicillium chrysogenum* | *Rhizopus stolonifer* |
|---|---|---|---|---|---|---|
| Fructose | 82 | 86 | 84 | 87 | 86 | 86 |
| Galactose | 79 | 87 | 82 | 86 | 80 | 84 |
| Sucrose | 79 | 83 | 81 | 85 | 81 | 83 |
| Maltose | 77 | 84 | 80 | 86 | 80 | 82 |
| Lactose | 75 | 84 | 78 | 88 | 81 | 83 |
| CMC | 69 | 82 | 76 | 78 | 73 | 78 |
| Pectin | 75 | 85 | 78 | 86 | 84 | 84 |
| Starch | 67 | 80 | 68 | 79 | 72 | 76 |
| Control | 75 | 84 | 80 | 84 | 80 | 82 |

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

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

| Carbon sources | *Alternaria alternata* | *Aspergillus niger* | *Colletotrichum gloeosporioides* | *Fusarium oxysporum* | *Phoma caricae* | *Phytophthora nicotiana* |
|---|---|---|---|---|---|---|
| Fructose | 84 | 88 | 82 | 85 | 84 | 85 |
| Galactose | 81 | 89 | 80 | 84 | 78 | 84 |
| Sucrose | 81 | 85 | 79 | 83 | 79 | 83 |
| Maltose | 79 | 86 | 78 | 84 | 78 | 84 |
| Lactose | 77 | 86 | 76 | 86 | 79 | 83 |
| CMC | 71 | 84 | 74 | 80 | 71 | 77 |
| Pectin | 77 | 88 | 76 | 81 | 72 | 81 |
| Starch | 69 | 82 | 66 | 77 | 70 | 75 |
| Control | 77 | 86 | 78 | 82 | 78 | 80 |

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

Table 3. Effect of nitrogen sources on pectinase 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 | 84 | 88 | 77 | 85 | 84 | 88 |
| Calcium nitrate | 81 | 89 | 78 | 84 | 78 | 83 |
| Sodium nitrite | 81 | 85 | 79 | 83 | 79 | 82 |
| Urea | 79 | 86 | 78 | 84 | 78 | 81 |
| Ammonium oxalate | 77 | 86 | 76 | 86 | 79 | 81 |
| Ammonium nitrate | 71 | 84 | 74 | 80 | 71 | 73 |
| Ammonium phosphate | 77 | 83 | 76 | 81 | 72 | 74 |
| Ammonium sulphate | 69 | 82 | 66 | 77 | 70 | 76 |
| Control | 77 | 86 | 78 | 82 | 78 | 80 |

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

Table 4. Effect of nitrogen sources on pectinase 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 | 78 | 66 | 53 | 74 | 81 | 62 |
| Calcium nitrate | 82 | 64 | 52 | 66 | 64 | 64 |
| Sodium nitrite | 84 | 74 | 56 | 76 | 66 | 66 |
| Urea | 82 | 76 | 52 | 56 | 68 | 56 |
| Ammonium oxalate | 72 | 75 | 54 | 68 | 70 | 54 |
| Ammonium nitrate | 76 | 66 | 48 | 62 | 76 | 68 |
| Ammonium phosphate | 78 | 64 | 54 | 76 | 80 | 72 |
| Ammonium sulphate | 77 | 72 | 55 | 65 | 81 | 74 |
| Control | 80 | 70 | 58 | 72 | 78 | 70 |

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

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