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Effect of Supplementation of Carbohydrates and Nitrogen Sources on Pectinase Production In Tomato Fungi

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ABSTRACT

Tomato (Lycopersicon esculentum Mill.) is one of the common vegetables grown all over the country extensively almost the year round. The crop is reported to be affected by about twenty diseases of microbial origin. Among them, the fungal pathogens have been found to affect and damage severely the tomato fruits both in field at different developmental stages as well as in the market during storage. This may result in the qualitative and quantitative loss of tomato fruits. The fungi are known to produce different hydrolytic enzymes during pathogenesis. These enzymes degrade the food contents. During the present investigation, studies were made on the effect of supplementation of different carbohydrates and nitrogen sources on pectinase production in the fungi isolated from tomato fruits. These factors were found to affect the pectinase production in the fungi isolated from tomato fruits.

KEYWORDS Carbohydrates, nitrogen sources, pectinase production, tomato fungi.

INTRODUCTION:

Tomato (***Lycopersicon esculentum*** Mill.) is one of the common vegetables grown all over the country extensively almost the year round. The crop is reported to be affected by about twenty diseases of microbial origin. Among them, the fungal pathogens have been found to affect and damage severely the tomato fruits both in field at different developmental stages as well as in the market during storage. This may result in the qualitative and quantitative loss of tomato fruits.

It is the well known fact that the fungi produce different hydrolytic enzymes during pathogenesis. The hydrolytic enzymes produced by the fungi like cellulases, pectinases, amylases, lipases and proteases are known to degrade food contents. Sreekantiah ***et al***. (1971) found that, ***Alternaria alternata, Fusarium solani*** f.sp***. minus, Pleospora infectoria*** and ***Alternaria solani*** were capable of producing all the four kinds of hydrolytic enzymes, viz., pectinase, cellulase, amylase and proteinase. Balsubramanian (1972) reported that, protease along with cellulase and pectinase was found to be effective in infection by ***Rhizopus stolonifer*** within the tissue. Mehta ***et al***. (1974) found that, during pathogenesis of tomato fruits, ***Alternaria*** sp. produce pectolytic and celluloytic enzymes. They also reported that, polygalacturonase and pectin methyl galacturonase are found to play important role in pathogenesis due to ***Alternaria solani*** and in ***A. tenuis*** (***A. alternata***) infection. Ramasami and Shanmugam (1976) studied pectolytic and celluloytic enzymes of ***Rhizoctonia batatticola in vitro*** and ***in vivo***. . Hasija and Batra (1981) recorded that, ***Phoma destructiva*** produced pectin transliminase and polygalacturonase in diseased tomato fruits, while pectin methyl esterase, pectin methyl galacturonase occurred in both healthy and diseased tissue. Hasija and Batra (1984) found that, ***Phoma destructiva*** causing fruit rot of tomato fruits produced all types of pectic enzymes (PME, PMG, PG and PGTE ***in Vitro***).

In the present investigation, effect of supplementation of different carbohydrates and nitrogen sources was studied on the pectinase production in the fungi isolated from tomato fruits.

MATERIAL AND METHODS:

Production of pectinase:

For the production of pectinase i.e. pectin methyl galacturonase (PMG), the fungi were grown in a liquid medium composed of Pectin - 1%, KNO_3 - 0.25%, KH_2PO_4 – 0.1%, and $MgSO_4$. $7H_2O$ - 0.05%, pH -5.0. Twenty five ml of the medium was taken in 100 ml conical flasks and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with 1 ml standard spore suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 days at 25 °C. On 7th day, the flasks were harvested by filtering the contents through Whatmann No. 1 filter paper. The filtrates were collected in pre-sterilized bottles and termed as crude enzyme preparations.

Enzyme assay (Viscometry):

The Ostwald's viscometer was thoroughly cleaned with distilled water and dried before use. Six ml of 1% pectin in 2 ml of 0.2 M acetate buffer pH 5.2 and 4 ml of enzyme source were taken in viscometer and were thoroughly mixed and incubated at 25°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:

Per cent loss of viscosity = (To-Tx)/(To-Tw) x 100

Where To = 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:

Table 1: Effect of carbohydrates on production of pectinase in tomato fungi

Carbohydrates (0.5% conc.)	Fungi					
	Aso	Clu	Gca	Rso	Fox	Phy
	% Viscosity loss after 40 minutes					
Monosaccharides						
Glocose	72.2	51.2	84.5	48.0	73.3	87.1
Fructose	71.7	47.3	80.3	54.1	74.8	68.1
Galactose	22.2	27.5	71.2	61.3	54.3	71.2
Mannose	34.3	29.3	60.1	58.1	59.1	48.0
Xylose	27.5	41.1	61.2	49.5	53.7	62.0
Disaccharides						
Sucrose	40.5	57.6	47.8	67.8	58.1	85.2
Maltose	30.1	37.2	40.2	56.8	48.7	67.8
Lactose	20.1	50.1	51.5	49.7	60.2	70.3
Polysaccharides						
CMC	21.7	30.7	37.8	47.5	41.3	41.2

Pectin	68.3	61.5	82.5	65.0	67.3	83.3
Starch	31.8	31.2	45.2	50.1	41.7	32.7

Table 2: Effect of nitrogen sources on production of pectinase in tomato fungi

Nitrogen sources (0.25% conc.)	Fungi					
	Aso	Clu	Gca	Rso	Fox	Phy
	% Viscosity loss after 40 minutes					
Nitrate forms						
Sodium nitrate	50.1	00.0	37.5	00.0	39.3	62.1
Calcium nitrate	39.1	47.5	61.1	47.0	42.0	58.7
Nitrite forms						
Sodium nitrite	51.2	37.8	38.7	41.2	31.2	41.8
Potassium nitrite	48.7	39.2	42.9	32.8	41.1	43.1
Ammonium forms						
Ammonium oxalate	37.9	41.5	69.3	53.9	48.7	64.5
Ammonium nitrate	70.9	67.9	84.7	69.5	73.3	87.2
Ammonium phosphate	51.7	41.2	77.5	57.1	53.8	71.3
Ammonium sulphate	49.3	51.9	73.2	52.3	49.3	74.8
Ammonium molybdate	30.1	34.7	49.3	41.2	43.8	54.7
Amide form						
Urea	00.00	00.00	40.0	00.0	47.1	48.2
Organic forms						
Gelatin	40.1	41.0	71.8	57.1	42.1	59.3
Peptone	70.7	63.3	88.5	68.3	70.7	84.9
Casein	41.8	48.0	60.2	40.2	42.0	70.1
Control	68.3	60.5	82.5	65.0	67.1	83.3

Aso - *Alternaria solani* Clu - *Curvularia lunata*
Gca - *Geotrichum candidum* Rso - *Rhizoctona solani*
Fox - *Fusarium oxysporum* Phy - *Phytophthora* sp.

RESULTS AND DISCUSSION:

In order to study effect of supplementation of carbohydrates on pectinase production, fungi were grown on basal media supplemented with different carbohydrates at 0.5% concentration. From table 1, it is clear that, among monosaccharides, glucose was found to stimulate pectinase production in ***Alternaria***, ***Geotrichum candidum***, ***Fusarium oxysporum*** and ***Phytophthora*** sp., while fructose was stimulatory in case of ***Alternaria solani*** and ***Fusarium oxysporum***. Galactose, mannose, xylose, starch and carboxy methyl cellulose (CMC) were found to be inhibitory for pectinase production in all fungi. All the disaccharides inhibited pectinase production, except sucrose in ***Rhizoctonia solani*** and ***Phytophtora*** sp. Damle (1952) reported stimulation of pectinase production by fructose, glucose, starch and sucrose in ***Pythium*** sp. and ***Botrytis cinera***. Mantri (1969) also reported stimulation by glucose in ***Phytophthora rubra***. Glucose concentration above a certain level reduced enzyme secretion (Deshpande, 1960). Glucose was reported to be inhibitory for pectinase production in ***Alternaria tenuissima*** (Pandey and Gupta, 1966). Increasing concentrations of glucose and sucrose were inhibitory in case of ***Botrytis cinera*** and ***Phythium debaryanum*** (Ashour, 1955). Inhibition was caused by galactose in ***Fusarium oxysporum*** f.sp. ***lycopersici*** (Biehn and Dimond, 1971), glucose in ***Alternaria compacta*** (Punde, 1972) and arabinose, mannose, glucose, galactose, sucrose and raffinose in case of ***Penicillium expansum*** (Spalding and Baki, 1973).

In order to study the effect of supplementation of nitrogen sources other than potassium nitrate on pectinase production, different nitrogen sources at 0.25% concentration were added to the pectin nitrate medium. The medium with potassium nitrate served as control. From table 2, it is clear that, all the nitrate and nitrite form were inhibitory for pectinase production in all fungi. Sodium nitrate was completely inhibitory in case of ***Curvularia lunata*** and ***Rhizoctonia solani.*** Among the ammonium forms, only ammonium nitrate stimulated pectinase production in all fungi and other forms were inhibitory. Urea was found to be inhibitory for pectinase production in all fungi. Urea completely inhibited pectinase activity in ***Alternaria Solani, Curvularia lunata*** and ***Rhizoctonia Solani***. All the organic forms except peoptone were proved inhibitory for pectinase production in all fungi. Effect of different nitrogen sources on pectinase production has been reported by several workers. Peptone for ***Rhizoctonia solani*** (Deshpande,1960) ammonium sulphate and ammonium nitrate for some fungi including ***Penicillium*** sp. (Chatterji and Basu, 1960), peptone, ammonium nitrate and ammonium sulphate for ***Phytophthora rubra*** (Mantri, 1969), peptone for ***Aspergillus niger*** (Mukherjee and Majumdar, 1971), ammonium sulphate for ***Phoma betae*** (Bugbee,1972), ammonium sulphate, ammonium nitrate and potassium nitrate for ***Aspergillus sp.*** (Sreekantaiah ***et al***., 1973) were found to be stimulatory for pectinase production. On the other hand, potassium nitrate was inhibitory for pectinase production in ***Pythium*** sp. and ***Botrytis cinera*** (Damle, 1952). Gupta and Rautela (1964) found ammonium nitrogen better than nitrate nitrogen for pectinase production in ***Penicillium expansum***.

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