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Original Research Paper

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INFLUENCE OF SUPPLEMENTATION OF CARBOHYDRATES AND NITROGEN SOURCES ON PROTEASE PRODUCTION IN SOME 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 influence of supplementation of some nutritional factors viz. carbohydrates and nitrogen sources on protease production in some fungi isolated from tomato fruits. These factors were found to affect the protease production in the fungi.

KEYWORDS : Carbohydrates, nitrogen sources, protease 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.

It is well established fact that, growth of microorganisms is directly or indirectly related to their metabolic activities. Therefore, in the present investigation, the factors which control the growth of microorganisms like carbohydrates and nitrogen sources were studied for their effect on the protease production in some fungi isolated from tomato fruits.

MATERIAL AND METHODS:

a) Production of protease:

Production of protease was made by growing the fungi on liquid medium containing Glucose – 10 gm, Gelatin – 10 gm, K_2HPO_4 – 1.0 gm, $MgSO_4.7H_2O$-0.5 gm and Distilled Water – 1000 ml, pH-5.5. 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.

b) Enzyme assay (Cup plate method):

The protease activity was studied by Cup plate method (Hislop *et al*., 1982). A basal medium was prepared by adding 2% agar and 1% gelatin and pH of was adjusted at 5.0. Then, it was sterilized at 15 lbs pressure for 20 minutes. About 15 ml of the medium was poured in pre-sterilized Petri plates under aseptic conditions. On solidification, 6 mm diameter cups/cavities were made in the centre of each of the agar plate with a sterilized cork borer. The cups/cavities were filled carefully with about 0.5 ml of culture filtrate (crude enzyme preparation). The plates were incubated at 25°C for 24 hours. Then the plates were flooded with 15% mercuric chloride in 1N HCl. After 10 minutes of standing, a clear transparent zone indicated the hydrolysis of gelatin by the extracellular proteolytic enzymes, whereas the rest of the regions of the Petri plates became opaque due to coagulation of gelatin (protein) by mercuric chloride. Diameter of clear zone was used as a measure of protease activity, while non-appearance of clear zone was considered to be due to absence of protease in the culture filtrates.

RESULTS AND DISCUSSION:

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

Carbohydrates (0.5% conc.)	Fungi					
	Aso	Clu	Gca	Rso	Fox	Phy
	Activity zone (mm)					
Monosaccharide						
Glucose	15	17	17	15	16	17
Fructose	09	16	17	12	11	15
Galactose	13	15	12	13	15	15
Mannose	16	21	22	17	15	12
Xylose	13	14	18	12	16	13
Disaccharides						
Sucrose	23	20	25	20	19	18
Maltose	18	19	20	19	20	17
Lactose	13	12	12	11	14	15
Polysaccharides						
CMC	14	13	09	12	10	12
Pectin	18	21	25	12	13	12
Starch	20	14	15	13	14	12

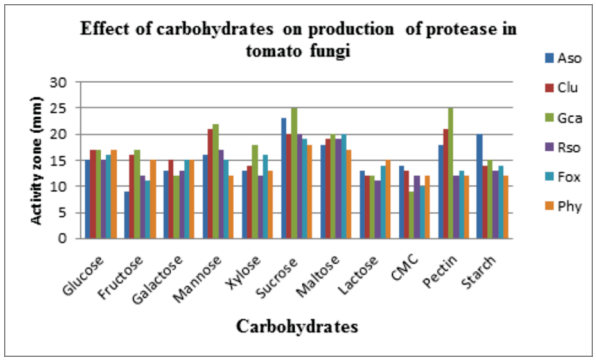
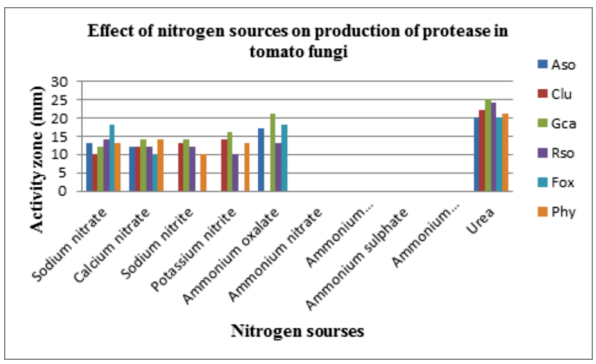


Table 2: Effect of nitrogen sources on production of protease in tomato fungi

Nitrogen sources (0.25% conc.)	Fungi					
	Aso	Clu	Gca	Rso	Fox	Phy
	Activity zone (mm)					
Nitrate forms						
Sodium nitrate	13	10	12	14	18	13
Calcium nitrate	12	12	14	12	10	14
Nitrite forms						
Sodium nitrite	--	13	14	12	--	10
Potassium nitrite	--	14	16	10	--	13
Ammonium forms						
Ammonium oxalate	17	--	21	13	18	--
Ammonium nitrate	--	--	--	--	--	--
Ammonium phosphate	--	--	--	--	--	--
Ammonium sulphate	--	--	--	--	--	--
Ammonium molybdate	--	--	--	--	--	--
Amide form						
Urea	20	22	25	24	20	21
Organic forms						
Gelatin	20	18	22	18	16	19
Peptone	20	17	21	18	18	19
Casein	17	13	18	16	18	18
Control	18	16	20	17	16	16



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

In order to study effect of supplementation of carbohydrates on perotease production, fungi were grown on basal media supplemented with different carbohydrates at 0.5% concentration. The fungi showed variation in their selection for carbohydrates to stimulate protease production. From table 1, it is clear that, among monosaccharides, fructose and glucose inhibited protease production in all fungi at some extent. Mannose stimulated protease production in *Curvularia lunata, Geotrichum candidum* and *Rhizoctona solani*. Xylose inhibited the enzyme production in all fungi, except *Geotrichum candidum* and *Fusarium oxysporum*. Sucrose and maltose stimulated protease production in all the fungi. Lactose and caboxymethyl cellulose inhibited protease production in all fungi. Pectin was stimulatory to *Alternaria solani*, *Curvularia lunata* and *Geotrichum candidum*, while starch was inhibitory in case of all the fungi, except *Alternaria solani*. The effect of carbohydrates on protease production in fungi was studied by different workers. Glucose in case of *Aspergillus flavus* (Srinivasan *et al*., 1990) and *Aspergillus niger* (Singh *et al*., 1994), maltose in case of *Aspergillus terricola* (Imshentskii and Popova, 1970), Lactose in case of *Fusarium* sp. and *Alternaria* sp. (Egorov *et al*., 1971), *Aspergillus flavus* (Malathi and Chakraborty, 1991 and Mulimani and Patil, 1999) were found to be stimulatory for protease production. Fructose and sucrose in *Alternaria alternata* (Patil and Shastri, 1982) and fructose in *Alternaria alternata, Macrophomina phaseolina* and *Rhizoctonia solani* (Bhikane, 1988) stimulated protease production

In order to study the effect of supplementation of nitrogen sources on protease production, different nitrogen sources at 0.25% concentration were added to the medium. From table 2, it becomes clear that, all the nitrite, nitrate and ammonium forms except sodium nitrate for *Fusarium oxysporum*, ammonium oxalate for *Geotrichum candidum* and *Fusarium oxysporum* proved inhibitory for protease production Urea, gelatin and peptone stimulated protease production in all the fungi. Casein inhibited protease production in all the fungi, except *Fusarium oxysporum* and *Phytophthora* sp. Similar type of variations in requirement of nitrogen sources for protease production were reported by various workers. Potassium nitrate and sodium nitrate were stimulatory for protease production in *Alternaria alternata* (Patil and Shastri, 1982), *Alternaria alternata, Macrophomina phaseolina* and *Rhizoctonia solani* (Bhikane, 1988), *Aspergillus flavus* (Srinivasan *et al*., 1990) and *Aspergillus niger* (Singh *et al*., 1994).

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