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# STUDIES ON EFFECT OF PHYSICAL FACTORS ON PROTEASE 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 physical factors like incubation period, temperature and pH on protease production in some fungi isolated from tomato fruits. These factors were found to affect the protease production in the fungi.

**KEYWORDS :** Physical factors, 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 incubation period, temperature and pH 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 7$^{th}$ 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 incubation period on protease production in tomato fungi**

| Incubation period (Days) | Fungi | | | | | |
|---|---|---|---|---|---|---|
| | Aso | Clu | Gca | Rso | Fox | Phy |
| | Activity Zone (mm) | | | | | |
| 4 | 19 | -- | 12 | 10 | 14 | 16 |
| 8 | 20 | 16 | 13 | 14 | 16 | 16 |
| 12 | 19 | 16 | 14 | 14 | 16 | 15 |
| 16 | 17 | 15 | 15 | 15 | 13 | 14 |
| 20 | 17 | 13 | 14 | 14 | 14 | 13 |

**Table 2: Effect of temperature on protease production in tomato fungi**

| Temperature (°C) | Fungi | | | | | |
|---|---|---|---|---|---|---|
| | Aso | Clu | Gca | Rso | Fox | Phy |
| | Activity Zone (mm) | | | | | |
| 5 | -- | -- | -- | -- | -- | -- |
| 10 | 11 | -- | -- | -- | -- | -- |
| 15 | 11 | 12 | 13 | -- | 13 | 14 |
| 20 | 14 | 15 | 16 | -- | 14 | 15 |
| 25 | 17 | 15 | 17 | 16 | 15 | 17 |
| 30 | 15 | 17 | 17 | 16 | 15 | 16 |
| 35 | 14 | 14 | 16 | 16 | 16 | 14 |
| 40 | 13 | 12 | 14 | 13 | 14 | 12 |

**Table 3: Effect of pH on protease production in tomato fungi**

| pH | Fungi | | | | | |
|---|---|---|---|---|---|---|
| | Aso | Clu | Gca | Rso | Fox | Phy |
| | Activity Zone (mm) | | | | | |
| 3.0 | -- | -- | -- | -- | -- | -- |
| 3.5 | -- | 08 | 20 | -- | -- | -- |
| 4.0 | 24 | 16 | 21 | -- | 13 | -- |
| 4.5 | 24 | 21 | 22 | -- | 13 | 14 |
| 5.0 | 19 | 19 | 21 | -- | 15 | 16 |
| 5.5 | 17 | 18 | 21 | 12 | 15 | 16 |
| 6.0 | 16 | 18 | 18 | 14 | 16 | 16 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **6.5** | 13 | 17 | 16 | 18 | 15 | 14 |
| **7.0** | 13 | 13 | 16 | 17 | 15 | 13 |
| **7.5** | 13 | 12 | 14 | 16 | -- | 12 |
| **8.0** | 13 | 12 | 14 | 10 | 11 | 10 |

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

In order to find out optimum incubation period for pectinase production, the culture filtrates of fungi from 4 to 20 days of incubation were analysed for protease activity. From table 1, it is clear that, all the fungi except *Curvularia lunata* showed protease production on 4$^{th}$ day of incubation. Protease production increased gradually with increase in incubation period upto 12 days in all the fungi, except *Geotrichum candidum* and *Rhizoctonia solani*, which showed maximum protease activity after 16 days. After that, there was decrease in protease production in all the fungi. Incubation period is recorded two days in case of *Aspergillus flavus* (Malathi and Chakraborty, 1991). *Alternaria alternata* required incubation period of 4-5 days (Patil and Shastri, 1982). Micro-organisms which required optimum period more than a week for production are *Fusarium oxysporum* and *Rhizoctonia solani* (Charya and Reddy, 1982), *Aspergillus niger* (Ashour *et al*., 1996) and *Penicillium* sp. (Singh and Saxena, 1988). Sharma and Saxena (1981) recorded 4-5 days of incubation period for protease production in *Chaetomium globosum* and *Fusarium moniliforme*.

The protease production was studied at eight different temperatures. From table 2, it is clear that, except *Alternaria solani* at 10 $^0$C, all the fungi were unable to produce the protease at and below 10 $^0$C. It was interesting to note that, *Rhizoctonia solani* could not produce protease below 25 $^0$C temperature. Optimum temperature range was and 25-30 $^0$C for *Alternaria solani* and *Phytophthora* sp., 20-30 $^0$C for *Curvularia lunata* and *Geotrichum candidum* and 25-35 $^0$C for *Rhizoctonia solani* and *Fusarium oxysporum*. Fungi having optimum temperature range between 20-30$^0$C are *Alternaria alternata* (Patil and Shastri, 1982) and *Alternaria tenuissima* (Jonsson, 1968) and *Rhizopus oryzae* (Banerjee and Bhattacharya, 1992). The fungus having optimum temperature range between 35$^0$C -40$^0$C is *Aspergillus* sp. (Nehra *et al*., 1998).

The protease production was studied at eleven different pH values. It is clear from table-3 that, all the fungi except *Curvularia lunata* and *Geotrichum candidum* could not produce protease below pH 3.5. Above pH 6.0, all the fungi showed decrease in protease production except *Rhizoctonia solani*, The optimum pH was 4.0-5.5 for *Alternaria solani* and *Geotrichum candidum*, 4.5-6.0 for *Curvularia lunata*, 6.5 for *Rhizoctonia solani*, 6.0 for *Fusarium oxysporum* and 5.0-6.0 for *Phytophthora* sp. Acidic pH is required for protease production in *Penicillum* sp. (Singh and Saxena, 1988), *Fusarium oxysporum* (Rajamani, 1990), while alkaline pH is required in *Alternaria tenuissima* (Jonsson, 1968) and *Aspergillus* sp. (Nehra *et al*., 1998).

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