ORIGINAL RESEARCH PAPER

Botany

PROTEASE PRODUCTION IN TOMATO FUNGI IN RELATION TO DIFFERENT CULTURE MEDIA AND AMINO ACIDS

KEY WORDS: Protease production, tomato fungi, culture media, amino acids.

C. S. Swami Department of Botany, Dayanand Science College, Latur – 413 512 (MS)

N.M. Ghangaonkar* Department of Botany, C.T.Bora College, Shirur, Dist: Pune (MS), 412210 *Corresponding Author

ABSTRACT

Tomato (Lycopersiconesculentum 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 protease production in some fungi isolated from tomato fruits in relation to different culture media and amino acids. These factors were found to affect the protease production in thefungi.

INTRODUCTION:

Tomato (Lycopersiconesculentum 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. Sreekantiahet al. (1971) found that, Alternariaalternata, Fusariumsolanif.sp. minus, PleosporainfectoriaandAlternariasolani 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 Rhizopusstolonifer within the tissue.

It is well established fact that, growth of microorganisms is directly or indirectly related to their metabolic activities. Therefore, in the presentinvestigation, the factors which control the growth of microorganisms like different culture media and amino acidswere 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, K2HPO4 – 1.0 gm, MgSO4.7H2O-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 oC. 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 (Hislopet 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 25oC 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: Production of protease in tomato fungi on different culture media

Fungi	Media				
	Non-substrate	Substrate			
	Glucose nitrate	Glucose gelatin	Gelatin broth	Glucose asparagine	Glucose peptone
	Activity zone (mm)				
Alternariasolani	19	18	18	16	20
Geotrichum candidum	20	19	18	17	20
Fusariumroseum	15	15	13	14	15
Fusariumoxysporum	16	17	15	13	15
Phomadestructiva	15	16	16	15	14
Rhizoctoniasolani	16	16	16	14	15
Phytophthorasp.	18	17	18	16	17
Cladosporiumfulvum	14	15	15	19	16
Curvularialunata	12	16	17	16	14
Aspergillusniger	17	20	18	18	16

Aspergillusflavus	20	22	21	18	22
Penicilliumexpansum	24	23	22	20	21
Rhizopusstolonifer	22	16	18	15	11

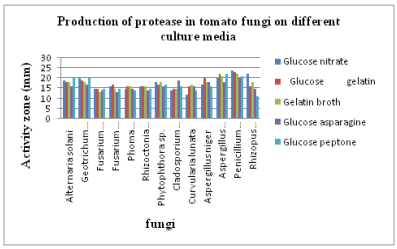
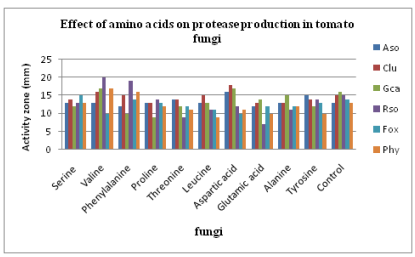


Table 2: Effect of amino acids on protease production in tomato fungi

Amino acids (0.02% conc.)	Fungi					
	Aso	Clu	Gca	Rso	Fox	Phy
	Activity zone (mm)					
Serine	13	14	12	13	15	13
Valine	13	16	17	20	10	17
Phenylalanine	12	15	10	19	14	16
Proline	13	13	09	14	13	12
Threonine	14	14	12	09	12	11
Leucine	13	15	13	11	11	09
Aspartic acid	16	18	17	12	10	11
Glutamic acid	12	13	14	07	12	10
Alanine	13	13	15	11	12	12
Tyrosine	15	14	12	14	13	10
Control	13	15	16	15	14	13



Aso-	AlternariasolaniClu -		Curvularialunata
Gca-	GeotrichumcandidumRso -		Rhizoctonasolani
Fox -	FusariumoxysporumPhy -		Phytophthora sp.

In order to know the constitutive and adaptive nature of protease production in the fungi, they were grown on Glucose nitrate medium (non-substrate) and different substrate supplemented media. The culture filtrates were tested for protease production. From Table 1, it is clear that, all the fungi were found to be capable of producing protease even in the non-substrate medium. The protease production on Glucose nitrate medium was maximum in case of Penicilliumexpansum, Rhizopusstolonifer, Geotrichumcandidumand Aspergillusflavus. Glucose gelatin favouredprotease production in in all the fungi, except Alternariasolani,Geotrichumcandidumand Phytophthora sp. Gelatin broth was favourable for Phomadestructiva, Rhizoctoniasolani, Phytophthorasp. and Curvularialunata. Glucose asparagine was found to be stimulatory for protease production as campared to that on non-substrate medium in case of Cladosporiumfulvum, Curvularialunata and Aspergillusniger. Stimulation of protease production in case of Alternariasolani, Cladospori umfulvum, Curvularialunata and Aspergillusnigerwas found in Glucose peptone medium, while the medium was inhibitory for most of the fungi, except Geotrichumcandidum, in which the activity was unaffected. All the fungi isolated from tomato fruits produced protease in the medium with or without substrate. Protease production in absence of protein indicates constitutive nature of protease production in them. The fungi produced more protease on substrate containing medium than that of non-substrate medium. The constitutive nature of protease production was reported in Rhizoctoniasolani, Macrophominaphaseolina, Fusariumoxys porumandAspergillusflavus (Bhikane, 1988).

Effect of amino acids at 0.02 % concentration on protease production in some fungi isolated from tomato fruits was also studied. From Table 2, it is clear that, all the amino acids were inhibitory for protease production, except tyrosine and alanine for Alternariasolani, serine forFusariumoxysporum, proline for Rhizoctonasolani, valine for all fungi exceptFusariumoxysporum and Alternariasolani, leucine for Alternariasolani andCurvularialunata, aspartic acid for Alternariasolani, Curvularialunataand Geotrichumcandidum. This indictes that, different fungi need different type of amino acids during protease synthesis. Some amino acids were found to inhibit protease production in some fungi. Stimulatory nature of amino certain acids in protease production have also been reported by various workers. Tyrosine and tryptophan in Alternariaalternata(Patil and Shastri, 1982), arginine and cysteine in Fusariumoxysporum, Rhizoctonasolaniand MacrophominaphaseolinaBhikane, 1988)were stimulatory for protease production. Waghmare (1996) reported protease inhibition by asparagine, tyrosine and lysine in Fusariumdimerum, serine in Fusariumoxysporum, tyrosine in Fusariumroseumand lysine in Fusariumsemitectum.

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