Observation on Quantitative and Qualitative Variability in Extracellular Enzymes of Certain Isolates of Genus *Alternaria*

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Research Article

Abstract: A few extracellular enzymes of the isolates of *AlternariA. alternata* and *AlternariA. triticina* were viscometrically determined, Quantitative variability was noticed in PG, PME, PGTE, PMTE, PMG, cellulase and protease in screction in the culture medium by various isolates. Absence of PME activity in the culture filtrates of a few isolates of the same species was an interesting observation. Present findings point towards the fact that isolates of the same morphological species may differ in bio-chemical level.

Key words: Extracellular enzymes, *Alternaria, alternata, Alternaria, triticina.*

Introduction

The importance of vegetables in daily human diet is recognized all over the world. Vegetable are rich and comparatively cheaper source of vitamins and minerals. Consumption of these vegetables enhances taste, palatability, appetite and provides fibers for digestion. Vegetables play a key role in neutralizing all acids produced during digestion of fatty acids [1 & 3].Even an urban householder observes in the course of his domestic chores that common vegetables are at times rottened and diseased. A good edible part of these vegetables is lost due to unsatisfactory conditions of transit and marketing [12, 29, 30 & 31]. Solanaceous fruits like so many other Fruits become soft and pulpy after attack of pathogens. Depression of tissue is common symptom of soft rot diseases. This depression in the tissue is due to collapse of cell wall skeleton. These symptoms are followed by maceration with the advancement of growth of pathogen. Maceration is due to degradation of middle lamella, which is made up of pectic substance [23]. Along with the pectic substance cellulose is also formed main skeleton of cell wall. In the degradation of pectic and cellulolytic substances pectic and cellulase enzyme play an importance role [4, 5, 8, 15, 11, 18, 20, 27 & 33]. Protein is also present in middle lamella a lay an important role in cell wall skeleton formation. The protein of middle lamella is hydrolysed by proteolytic enzymes [16 & 19]. These pectic, cellulolytic and protease enzymes degrade the skeleton of cell wall & convert complex molecules in to simple molecules. These simple released molecules after hydrolysis of cell wall material is used as nourishment for pathogen. Thus extracellular enzymes play major key role in disease development by degradation of parenchymatous tissue of host [15, 26 & 32]. These extracellular enzymes are also secreted by pathogenic fungal organism in culture filtrates [16, 33, 36, & 39]. Aim of this study was to observed variability in secretion of extracellular enzymes of different isolates of morphologically same genus Alternaria.

Material and Methods

Diseased solanaceous fruits, showing symptoms like soft rot, water soaked area and sunkened blackish brown lesions with concentric rings, were collected from vegetable market in separate polythene bags and were collected and brought to the laboratory for further study.

Isolation, Punification and Maintenance of Pathogen

Diseased fruits as such or in small pieces were surface sterilized by 1% mercuric chloride solution for about half to one minute [21]. Surface sterilized fruits were washed with distilled sterilized water. Surface sterilized pieces were inoculated on petriplates containing potato dextrose agar. Inoculated petriplates were incubated at 28^0C to 30^0C temperature. Isolated fungal pathogen was purified by repeated subculturing. Purified pathogens were taken in to stock cultures on agar slant. During all these operation Perfect aseptic conditions were maintained [17]. For pathogenicity tests isolated fungal organisms were inoculated on fresh and healthy solanaceous fruits and the reisolated [18, 19, 26 &37].

Enzymes Assay

Enzymes extracts were obtained by growing pathogens in cole medium [13]. Culture filtrates were used for assay the activity of Polygalacturonase (PG), Pectin methyl esterase (PME), Polymethyl Galacturonase (PMG), Polygalacturonate transeliminase (PGTE), Pectin methyl transeliminase (PMTE), Protease and cellulase.

Viscometric study were made for the detection for PG, PMG, PGTE, PMTE, Protease and cellulase by using standard viscommetric procedure [18,19,20 & 39]. Activity of PME was measured by continuous titration method [18]. Component of reaction mixture for enzyme assay are mentioned in Table 1.

Name of the enzyme to be detected	Components of reaction mixture in sequence				
	Substrate	Distilled water	Buffer	Enzyme Extract	Temperature of reaction
PG	3.5 ml. 1.2 % NaPP	1 ml.	Macllvaine PH 4.6 1.5 ml.	1.5 ml.	30 ± 1^0C
PGTE	3.5 ml. 1.2 % NaPP	1 ml.	Macllvaine PH 8 1.5 ml.	1.5 ml.	30 ± 1^0C
PMTE	3.5 ml. 1.2 % pectin	1 ml.	Macllvaine PH 4.6 1.5 ml.	1.5 ml.	30 ± 1^0C
PMG	3.5 ml. 1.2 % pectin	1 ml.	Macllvaine PH 8 1.5 ml.	1.5 ml.	30 ± 1^0C
Cellulase	3.5 ml. 0.5 % Carbory methyl cellulose	1 ml.	Macllvaine PH 5.6 1.5 ml.	1.5 ml.	30 ± 1^0C
Protease	3.5 ml. 1.2 % gelatin	1 ml.	Boric acid Borax PH 8.5 1.25 ml.	2.0 ml.	40 ± 1^0C
PME	3.5 ml. 1.2 % pectin	1 ml.	PH 5.5	4.5 ml.	30 ± 1^0C

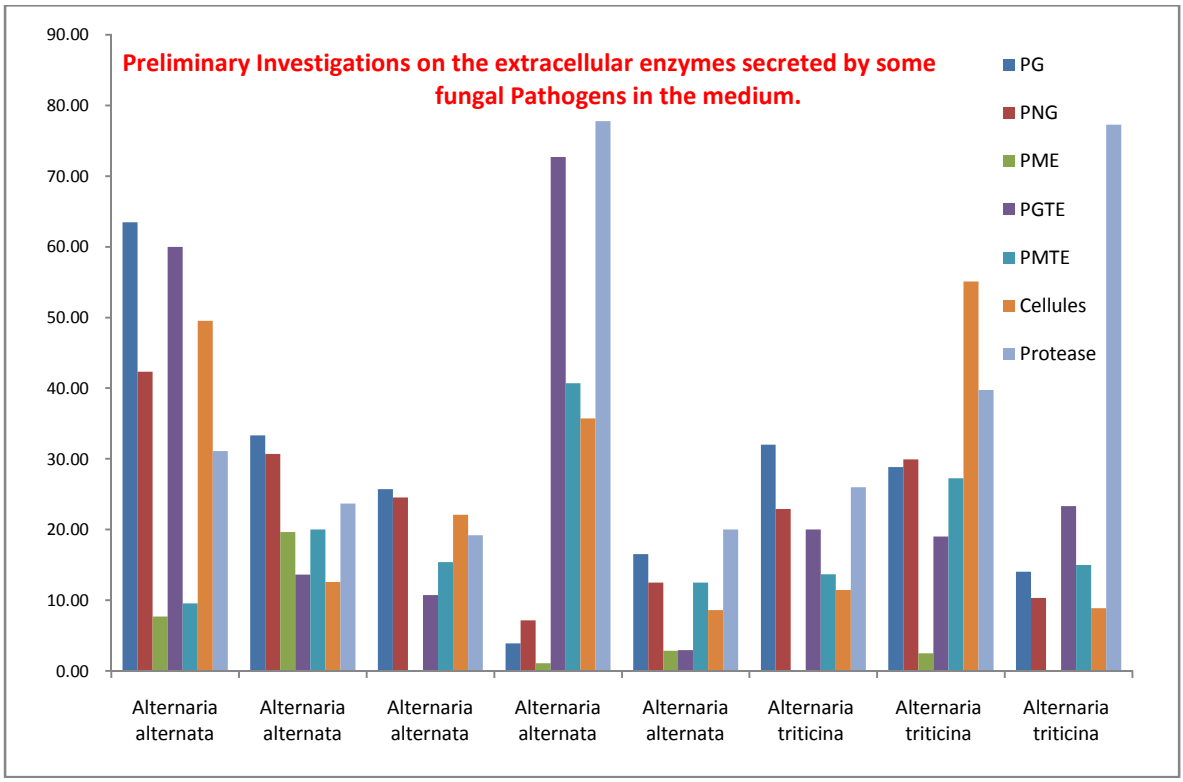
Result

All the isolates of genus *Alternaria* tested were able to produced extracellular enzymes in smaller or greater quantity except PME in few cases. Results are tabulated in Table-2.

1) ***Polygalacturonase (PG)***- Secretion of this enzyme observed in all isolates of *Alternaria* Maximum amount was seen in *AlternariA. alternata* Isolate No. 1 as compared to other isolates. Similarity Maximum amount of this enzyme was observed in *A. triticina* Isolate No. 6 as compared to other isolates of *A. triticina*.

Table 2: Preliminary Investigations on the extracellular enzymes secreted by some fungal Pathogens in the medium

S. No.	IMI No.	Iso-late	Name of Pathogen	Ph				Enzymes						
				Tm total	4th day	7th day	9th day	PG	PNG	PME	PGTE	PMTE	Cellules	Protease
1.	203393	1	Alternari*A. alternata*	5.2	4.5	7.7	7.8	63.48	42.31	7.70	60.00	9.57	49.53	31.11
2.	203394	2	Alternari*A. alternata*	5.2	5.0	5.2	5.2	33.33	30.70	19.64	13.61	20.00	12.57	23.68
3.	203395	3	Alternari*A. alternata*	5.2	5.0	5.2	5.2	25.70	24.54	--	10.71	15.38	22.11	19.18
4.	226407	24	Alternari*A. alternata*	5.2	5.2	7.8	7.9	3.91	7.14	1.07	72.72	40.71	35.71	77.77
5.	203837	5	Alternari*A. alternata*	5.2	5.1	5.2	5.2	16.51	12.50	2.86	2.94	12.50	8.59	20.00
6.	203838	6	Alternari*A. triticina*	5.2	5.0	5.2	5.4	32.00	22.93	--	20.00	13.69	11.46	25.99
7.	203900	7	Alternari*A. triticina*	5.2	5.0	5.2	6.2	28.82	29.91	2.50	19.03	27.27	55.09	39.77
8.	203808	8	Alternari*A. triticina*	5.2	5.1	5.3	8.0	14.05	10.30	--	23.33	15.00	8.88	77.27



2) ***Pectin Methyl esterase***- PME considerable amount of PME was secreted by *A. alternaria* Isolate No.2. Insignificant amount of this enzyme was found in *A. alternaria* Isolate No. 1, 2, 4 & 5 and *A. triticina* Isolate No.7, No. secretion of this enzyme was observed in *A. alternata* Isolate No. 3 and *A. triticina* Isolate No. 6, 8 & 9.
3) ***Polymethyl Transeliminase (PMTE)-*** Maximum amount of PMTE was secreted by *AlternariA. alternata* Isolate No.24 as competed to other isolates of *A. alternata*. Similarly secretion of this enzyme was more in *A. triticina* isolate No.7 in comparison to other isolate of *A. triticina*.
4) ***Polymethylgalacturonase (PMG)***- Maximum secretion of PME was observed in *A. alternata* isolate No.1 as compared to four other isolates of *A. alternata*. Considerable amount of secretion of this enzyme was observed in *A. triticina* isolate No.7 in comparision to other isolates.
5) ***Polygalacturonase Trans***- Eliminase PGTE Maximum amount of PGTE was secreted by *A. alternata* isolate No. 24 as compared to other isolates of *A. alternata* & *A. triticina*.
6) ***Cellulase***- It is significant to note that out of five isolates of *A. alternata*, isolate No. 1 was strongly cellulolytic similarly; secretion of cellulose was more in *A. triticina* isolate No. 7 as compared to other isolates of same species.
7) ***Protease***- Maximum amount of protease was secreted by *A. alternata* isolate No. 4 as compared to other isolates. Similarly maximum secretion was observed in *A. triticina* isolate no. 8 as compared to other isolates of same species.

Discussion

Solanaceous fruits, like many other fruits become soft and pulpy after the attack of pathogenic fungi. The mechanism by which healthy parenchymatous tissue is converted in to soft and pulpy mass by pathogens remain almost completely unknown until later part of 19th century. In 1886 first time concluded that something moves in advance to the growth of fungal hyphae. [14]. Thus biochemical substances later on recognized as enzymes responsible for degradation of soft parenchymatous tissue of host [4, 8, 33, 39]. Many pathogenic and saprophytic organisms produce extracellular enzymes for degradation of cell wall material. Complete mechanism of working of enzymes on cell wall skeletion is well understood [14]. Pectic substances are hydrolyzed by pectic enzymes. Pectinmethylesterase liberates methyl group from pectin and convert it in to pectic acid which is hydrolyzed by polygalacturonase enzyme in to simple units. Cellulose is degraded by successive enzymatic action of cellulase secreted by microorganisms. The larger molecules of cellulose are hydrolysed in to simple and smaller units which are converted in to glucose [40]. Protein is

hydrolysed by protease enzyme in to simple structure [2, 22, 38]. Culture filtrates of pathogenic fungi tested in present study contain pectic, cellulolytic and proteolytic enzymes, which are capable of degrading cellulose, pectic, substance and proteolytic substances of cell wall of parenchymatous tissue of host converts healthy tissue. The action of these enzymes on parenchymatous tissue of host converts healthy tissue in to soft & pulpy mass. Association of extracellular enzymes with diseased tissue has also been confirmed [4, 5, 21, 34]. Role of these enzymes were also confirmed in pathogenesis [37]. It is significantly interesting to note that there is variation in secretion of extracellular enzymes quantitatively as well as qualitatively among the isolates of *A. alternata* and *A. triticina* of same morphological species. There are certain reports in favour of present conclusions. High degree of variability in production of extracellular enzymes of different isolates of same genus. *Puccinia graminis* (10). *Polyporus* Spp [37], *Fusarium oxysparum* [7], *Cochliobolus carbonum* [36], *Accremorium* Sp [24]. Variation is enzyme secretion by pathogenic fungal of same genus was also observed. [25,28]. Therefore quantitative & qualitative variations at Enzymative level among the isolates of same species suggest. The rethinking of status of species constructed only on the basic of morphological characters.

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