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Periodic Research

Studies on Pectinolytic, Cellulolytic and Macerating Enzymes Activities of Storage Fungi in Ginger

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Abstract

Fusarium solani and *Pythium aphanidermatum* cause severe ginger rotting during storage. Extracellular polygalacturonase (PG), polygalacturonase transeliminase (PGTE), pectin transeliminase (PTE) and cellulolytic enzyme (Cx) were evaluated under *in vitro* and *in vivo* conditions. The test pathogens showed higher PG, PGTE and PTE enzyme activity *in vitro* compared to *in vivo* except cellulolytic enzymes, whereas complete maceration was recorded at 4 h of incubation.

Keywords: Pectinolytic, Cellulolytic, Macerating Enzymes, Storage Rot, Ginger

Introduction

Ginger (*Zingiber officinale* Rosc.) is an important spice crop, which is known for its medicinal properties. In India large portion of the ginger production is consumed domestically as green ginger or dried ginger in many culinary preparations. Ginger has high medicinal value and pharmaceutical uses as carminative rubefiacient stimulation in alcoholic, gastritis, dyspepsia, flatulent or, colic. An Enzymes production has significance in plant disease especially of rot type's fungi where they play an important role in pathogenesis by loosening of cell walls through maceration (Sharma and Dohroo 1985 and Hamed 1999). A wide range of prokaryotic and eukaryotic micro-organisms have the potential to produce cell-wall degrading enzymes when chitin or isolated fungal cell wall material was present in the growth medium (Jijaki and Lepoivre 1998; Giuliano et al 2001).*F. solani* and *P. aphanidermatum* produces extracellular polygalacturonase (PG), polygalacturonase transeliminase (PGTE), pectin transeliminase (PTE) and cellulolytic enzyme (Cx) (Cheng Jie et al 1998; Gao et al 2000). Therefore, a study was made on production of enzymes by storage rots causing fungi.

Materials and Methods

The rhizome rot pathogens *Fusarium solani* and *Pythium aphanidermatum* were isolated and purified from rotten storage ginger rhizomes and was maintained on PDA, Corn Meal Agar medium. Pectinolytic and cellulolytic enzyme activity was studied by growing *F. solani* and *P. aphanidermatum* on Richard's medium at 28 ± 2 C for 7d. A separate control was maintained using basal medium. The mycelial mat was harvested using filter paper and cultural filtrate was centrifuged at 5000 rpm for 30 min to separate spores and then filtrate was dialyzed in double distilled water at 4 ºC for 24 h by changing water at every 8 h. For the estimation of pectinolytic and celluloytic enzymes, sucrose of Richard's medium was replaced by citrus pectin (1%) and carboxyl methyl cellulose (1%), respectively. The control was maintained using sucrose in medium.

Pectinolytic activity was estimated in vitro using reaction mixture consists of 5 ml sodium polypectate (1.2%) at pH 8.7 in boric acid borax buffer, 2 ml of borate buffer (8.7pH) and 2 ml culture filtrate for PGTE (Albersheim et al 1960), while PTE was assayed by reaction mixture, pectin (1.2 %) in boric acid borax buffer (pH 8.7) (Ayers et al 1966). However, reaction mixture consists of 5 ml sodium polypectate (0.75 %) acetate buffer (pH 4.6) and 2 ml sodium acetate acetic acid buffer (pH 4.6) used for PG enzyme activity (Bateman, 1966).The Cellulolytic activity in vitro was estimated by the make use of reaction mixture consists 5 ml carboxyl methyl cellulose (0.5 %) in acetate buffer (pH 5.6), 2 ml acetic acid acetate buffer (pH 5.6) and 2 ml of enzymes preparation (Reddy and Mahadevan, 1967). Treatments were replicated thrice and reaction mixtures viscosity (%) loss due to enzyme activity was recorded at interval of 5, 10, 15, 30, 60, 120 and 240 min in water bath (30 C) by viscometeric method (Albersheim et al 1960; Ayers et al 1966; Bateman, 1966 and Reddy and Mahadevan, 1967). At

the same time, pectinolytic and cellulolytic activities were assessed *in vivo* by steeping uniform sized healthy ginger rhizomes in spore suspension (1 X 10^5 spore /ml) of *F. solani* and *P. aphanidermatum,* separately and incubated at 28 ± 2 C for 12 h. The sterilized water dipped rhizomes served as control. Infected rhizomes were blended in distilled water (w/v) in warring blender for 10 min at 4 C and filtered through muslin cloth. The crude enzyme clear supernatant was obtained by centrifugation (5000 rpm) for 20 min at 4 C. A set of healthy rhizomes inoculated with plain agar served as control (Prasad et al 1988).While, macerating enzymes activity was recorded using method given by Brown, (1915).

Results and Discussion

The per cent loss in viscosity was higher *in vitro* as compared to rhizomes inoculated with *F. solani in vivo* condition except cellulolytic enzymes (Cx), which showed more per cent loss in viscosity *in vivo* conditions. The highest enzymatic activity of PG expressed as per cent loss in viscosity was 84.09 % in 240 min, while it was lowest (28.27 %) in 5 min *in vitro* condition. *In vivo* condition same trend was observed. It was observed that PGTE activity was higher at 240 min in both *in vitro* and *in vivo* condition (76.73 and 72.23 % loss, respectively). The PTE activity was maximum *in vivo* compare to *in vitro* condition. Lowest Cx activity *in vitro* 30.83 % was observed in 5 minutes, while it was 37.64 % loss in viscosity under *in vivo* conditions. The enzymes activity was increased with time. Results of present study are in consonance with phase of observed by (Baayen et al 1997 and Gao et al 2000).

Pythium Aphanidermatum

Table 1 also indicates that the highest enzymatic activity of PG expressed as per cent loss in viscosity was 77.37 % at 240 minutes while it was lowest 22.38 % in 5 min *in vitro* conditions. *In vivo* conditions same method was used. It was observed that PGTE activity was highest at 240 min *in vitro* and in vivo 82.67 and 67.82 % loss in viscosity respectively. Table 2 also showed that the PTE activity was highest at 75.82 % while it was 79.12 % *in vivo*. Lowest Cx activity *in vitro* (28.54 %) was observed in 5 minutes, while it was 36.62 % loss in viscosity under *in vivo* conditions. Similar trend was also observed by Indrasenan and Paily (1982) in case of soft rot of ginger caused by *P. aphanidermatum*.

Estimation of Macerating Enzymes

The complete maceration were observed at 24 h incubation in both *P. aphanidermatum* and *F solani in vivo* while it was lesser at 4h in vitro 4 h incubation showed complete maceration in *P. aphanidermatum* but fairly good maceration was observed in *F. solani.*(Table 2). The similar results were reported earlier on ginger and maize crop by Sharma and Dohroo (1985) and Chen Jie et al (1998) respectively. On the basis of present investigation we can conclusively stated that both the pathogenic fungi produce PG,PGTE,PTE , Cx and macerating enzymes activity in vitro and in vivo condition.

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Table 1. Pectinolytic and Cellulolytic Enzymes Production *in Vitro* and *in Vivo* by Fusarium Solani and *Pythium Aphanidermatum* (Expressed in % Loss in Viscosity)

Time (Min.)	*In vitro*								*In vivo*							
	PG		PGTE		PTE		Cx		PG		PGTE		PTE		Cx	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
5	28.27	22.38	29.96	39.38	24.48	22.08	30.83	28.54	26.41	28.13	22.23	18.61	23.20	23.89	37.64	36.62
10	49.20	38.61	39.45	52.07	39.05	41.11	43.80	43.82	43.48	45.08	36.87	33.04	38.67	37.35	47.78	46.40
15	62.69	51.20	54.32	63.60	57.68	53.17	54.26	50.34	55.62	58.58	48.24	43.02	58.54	58.58	57.19	54.89
30	71.43	61.75	61.21	71.08	67.47	63.58	61.24	58.73	65.22	68.41	55.06	53.49	69.07	66.37	65.31	64.58
60	71.99	70.67	74.58	76.30	73.31	72.55	64.34	65.42	75.65	76.59	67.18	60.62	75.07	75.22	73.07	74.98
120	82.15	75.38	74.50	79.78	77.69	74.66	68.22	69.84	79.13	80.45	70.21	65.59	78.27	76.99	78.23	78.16
240	84.09	77.37	76.73	82.67	78.78	75.82	70.55	73.22	81.74	82.95	72.23	67.82	79.47	79.12	82.29	80.53
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.06	8.20	3.82	5.00	3.92	5.32	6.09	11.42
SEm ±	0.25	0.15	0.29	0.21	0.199	0.91	0.45	0.49	0.26	0.18	0.25	0.20	0.30	0.13	0.31	0.31
CD(P=0.05)	2.57	0.45	0.75	0.60	2.57	2.68	2.57	1.44	2.42	0.52	0.74	0.60	0.87	0.38	0.90	0.93
CV %	0.93	0.62	1.10	0.80	0.78	3.68	1.48	2.02	1.10	0.74	0.93	0.92	1.84	0.59	1.29	1.40

Average of three replication; Data were angular transformed before analysis.
F= *Fusarium solani* ; P= *Pythium aphanidermatum*

Table 2. Macerating Enzymes Production *in Vitro* and *in Vivo* by *F. Solani* and *P. Aphanidermatum*

Pathogens	*In vitro*		*In vivo*	
	Macerating rating (h)		Macerating rating (h)	
	4h	24 h	4 h	24 h
Fusarium solani	4	5	2	5
P. aphanidermatum	5	5	3	5