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Full Length Research Paper

Characterization of polygalacturonases from fruit spoilage *Fusarium oxysporum* and *Aspergillus tubingensis*

Ahmed R. Al-Najada[1], Rashad R. Al-Hindi[1] and Saleh A. Mohamed[2,3]*

[1]Biology Department, Faculty of Science, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia.
[2]Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia.
[3]Molecular Biology Department, National Research Center, Dokki, Cairo, Egypt.

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We reported the partial purification and characterization of polygalacturonases from fruit spoilage *Fusarium oxysporum* and *Aspergillus tubingensis* isolated from banana and peach, respectively. By using diethylaminoethyl (DEAE)-Sepharose column, one and two forms of polygalacturonases were separated from *F. oxysporum* (PGase) and *A. tubingensis* (PGaseI and PGaseII), respectively. The polygalacturonases examined had higher affinity toward various polygalacturonic acids and pectins. The apparent K_m and V_{max} values were reported for the enzymes. Acidic pH optima (4.0 to 6.0) was also reported for the enzymes. Optimal temperature and thermal stability of the enzymes showed a range from 40 to 60°C. The effect of metal cations on the enzymes was studied. The most chemical compounds caused moderate inhibitory effect except benzoic and citric acids which had strong inhibitory effect on the polygalacturonases. The benzoic and citric acids were used as antifungal compounds for *F. oxysporum* and *A. tubingensis*. The citric acid was found to be more effective against fungal growth than benzoic acid.

Key words: Fruit, spoilage, *Fusarium oxysporum*, *Aspergillus tubingensis*, polygalacturonase.

INTRODUCTION

Pectin is an important component of middle lamella and primary cell wall of higher plants. Pectins are high molecular weight acid polysaccharide primary made up of α (1−4) linked D-galacturonic acid residues (Torres-Fanela et al., 2003). Pectinases are enzyme group that degrade pectic substances and are classified according to their mechanism of action in methylesterases (EC.3.1.11.1) that remove methoxyl groups from highly or partially esterified galacturonan. Polygalacturonases catalyse the hydrolysis of the glycosidic bonds in a random fashion (endopolygalacturonase-EC.3.2.1.15) or from nonreducing end of homogalacturonan releasing galacturonic or digalacturunic acid residues (exopoly-glacturonases EC.3.2.1.67 and EC 3.2.1.82) (Alkorta et al., 1998).

*Corresponding author. E-mail: saleh38@hotmail.com.

Pectinases are naturally produced by many organisms, including bacteria, fungi, yeasts, insects, nematodes, protozoan and plants. Pectinase production has been reported from bacteria including streptomycetes (Beg et al., 2000). Pectinases are the first enzymes to be secreted by fungal pathogens when they attack plant cell walls (Collmer and Keen, 1986; Idnurm and Howlett, 2001). Pectinases are essential for fungal pathogens that do not have specialized penetration structures as well as for necrotrophic pathogens during the late stages of the invasion process (De Lorenzo et al., 1997). Secretion of pectinases during infection to the plants has been reported from various plant pathogenic fungi such as *Fusarium oxysporum, Botrytis cinerea, Sclerotinia sclerotiorum* (Di Pietro and Roncero, 1998; Garcia-Maceira et al., 2001; ten Have et al., 2001; de las Heras et al., 2003; Li et al., 2004), from non-pathogenic fungus Rhizoctonia AG-G (Machinandiarena et al., 2005) and from several yeasts (da Silva et al., 2005).

Polygalacturonases are important pathogenicity factors for fungi such as *Aspergillus flavus*, *Alternaria citri* and *Claviceps purpurea*, and for bacteria such as *Agrobacterium tumefaciens* and *Ralstonia solanacearum* (Di Matteo, 2006). The characterization of purified polygalacturonases is an important area of research since it focuses on being able to distinguish between the enzymatic complex components of the substrate degradation mechanism, optimum conditions for enzymatic activity, and the regulation of enzyme by inhibitors (Gummadi and Panda, 2003; Pedrolli et al., 2009). Previously, we isolated and identified *F. oxysporum* and *Aspergillus tubingensis* from banana and peach, respectively, and screened their polygalacturonases (Al-Hindi et al., 2011). In this work, the partial purification and characterization of polygalacturonases produced by *F. oxysporum* and *A. tubingensis* were studied. In addition, the effect of benzoic and citric acids on the development of these fungi was evaluated.

MATERIALS AND METHODS

Fruit spoilage fungi

F. oxysporum and *A. tubingensis* were isolated and identified from banana and peach as previously described (Al-Hindi et al., 2011).

Cultivation of fungi

F. oxysporum and *A. tubingensis* were inoculated under aseptic conditions in 250 ml Erlenmeyer flasks containing 5% banana and peach peels, respectively. The inoculated flasks were incubated at 28°C with shaking on a rotary incubator shaker at 150 rpm for five days. The cell-free broth was recovered by filtration using a polyamide tissue. The cell-free broth was subjected to dialysis against 20 mM Tris-HCl buffer, pH 7.2 over night. The dialyzate was centrifuged at 10,000 rpm for 12 min and the supernatant was designated as crude extract.

Partial purification of polygalacturonases

Crude extracts from *F. oxysporum* and *A. tubingensis* were separately loaded on a diethylaminoethyl (DEAE) - Sepharose CL-6B column (10 x 1.6 cm i.d.) equilibrated with 50 mM Tris-HCl buffer, pH 7.2. The enzyme was eluted with a stepwise gradient from 0.0 to 0.3 M NaCl in the same buffer. Fractions in 3 ml volume were collected at a flow rate of 60 ml/h. The eluted fractions were monitored at 280 nm for protein and assayed for enzyme activity. Protein fractions exhibiting enzyme activity were pooled.

Polygalacturonase activity assay

Polygalacturonase (EC 3.2.1.15) activity was assayed according to Miller (1959). The reaction mixture (0.5 ml) contained 1% polygalacturonic acid, 0.05 M sodium acetate buffer pH 5.5 and a suitable amount of enzyme. Assay was carried out at 37°C for 1 h. Then, 0.5 ml dinitrosalicylic acid reagent was added and heated in a boiling water bath for 10 min. After cooling to room temperature, the absorbance was measured at 560 nm. One unit of enzyme activity was defined as the amount of enzyme which liberated 1 µmol of galacturonic acid per hour under standard assay conditions.

Protein determination

Protein was determined according to Bradford (1976) and bovine serum albumin was used as standard.

Characterization of polygalacturonase

K_m

The k_m value was determined from Lineweaver-Burk plot by using polygalacturonic acid concentrations from 2 to 6 mg/ml.

Optimum pH

Enzyme activity was determined at various pH using 50 mM each of sodium acetate (pH 4.0 to 6.0) and Tris-HCl (6.5 to 8.5). The maximum activity was taken as 100% and percentage relative activity were plotted against different pH values.

Optimum temperature

Enzyme activity was determined at a temperature range of 20 to 70°C. The maximum activity was taken as 100% and percentage relative activity were plotted against different temperatures.

Thermal stability

The enzyme was incubated at a temperature range of 20 to 70°C for 15 min prior to substrate addition. The % relative activity was plotted against different temperatures.

Effect of metal ions

The enzyme was incubated with 2 mM solution of Co^{2+}, Ca^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and Hg^{2+} for 15 min prior to substrate addition. The enzyme activity without metal ions was taken as 100% and percentage relative activity was determined in the presence of metal ions.

Effect of chemical compounds

Enzyme activity was determined in the presence of phenylmethanesulfonylfluoride (PMSF), *p*-HMB, β-mercaptoethanol, trypsin inhibitor, 1,10 phenanthroline, ethylenediaminetetraacetic acid (EDTA), sodium citrate, sodium oxalate, sodium benzoate, benzoic acid, gallic acid, tannic acid and citric acid at a concentration of 5 mM. The enzyme activity without chemical compound was taken as 100% and percentage relative activity was determined in the presence of chemical compound.

Effect of benzoic and citric acids on the growth of fruit spoilage fungi

Benzoic and citric acids were used at the concentration of 5 mM as antifungal compounds. The fruits were divided into four groups: 1) injured banana and peach without acid, 2) injured banana and peach with each acid, 3) injured banana and peach with *F.*

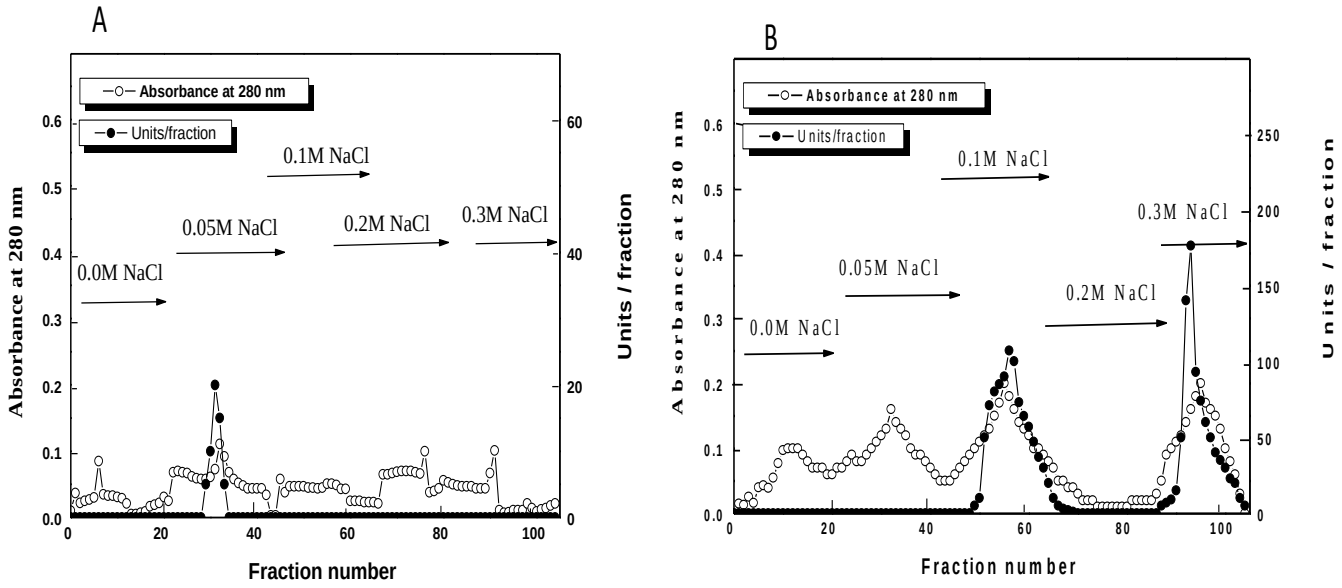

Figure 1. Elution profiles for the chromatography of polygalacturonases from *F. oxysporum* (a) and *A. tubingensis* (b) crude extracts on DEAE-Sepharose column (10 × 1.6 cm i.d.).

Table 1. Partial purification of polygalacturonases from *F. oxysporum* and *A. tubingensis*.

Purification step	Total units*	Total protein (mg)	Specific activity (unit/mg protein)	Fold purification	Recovery (%)
Crude extract					
F. oxysporum	140	0.32	434	1.00	100
A. tubingensis	5000	4.5	1111	1.00	100
DEAE-Sepharose					
F. oxysporum PGase	48	0.034	1411	3.25	32
A. tubingensis PGaseI	1011	0.051	19823	17.8	20
PGaseII	675	0.036	18750	16.8	13

*One unit of enzyme activity was defined as the amount of enzyme which produced one µmol galacturonic acid per h under standard assay conditions.

oxysporum and *A. tubingensis,* respectively and 4) injured banana and peach with each acid and *F. oxysporum* and *A. tubingensis*, respectively. The development of spoilage fungi was observed after three, five and seven days intervals of incubation at 28°C.

RESULTS AND DISCUSSION

Partial purification of polygalacturonases from *F. oxysporum* and *A. tubingensis*

By using DEAE-Sepharose column, one and two forms of polygalacturonase (PGase) were separated from *F. oxysporum* (PGase with specific activity 1411 units/mg protein) and *A. tubingensis* (PGaseI and PGaseII with specific activity 19,823 and 18,750 units/mg protein, respectively), respectively (Figure 1 and Table 1). Various numbers of PGases with different specific activities were reported from *Aspergillus niger* (710 units/mg protein) (Murad and Azzaz, 2011), *Trichoderma harzianum* (881 units/mg protein) (Mohamed et al., 2009), *Aspergillus carbonarius* (7000 units/mg protein) (Nakkeeran et al., 2011) and *A. flavus* (3000 units/mg protein) (Gewali et al., 2007). Low specific activity was reported for polygalacturonases from *F. moniliforme* (18.6 units/mg protein) (Niture and Pant, 2004) and *A. niger* NRRL3 (34.7 units/mg protein) (Fahmy et al., 2008).

Characterization of polygalacturonases from *F. oxysporum* and *A. tubingensis*

With regard to substrate specificity, a variety of polygalacturonic acids (PGAs) and pectins (citrus pectin with 7.8% degree esterification and apple pectin with 6% degree estrification) have been tried as substrates. The

Table 2. Substrate specificity of *F. oxysporum* PGase and *A. tubingensis* PGaseI and PGaseII.

Substrate	% Relative activity		
	F. oxysporum PGase	*A. tubingensis*	
		PGaseI	PGaseII
Polygalacturonic acid (Sigma)	100	100	100
Citrus pectin (7.8% esterification)	184	155	181
Polygalacturonic acid (BDH)	126	108	149
Apple pectin (6% estrification)	194	156	158

Each value represents the average of two experiments.

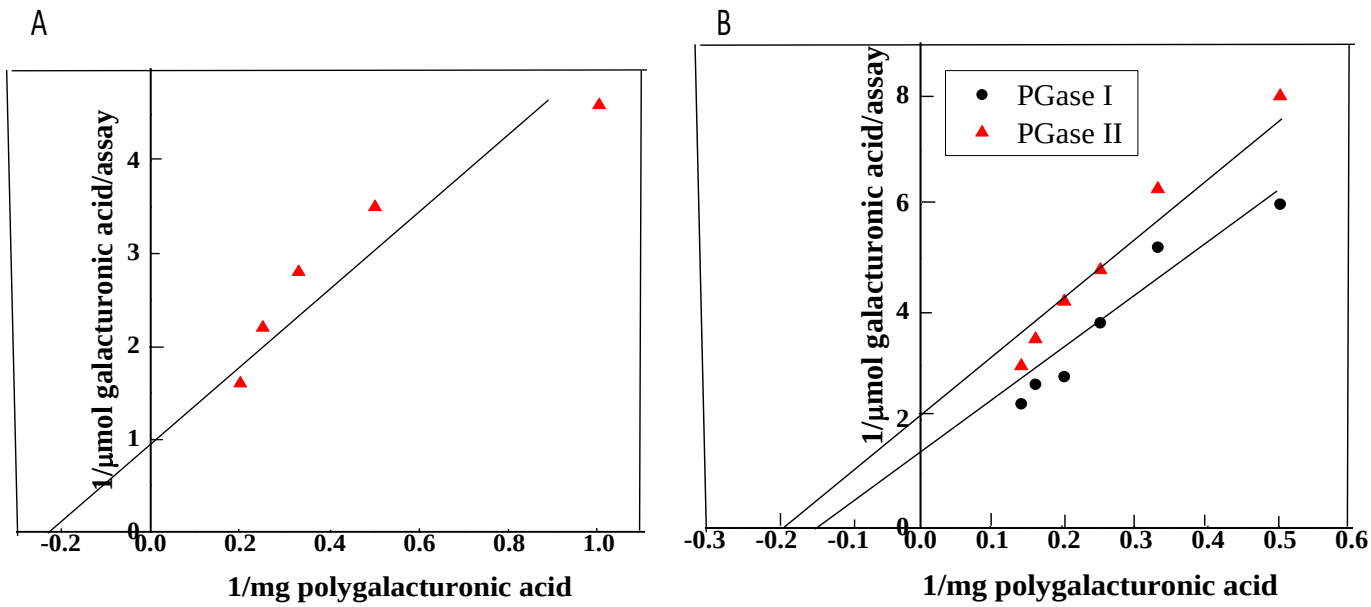

Figure 2. Lineweaver-Burk plot relating *F. oxysporum* PGase (a) and *A. tubingensis* PGaseI and PGaseII (b) reaction velocities to polygalacturonic acid as substrate concentrations. Each point represents the average of two experiments.

enzyme activities were compared to the activity with PGA (Sigma) which was regarded as a 100% activity. Higher relative activities were reported for *F. oxysporum* PGase (citrus pectin 184%, PGA (BDH) 126% and apple pectin 194%), and *A. tubingensis* PGaseI (citrus pectin 155 %, PGA (BDH) 108% and apple pectin 156%) and PGaseII (citrus pectin 181%, PGA (BDH) 149% and apple pectin 158%) (Table 2). The substrate specificity was reported for polygalacturonases from *Aspergillus giganteus* (citrus pectin 100%, PGA 94.9%, citrus pectin 51.9%, citrus pectin 25.5% and apple pectin 23.9%) (Pedrolli and Carmona, 2010), *A. niger* NRRL3 (citrus pectins with different esterification 41 to 97%) (Fahmy et al., 2008), *T. harzianum* (citrus pectins with different esterification 11 to 187%) (Mohamed et al., 2009) and *Mucor circinelloides* ITCC 6025 (citrus pectin 11.0 % and apple pectin 22 %) (Thakur et al., 2010).

The apparent K_m and V_{max} values were reported for *F. oxysporum* PGase (4.1 mg/ml and 5.5 µmol/ml) and *A. tubingensis* PGaseI (7.0 mg/ml and 0.69 µmol/ml) and PGaseII (5.2 mg/ml and 0.5 µmol/ml) (Figure 2). Various K_m and V_{max} values were reported for polyglacturonases from *F. moniliforme* NCIM 1276 (K_m 0.12 mg/ml and V_{max} 111.11 µmol/min/mg protein) (Niture et al., 2008), *Penicillium viridicatum* (K_m 1.30 mg/ml and V_{max} 1.76 to 2.07 µmol/min/mg protein) (Gomes et al., 2009), *A. giganteus* (K_m 3.25 and 1.16 mg/ml and V_{max} 669.6 and 602.8 µmol/min/mg) (Pedrolli and Carmona, 2010), *Paecilomyces variotii* (K_m 1.84 mg/ml and V_{max} 432 µmol/min/mg protein) (Damasio et al., 2010) and *A. niger* (SA6) (K_m 2.74 mg/ml and V_{max} 0.78 µmol/min/mg protein) (Buga et al., 2010).

Acidic pH optima have been reported for *F. oxysporum* PGase (pH 4.0) and *A. tubingensis* PGaseI and PGaseII (4.5 and 6.0, respectively) (Figure 3). Similar acidic pH optima were reported for polygalacturonase from *P. variotii* (pH 4.0) (Damasio et al., 2010), *Sclerotium rolfsii* (pH 4.5 to 5.0) (Schnitzhofer et al., 2007), *Mucor rouxii*

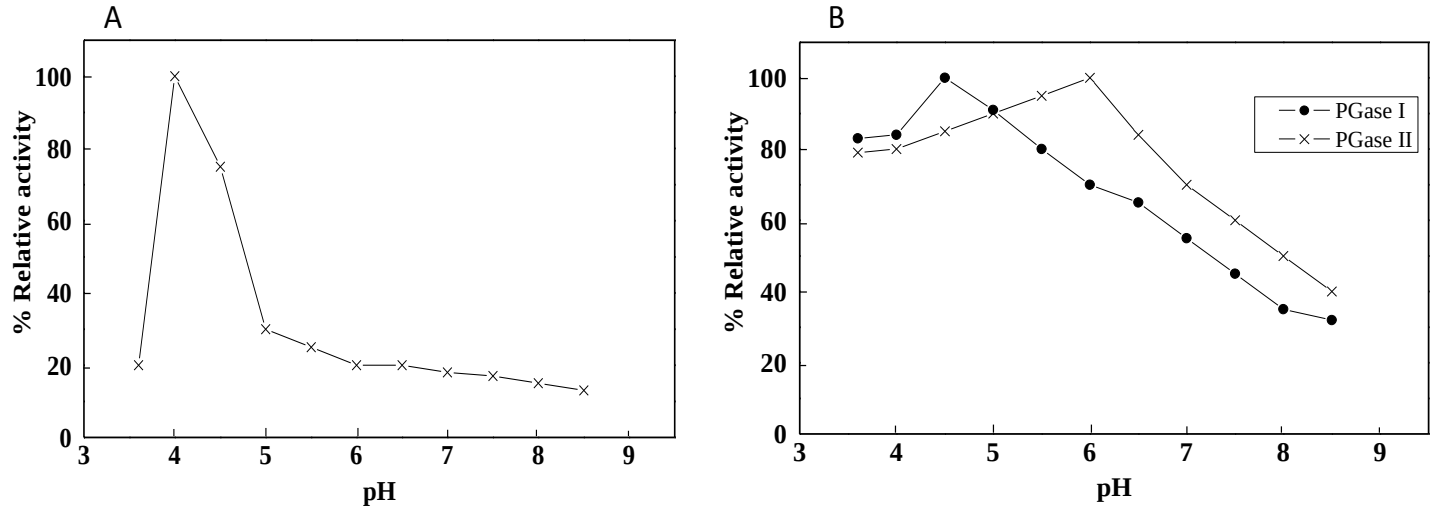

Figure 3. pH optima of *F. oxysporum* PGase (a) and *A. tubingensis* PGaseI and PGaseII (b). The reaction mixture contained in 0.5 ml were 2.5 mg polygalacturonic acid, suitable amount of enzyme and 50 mM sodium acetate buffer (pH 3.6 to 6.0), 50 mM Trsi-HCl buffer (6.5 to 8.5). Each point represents the average of two experiments.

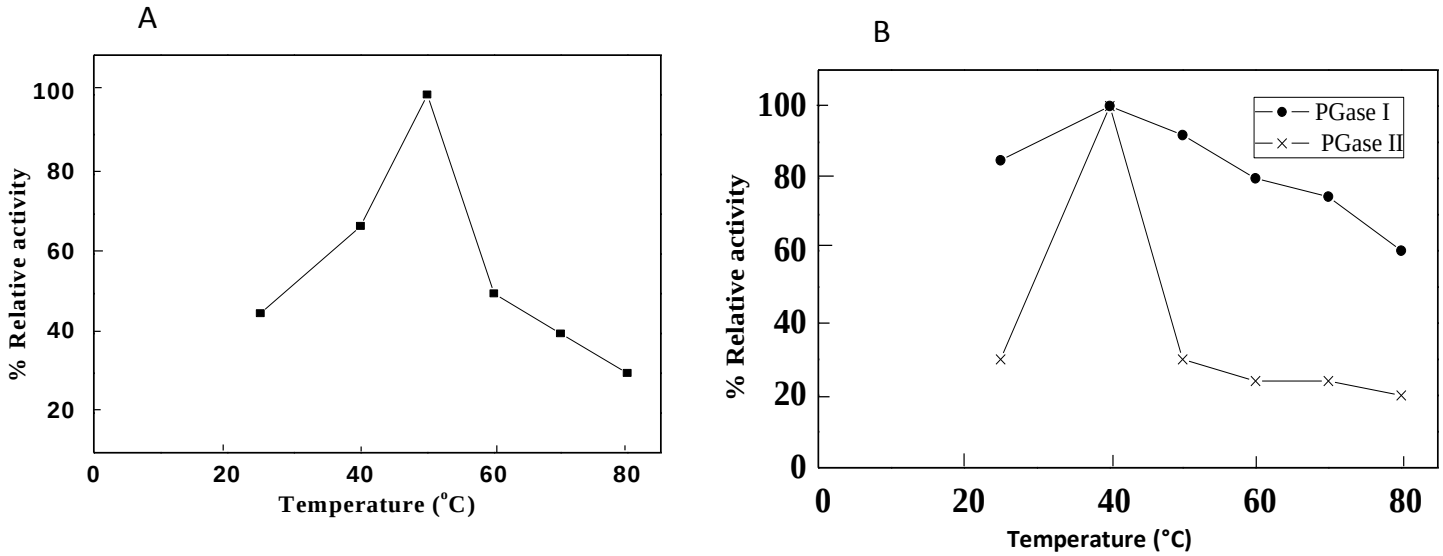

Figure 4. Temperature optima of *F. oxysporum* PGase (a) and *A. tubingensis* PGaseI and PGaseII (b). The enzyme activity was measured at various temperatures using the standard assay method as previously described. Each point represents the average of two experiments.

NRRL 1894 (pH 4.5) (Saad et al., 2007), *A. niger* (pH 3.8) (Khairnar et al., 2009), *Mucor circinelloides* ITCC 6025 (pH 5.5) (Thakur et al., 2010), *A. tubingensis* (pH 4.2) (Gewali et al., 2007) and *Aspergillus oryzae* (pH 5.0) (Riou et al., 1998).

Optimal temperatures of polygalacturonases for the examined species showed a range from 40 to 60°C (Figure 4). These optimal temperatures were similar to those observed for polygalacturonases from *Streptomyces erumpens* (50°C) (Kar and Ray, 2011), *Aspergillus sojae* (55°C) (Dogan and Tari, 2008), *T. harzianum* (40°C) (Nabi et al., 2003) and *A. niger* NRRL3 (40°C) (Fahmy et al., 2008). The temperature stability for *F. oxysporum* PGase and *A. tubingensis* PGaseI and PGaseII was detected up to 60, 40 and 50°C, respectively (Figure 5). Similar thermal stability was reported for polygalacturonases from *P. variotii* (45 to 55°C) (Damasio et al., 2010), *T. harzianum* (60°C) (Nabi et al., 2003) and *A. niger* (45 to 55°C) (Dogan and Tari, 2008).The effect of metal cations on the examined polygalacturonases at 2 mM was studied (Table 3). For *F. oxysporum* PGase, Zn^{2+}, Pb^{2+} and Hg^{2+} caused 12, 39

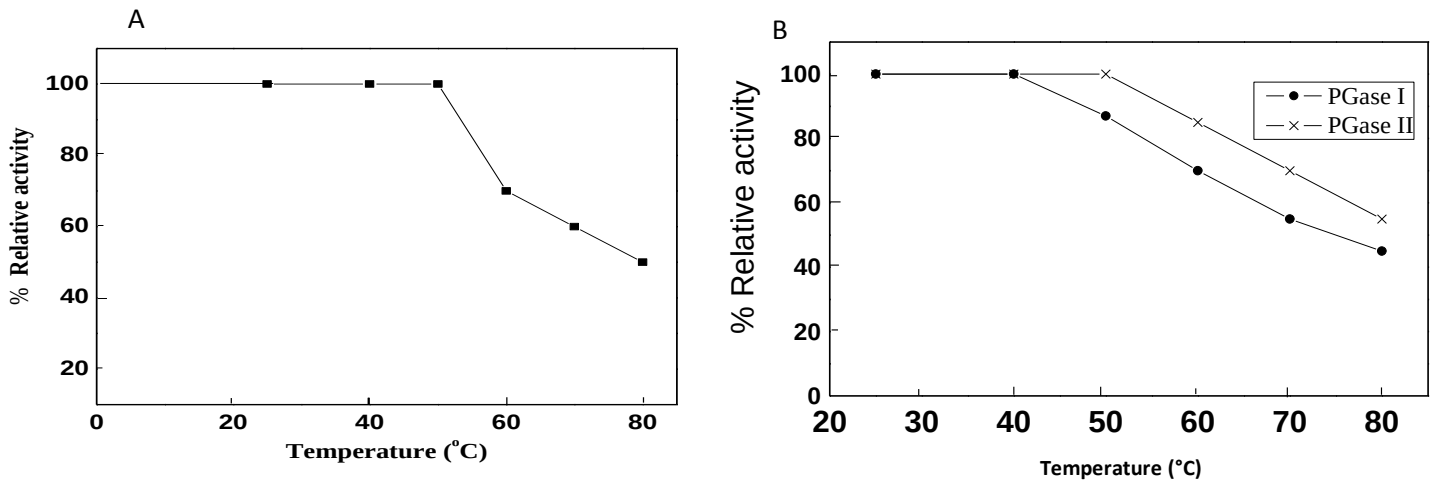

Figure 5. Effect of temperature on the thermal stability of *F. oxysporum* PGase (a) and *A. tubingensis* PGaseI and PGaseII (b). Each point represents the average of two experiments.

Table 3. Effect of 2 mM metal cations on *F. oxysporum* PGase and *A. tubingensis* PGaseI and PGaseII.

Metal cation	Relative acivity (%)		
	F. oxysporum PGase	*A. tubingensis*	
		PGaseI	PGaseII
Zn^{2+}	88	144	133
Pb^{2+}	61	88	61
Ca^{2+}	156	134	117
Hg^{2+}	68	49	71
Cu^{2+}	102	72	78
Co^{2+}	138	139	134
Ni^{2+}	132	98	167

Each value represents the average of two experiments.

and 32% inhibition, while Ca^{2+}, Co^{2+} and Ni^{2+} were found to activate the enzyme by 156, 138 and 132%, respectively. *A. tubingensis* PGaseI and PGaseII were activated by Zn^{2+}, Ca^{2+} , Co^{2+}, where Ni^{2+} only activated PGaseII. The other cations Cu^{2+}, Hg^{2+} and Pb^{2+} had partially inhibitory effect. In *P. viridicatum*, Ca^{2+} was foundto enhance the stability of polygalacturonase, while Hg^{2+}, Zn^{2+} and Cu^{2+} were strongly inhibited (Gomes et al., 2009). Co^{2+} and Cu^{2+} had no inhibitory effects on the polygalacturonase activity of *P. variotii* (Damasio et al., 2010). Polygalacturonases from *M. rouxii* (Saeed et al., 2007) and *A. niger* NRRL3 (Fahmy et al., 2008) were partially inhibited by Ca^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} and Hg^{2+}.

The effect of different compounds on the activities of the examined polygalacturonases was studied (Table 4). PMSF, *p*-HMB, β-mercaptoethanol, trypsin inhibitor, 1,10 phenanthroline, EDTA, sodium citrate, sodium oxalate, sodium benzoate, gallic acid and tannic acid had moderate and partial inhibitory effects on polygalcturonases from *F. oxysporum* and *A. tubingensis*, while benzoic and citric acids had strong inhibitory effects.

However, tannic acid was a potent inhibitor of polygalacturonase produced by *Alternaria alternata* (Kotwal, 1981). *P. oxalicum* polygalacturonase at the level of 5 mM was not inhibited by PMSF and EDTA (Yadav and Shastri, 2005). For *M. rouxii* polygalacturonase, EDTA stimulated the activity up to 125% (Saeed et al., 2007).

Effect of benzoic and citric acids on the growth of fungi

From the results, it can be concluded that benzoic and citric acids had strong inhibitory effect, as compared with other compounds examined, on the examined polygalacturonases. Therefore, the benzoic and citric acids were used as antifungal compounds for *F. oxysporum* and *A. tubingensis* loaded on the injured banana and peach, respectively (Figures 6 and 7). Comparing the

Table 4. Effect of 5 mM chemical compounds on *F. oxysporum* PGase and *A. tubingensis* PGaseI and PGaseII.

Chemical compound	*F. oxysporum* PGase	*A. tubingensis*	
		PGaseI	PGaseII
β-Mercaptoethanol	92	80	86
PMSF	95	69	72
Trypsin inhibitor	93	70	65
p-HMB	79	70	66
1,10 Phenanthroline	100	85	88
Benzoic acid	21	34	46
Gallic acid	78	98	116
Tannic acid	98	73	84
EDTA	93	82	78
Sodium benzoate	89	94	90
Sodium citrate	47	93	66
Sodium oxalate	48	83	61
Citric acid	13	35	28

Each value represents the average of two experiments. PMSF, phenylmethylsulfonyl fluoride; *p*-HMB, *p*-hydroxymercuribenzoic acid; EDTA, ethylenediamine tetraacetic acid.

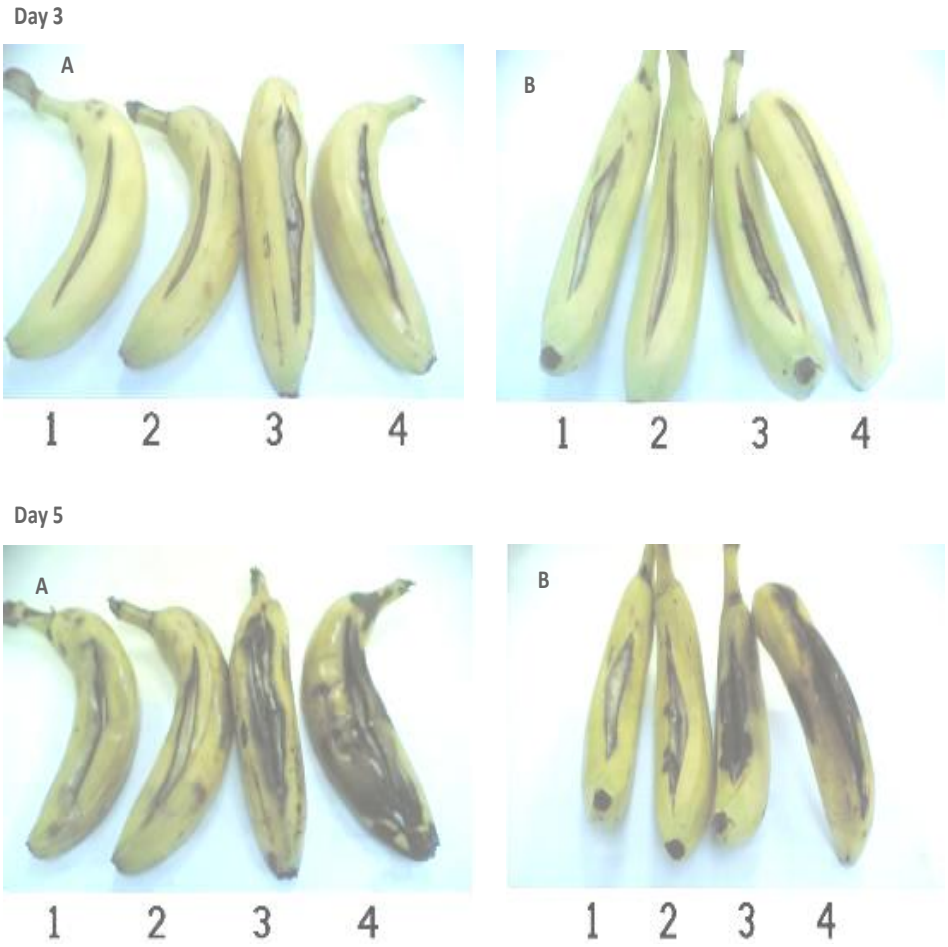

Figure 6. Effect of benzoic acid (a) and citric acid (b) on the development of *F. oxysporum* after three, five and seven days intervals of incubation at 28°C. 1) Injured banana without acid, 2) injured banana with acid, 3) injured banana with *F. oxysporum*, and 4) injured banana with acid and *F. oxysporum*.

Figure 6. Contd.

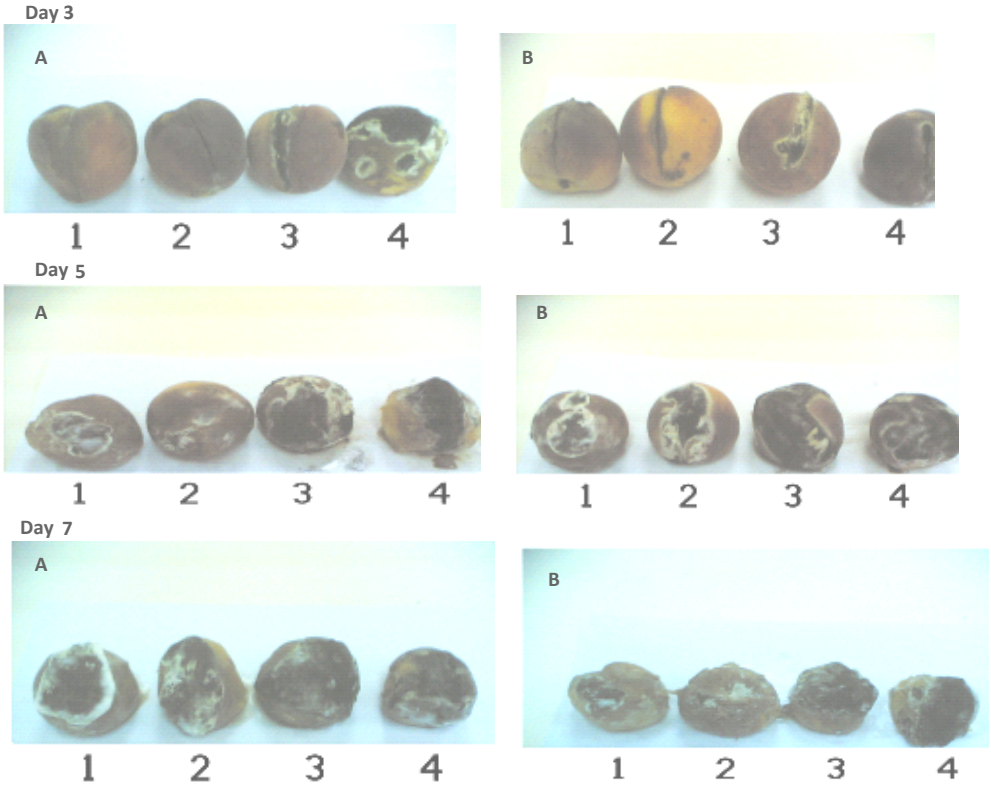

Figure 7. Effect of benzoic acid (a) and citric acid (b) on the development of *A. tubingensis* after three, five and seven days intervals of incubation at 28°C. 1) Injured peach without acid, 2) injured peach with acid, 3) injured peach with *A. tubingensis*, and 4) injured peach with acid and *A. tubingensis*.

effects of the two treatments, it appeared that citric acid was found to be more effective against fungal growth than benzoic acid. The reduction of fungal spoilage in hard peel banana treated with citric acid was higher as compared with the same fruits treated with benzoic acid.

However, the two acids had the same effect on fungal

spoilage of soft peel peach. Fruits treated with acids only, as compared with other treatments, showed more resistance to fungal spoilage development and less infection up to day three for soft peel peach, and up to day five for hard peel banana. The observation indicates that the formation of fungal spoilage in soft peel peach is faster than in hard peel banana. Similarly, benzoic acid was been found to be an antifungal agent (Pundir and Jain, 2010; Sofos et al., 1998), and is effective against *A. niger, A. flavus* and *Aspergillus fumigatus* (Doughari et al., 2007). Evaluation of inhibitory effects of citric acid on the growth inhibition of some important pathogenic fungi *in vitro Trichophyton mentagrophytes* var. *mentagrophytes*, *Candida albicans*, *A. fumigatus*, and *Malassezia furfur* was studied. The results demonstrate that citric acid had fungistatic and fungicidal activities against all pathogenic fungi tested, and its effect on filamentous fungi was higher than that on the yeasts (Shokri, 2011).

Conclusion

In conclusion, the study of the characterization of fungal polygalacturonases, especially the effect of citric and benzoic acids, which had strong inhibitory effect, may be used for the treatment of banana and peach spoilage fungi.

REFERENCES

Al-Hindi RR, Al-Najada AR, Mohamed SA (2011). Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. Afr. J. Microbiol. Res. 5: 443-448.

Alkorta I, Garbisu C, Llama MJ, Serra JL (1998). Industrial applications of pectic enzymes. Process Biochem. 33: 21-28.

Beg QK, Bhushan B, Kapoor M Hoondal GS (2000). Production and characterization of thermostable xylanase and pectinase from a *Streptomyces* sp. QG-11-3. J. Ind. Microbiol. Biotechnol. 24: 396-402.

Bradford MM (1976). A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Buga ML, Ibrahim S, Nok AJ (2010). Partially purified polygalacturonase from *Aspergillus niger* (SA6). Afr. J. Biotechnol. 9: 8944-8954

Collmer A, Keen N (1986). The role of pectic enzymes in plant pathogenesis. Ann. Rev. Phytopathol. 24: 383-409.

Da Silva EG, de Fatima Borges M, Medina C, Piccoli RH, Schwan, RF (2005). Pectinolytic enzymes secreted by yeasts from tropical fruits. FEMS Yeast Res. 5: 859-865.

Damasio A RL, da Silva TM, Maller A, Jorge JA, Terenzi HF, Polizeli MLTM (2010). Purification and partial characterization of an exo-polygalacturonase from *Paecilomyces variotii* liquid cultures. Appl. Biochem. Biotechnol. 160: 1496-1507.

De las Heras A, Patino B, Posada ML, Martinez MJ, Vazquez C, Gonzalez Jean MT (2003). Characterization and *in vitro* expression patterns of an exo-polygalacturonas encoding gene from *Fusarium oxysporum* f.sp. radicis lycopersici. J. Appl. Microbiol. 94: 856-864.

De Lorenzo G, Castoria R, Bellincampi D, Cervone F (1997). Fungal invasion enzymes and their inhibition. In: Carroll GC, Tudzynski P (Eds.), The Mycota V, Part B: Plant Relationship. Springer, Berlin, pp. 61-83.

Di Matteo (2006). Polygalacturonase-inhibiting protein (PGIP) in plant defence: a structural view. Phytochem. 67: 528-533.

Di Pietro A, Roncero MI (1998). Cloning expression and role in pathogenicity of pgI encoding the major extracellular endopolygalacturonase of the vascular wilt pathogen *Fusarium oxysporum*. Mol. Plant Pathol. 11: 91-98.

Dogan N, Tari C (2008). Characterization of three-phase partitioned exo-polygalacturonase from *Aspergillus sojae* with uniqu properties. J. Biochem. Eng. 39: 43-50.

Doughari JH, Elmahmood AM, Manzara S (2007). Studies on the antibacterial activity of root extracts of *Carica papaya* L. Afr. J. Microbiol. Res. 7: 37-41.

Fahmy AS, El-beih FM, Mohamed SA, Abdel-Gany SS, Abd-Elbaky EA (2008). Purification and characterization of an exo-polygalacturonase from *Aspergillus niger*. Appl. Biochem. Biotechnol. 149: 205-217.

Garcia-Maceira FI, Pietro D, Huertas-Gonzalez A, Ruiz-Roldan MC, Roncero MI (2001). Mole of a low molecular mass pectate lyase from *Fusarium moniliforme*. Biochem. J. 319: 159-164.

Gewali MB, Maharjan J, Thapa S, Shrestha JK (2007). Studies on polygalacturonase from *Aspergllus flavus*. Sci. World, 5: 19-22.

Gomes E, Leite RSR, da Silva R, Silva D (2009). Purification of an exopolygalacturonase from *Penicillium viridicatum RFC3* produced in submerged fermentation. Int. J. Microbiol. Article ID 631942, 8 pages, doi:10.1155/2009/631942.

Gummadi SN, Panda T (2003). Purification and biochemical properties of microbial pectinases-a review. Process Biochem. 38: 987-996.

Idnurm A, Howlett BJ (2001). Pathogenicity genes of phyto-pathogenic fungi. Mol. Plant Pathol. 2: 241-255.

Kar S, Ray RC (2011). Purification, characterization and application of thermostable exo-polygalacturonase from *Streptomyces erumpens* MTCC 7317. J. Food Biochem. 35: 133-147.

Khairnar Y, Vamsi KK, Boraste A, Gupta N, Trivedi S, Patil P, Gupta G, Gupta M, Jhadav A, Mujapara A, Joshi B, Mishra D (2009). Study of pectinase production in submerged fermentation using different strains of *Aspergillus Niger*. Int. J. Microbiol. Res.1: 13-17.

Kotwal S (1981). Studies on organisms infecting tropical fruits with special reference to *Alternaria alternata,* an indeginously isolated strain causing soft-rot, PhD Thesis, Nagpur University, Nagpur.

Li R, Rimmer R, Buchwaldt L, Sharpe AG, Seguin-Swartz G, Hegedus DD (2004). Interaction of *Sclerotinia sclerotiorum* with *Brassica napus*: cloning and characterization of endo- and exo-polygalacturonases expressed during saprophytic and parasitic modes. Fungal Gene Biol. 41: 754-765.

Machinandiarena MF, Wolski EA, Barrera V, Daleo GR, Andreu AB (2005). Characterization and in vitro expression patterns of extracellular degradative enzymes from non-pathogenic binucleate Rhizoctonia AG-G. Mycopathol. 159: 441-448.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428.

Mohamed SA, Al-Malki AL, Kumosani TA (2009). Characterization of a polygalacturonase from *Trichoderma harzianum* grown on citrus peel with application for apple juice. Aust. J. Basic Appl. Sci. 3: 2770-2777.

Murad HA, Azzaz HH (2011). Microbial pectinases and ruminant nutrition. Res. J. Microbiol. 6: 246-269.

Nabi NG, Asgher M, Shah AH, Sheikh MA, Asad MJ (2003). Production of pectinase by *Trichoderma harzianum* in solid state fermentation of citrus peel. Pakistan J. Agric. Sci. 40: 3-4.

Nakkeeran E, Umesh-Kumar S, Subramanian R (2011). *Aspergillus carbonarius* polygalacturonases purified by integrated membrane process and affinity precipitation for apple juice production. Bioresour. Technol. 102: 3293-3297.

Niture SK, Kumarb AR, Parabc PB, Panta A (2008). Inactivation of polygalacturonase and pectate lyase produced by pH tolerant fungus *Fusarium moniliforme* NCIM 1276 in a liquid medium and in the host tissue. Microbiol. Res. 163: 51-62.

Niture SK, Pant A (2004). Purification and biochemical characterization of polygalacturonase 11 produced in semi-solid medium by a strain of *Fusarium moniliforme*. Microbiol. Res. 159: 305-314.

Pedrolli DB, Carmona EC (2010). Purification and characterization of the exopolygalacturonase produced by *Aspergillus giganteus* in submerged cultures. J. Ind. Microbiol. Biotechnol. 37: 567-573.

Pedrolli DB, Monteiro AC, Gomes E, Carmona EC (2009). Pectin and pectinases: production, characterization and industrial application of

microbial pectinolytic enzymes. Open Biotechnol. J. 3: 9-18
Pundir RK, Jain P (2010). Screening for antifungal activity of commercially available chemical food preservatives. Int. J. Pharm. Sci. Rev. Res. 5: p. 2.
Riou C, Salmon J-M, Vallier M-J, Gunata Z, Barre P (1998). Purification, characterization, and substrate specificity of a novel highly glucose-tolerant β-glucosidase from *Aspergillus oryzae*. Appl. Environ. Microbiol. 64: 3607-3614.
Saad N, Briand M, Gardarin C (2007). Production, Purification and characterization of an endopolygalacturonase from *Mucor rouxii* NRRL 1894. J. Enzyme Microbiol. Technol. 41: 800-805.
Schnitzhofer W, Weber H-J, Vrsanska M, Biely P, Cavaco-Paulo A, Guebitz GM (2007). Purification and mechanistic characterisation of two polygalacturonases from *Sclerotium rolfsii*. J. Enzyme Microbiol. Technol. 40: 1739–1747.
Shokri H (2011). Evaluation of inhibitory effects of citric and tartaric acids and their combination on the growth of *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, *Candida albicans*, and *Malassezia furfur*. Comparative Clinical Pathology DOI: 10.1007/s00580-011-1195-6, Springer-Verlag London Limited.
Sofos JN, Beuchat LR, Davidson PM, Johnson EA (1998). Naturally occurring antimicrobials in Food. Regul. Pharmacol. Toxicol. 28: 71-72.
Ten Have A, Breuil WO, Wubben JP, Visser J, van Kan JA (2001). *Botrytis cinerea* endopolygalacturonase genes are differentially expressed in various plant tissues. Fungal Gene. Biol. 33: 97–105.
Thakur A, Pahwa R, Singh S, Gupta R (2010). Production, purification, and characterization of polygalacturonase from *Mucor circinelloides* ITCC 6025. Enzyme Res. Article ID 170549, 7.
Torres-Favela E, Aguilar C, Esquivel-Contreras CJ, Gustavo GV (2003). Pectinase. In Enzyme Technology. Asiatech Publisher Inc. Delhi., pp. 273-296.
Yadav S, Shastri NV (2005). Partial purification and characterization of a pectin lyase produced by *Penicillium oxalicum* in solid-state fermentation (SSF). Indian J. Biotechnol. 4: 501-505.