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Partial characterization of *Aspergillus fumigatus* polygalacturonases for the degumming of natural fibers

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SUMMARY

Aspergillus fumigatus strain 4, cultured on citrus pectin as the sole carbon source, produced polygalacturonases whose activity was optimum at 65 °C and pH 3.5–4.5. The enzymes presented a bimodal thermostability for 10 min, but not 60 min, of incubation. Polygalacturonases showed pH stability between 3.0 to 9.0. The enzymes were stable when stored at 4–6 °C for 90 days, but their activity was reduced by 24% when they were stored at 26–30 °C. Orange pulp was the best pectic carbon source tested for the production of pectinases capable of retting ramie fibers. The reutilization of these enzymes was possible, suggesting the viability of industrial use of pectinases for degumming ramie fibers.

INTRODUCTION

The fibers of ramie (*Bohemeria nivea*) are thickly covered with pectic substances which must be eliminated for the industrial utilization of the fibers. According to Rexová-Benková and Markovic [9], pectinases are important for retting plant tissues both at the beginning of the process and throughout the subsequent phases. Despite the difficulty in extracting natural fibers by enzymatic retting, attempts have been made using fungal pectinases from *Aspergillus versicolor* [5] and from *Alternaria, Fusarium* and *Botrytis* species, or commercial pectinases [10]. The objective of the present study was to evaluate the best conditions for the activity of polygalacturonases from a fungus and to observe the action of these enzymes in the process of ramie fiber retting.

MATERIALS AND METHODS

Microorganism and culture conditions

Aspergillus fumigatus strain 4 was isolated and the inoculum was produced by the method of Baracat et al. [2]. The fungus was cultured in a rotary shaker (150 rpm) for 24 h at 40 °C in 250-ml Erlenmeyer flasks containing 100 ml culture medium of the following composition: 2.0 g KH_2PO_4, 0.62 g K_2HPO_4, 1.1 g $MgSO_4 \cdot 7\ H_2O$, and 1.0 g $(NH_4)_2SO_4$ per liter, pH 6.3. The medium was sterilized

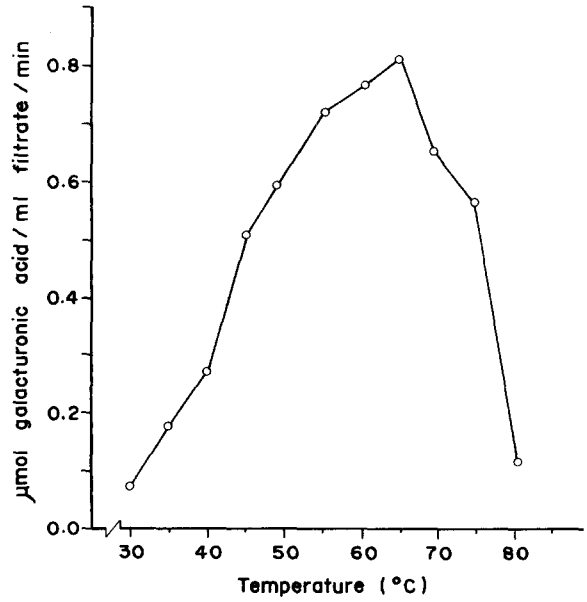

Fig. 1. Activity–temperature curve for polygalacturonases from *Aspergillus fumigatus* strain 4 grown on 0.5% citrus pectin at 40 °C and 150 rpm for 24 h.

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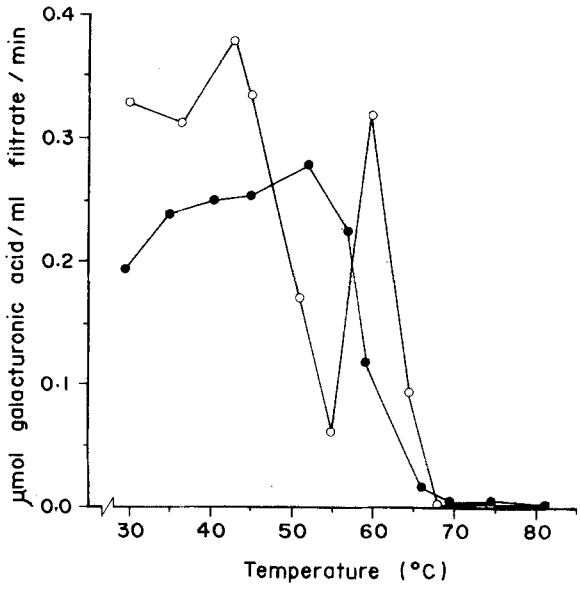

Fig. 2. Heat stability of polygalacturonases from *Aspergillus fumigatus* strain 4 grown on 0.5% citrus pectin at 40 °C and 150 rpm for 24 h, after 10 (○) and 60 min (●) of exposure to different temperatures.

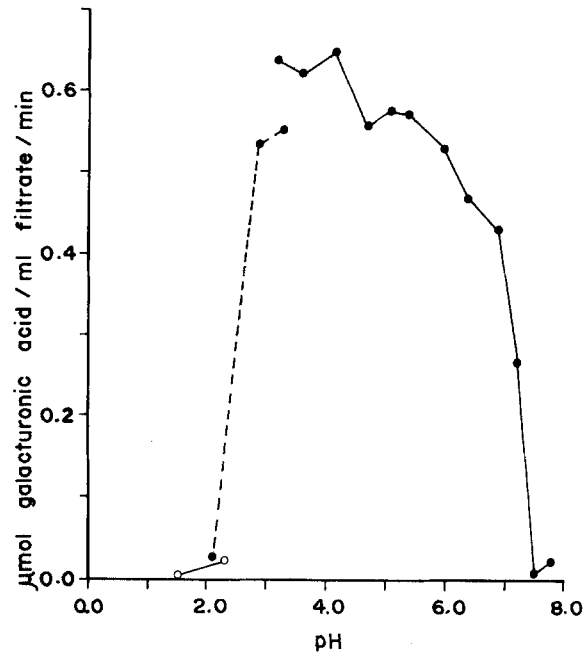

Fig. 3. Activity–pH curve for polygalacturonases from *Aspergillus fumigatus* strain 4, grown on 0.5% citrus pectin at 40 °C and 150 rpm for 24 h, in Clark and Lubs (○), glycine-HCl (● - - -●) and sodium acetate/Tris/monobasic potassium phosphate (●——●) buffers.

at 121 °C for 15 min. Tetracycline and rifamycin (20.0 mg/l) were added aseptically. Unsterilized citrus pectin (type 8106, Eskisa S.A.), 5.0 g/l, was added as the sole carbon source, except when otherwise indicated.

Polygalacturonase (PG) characterization

Since the intention was to test the industrial application of pectinases for natural fiber retting, the culture filtrate (400-mesh sieve) was used to test PG activity, without prior purification or concentration.

PG activity was evaluated from 30 to 80 °C by heating in a water bath. The heat stability of the enzyme was

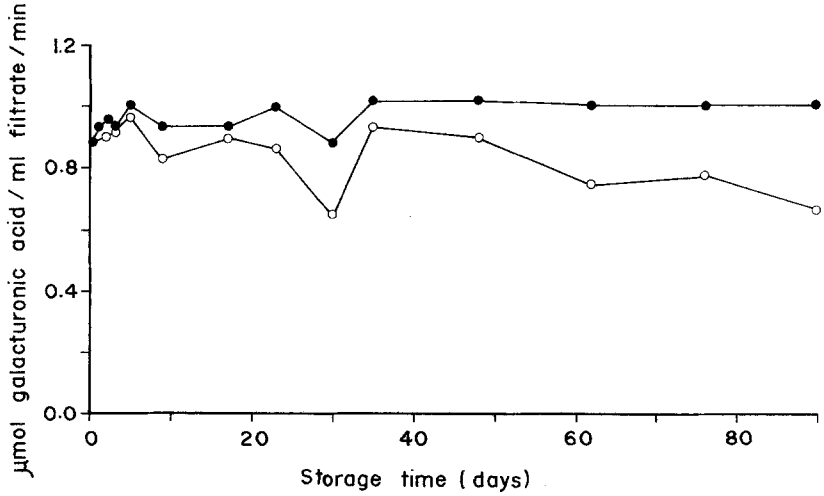

Fig. 4. Stability to storage at 6–10 °C (●) and 26–30 °C (○) of polygalacturonases from *Aspergillus fumigatus* strain 4 grown on 0.5% orange pulp at 40 °C and 150 rpm for 24 h.

TABLE 1

Polygalacturonase activity and reduction in weight of ramie fibers treated at 40 °C for 24 h with filtrates of *Aspergillus fumigatus* strain 4 grown on pectic carbon sources at 40 °C and 150 rpm for 24 h

Treatment	Polygalacturonase activity (μmol galacturonic acid/ml filtrate/min)	% Reduction in fiber weight
Control (without substrate)	–	3.03
Apple pectin (Sigma)	1.093	7.12
Citrus pectin (Sigma)	1.009	6.62
Citrus pectin (Eskisa)	0.765	7.03
Organde pulp	1.117	7.30

determined by heating the filtrates at the above temperatures for 10 or 60 min followed by rapid cooling in an ice bath.

Optimum pH values for PG activity were tested using the following buffer solutions: 0.2 M Clark and Lubs (pH 1.6–2.2), glycine-HCl (pH 2.2–3.6) and sodium acetate/Tris/monobasic potassium phosphate [1] (pH 3.5–8.0). The stability at different pH values was determined by maintaining the fungal filtrate in the sodium acetate/Tris/monobasic potassium phosphate buffer solution (pH 3.0–8.0) at 4–6 °C for 17 h.

PG stability during storage was determined using 0.5% orange pulp as the sole carbon source for fungal growth. The culture filtrates were maintained at 6–10 °C or 26–30 °C for 90 days and analyzed periodically. Formaldehyde (2.0 ml/l) was added as an antimicrobial agent.

Degumming of ramie fibers

Raw ramie fibers were boiled in tap water for 15 min and incubated with the filtrate of *A. fumigatus* strain 4 cultures grown on orange pulp at 40 °C for 24 h (4 g ramie/80 ml culture filtrate). The fibers were then dried at 70 °C. Variations in fiber dry weight and PG activity were determined in order to evaluate the action of the culture filtrates on ramie fibers.

Retting of ramie fibers by the culture filtrates at 40 °C was monitored for 24 h.

Citrus and apple pectin, and orange pulp, 0.5% (w/v) were used as the sole carbon source for the production of pectinases for ramie fiber retting.

Culture filtrates were obtained under the optimum conditions determined (orange pulp as carbon source, 45 °C, 150 rpm, 30 h) and used to ret ramie fibers. The enzymes were reutilized on raw ramie fibers two more times after replacement of the initial PG activity.

The experiments were carried out in triplicate.

Polygalacturonase assays

Extracellular polygalacturonases were determined by the method of Baracat et al. [3].

RESULTS AND DISCUSSION

The polygalacturonases (PG) of the thermophilic fungus *A. fumigatus* strain 4 were considered to be heat stable since their maximum activity was detected at 65 °C (Fig. 1). In general, the enzyme systems of thermophilic fungi are stable [4], permitting the action of the enzymes at high temperatures, a fact that may prevent the growth of pathogenic bacteria.

When maintained at different temperatures for 10 min, PG enzymes presented heat stability of bimodal behaviour, a fact that was not observed after 60 min of incubation at the same temperatures (Fig. 2). These results suggest the importance of determining enzyme thermostability at different incubation times. Bimodal behaviour has been

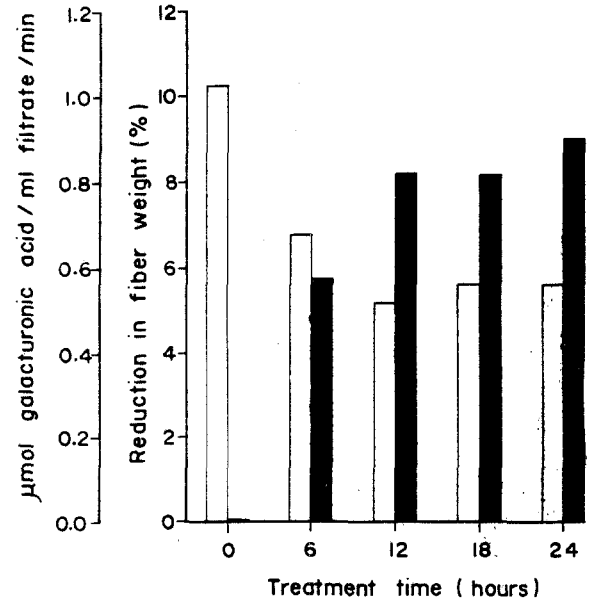

Fig. 5. Polygalacturonase activity (□) and reduction in weight of fibers (■) treated at 40 °C for different periods of time with filtrates of *Aspergillus fumigatus* strain 4 grown on 0.5% orange pulp at 40 °C and 150 rpm for 24 h.

TABLE 2

Reutilization of fungal filtrates obtained by culturing *Aspergillus fumigatus* strain 4 on 0.5% orange pulp at 45 °C and 150 rpm for 30 h, for weight reduction of ramie fibers treated at 40 °C for 24 h

Reutilization of the fungal filtrate	% Reduction in fiber weight
Control (boiled filtrate)	3.52
Treatment 1	8.25
Treatment 2	8.44
Treatment 3	7.79

observed for endo-PG from *Rhizopus* and *Mucor* [7,8], and the presence of inhibitors which are active at intermediate temperatures was suggested.

The maximum PG activities obtained between pH 3.5 and pH 4.5 (Fig. 3) were similar to those observed by Tani and Nanba [11] for PG from *Botrytis cinerea* (pH 4.3–5.5). These enzymes were found to be stable at pH values ranging from 3.0 to 9.0 (data not shown).

Polygalacturonases stored for 90 days presented greater stability when maintained at 6–10 °C (Fig. 4), losing 24% of their initial activity at 26–30 °C. Endo-PG from *Aspergillus awamori* presented 82% of its activity after 2 years at 5–8 °C [12] and pectinases from *Aspergillus carbanerius* had 92% activity after 37 days at 25–28 °C [6].

Pectic enzymes are important for both the initial and the subsequent phases of the processes of plant material retting [9], as can be seen from the data obtained here for ramie (Table 1). Orange pulp was considered to be the best source of pectic carbon among those tested. In addition to the high PG activity observed, orange pulp is a by-product of the fruit juice industry of Brazil and can be obtained at low cost. This material also contains natural substances such as ions or growth factors which may facilitate fungal growth and enzyme production. Further studies on orange pulp as carbon source are needed.

At the end of 24 h, the culture filtrate used to ret ramie fibers at 40 °C still had 53% of its initial PG activity (Fig. 5). The reutilization of these enzymes for raw ramie fibers was successfully evaluated three times (Table 2).

The present results suggest the viability of the use of fungal PG without purification or concentration (as done in the food industry) for the retting of raw ramie fibers, as well as the need to improve the process in order to minimize costs.

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