J Ind Microbiol Biotechnol (2008) 35:1001–1005
DOI 10.1007/s10295-008-0375-0

ORIGINAL PAPER

Optimization of culture conditions for the production of thermostable polygalacturonase by *Penicillium* SPC-F 20

Abraham Mathew · Abi N. Eldo · A. G. Molly

Received: 14 March 2008 / Accepted: 12 May 2008 / Published online: 27 May 2008

Abstract *Penicillium* strain isolated from citrus fruit was found to produce thermostable polygalacturonases. Optimization of process parameters resulted in high levels of enzyme production after 3 days of incubation at a pH of 5.0 at 30 °C in the presence of 1% pectin. The optimum temperature for enzyme activity was 60 °C and a pH of 5.5 was found to be the optimal pH. The enzyme showed a high level of thermostability in the presence of substrate with a residual activity of 48% after 2 h of incubation at 60 °C. A thermostable nature with a high pH range for activity makes it an industrially important enzyme.

Keywords Polygalacturonase · Thermostability · *Penicillium*

Introduction

Microbial pectinases have tremendous potential in fruit processing industries as to hydrolyze the pectic substances that are responsible for turbidity and undesirable cloudiness in fruit juices. They increase fruit juice yield, color and flavor, and promote antioxidant formation [1]. Pectinases are also used in textile processing, degumming of plant fibers, papermaking, coffee fermentation, and in treatment of wastewaters [2–4]. They are also used to prepare cell protoplast for studies in genetic engineering [5]. The worldwide consumption of pectinase has reached above 7×10^6 tons per year.

Polygalacturonase (E.C. 3.2.1.15) from microbial source is the most important pectinase used in industries. Thermostable polygalacturonases are of great significance as fruit juices are pasteurized at 50–60 °C [6]. High temperatures not only reduce microbial contamination but also increase the solubility of pectin and decrease the viscosity of the liquid [7–9]. Although a large number of organisms producing pectinases have been reported, selection of potential isolates remains a tedious task, especially when physiologically potential strains are to be obtained to achieve maximum yield [10]. Most of the commercial polygalacturonases produced are from *Aspergillus* species. In the light of growing importance and demand of the enzyme, a strain of *Penicillium*-producing thermostable polygalacturonase was isolated [11]. Optimization of the process parameters involved in the production of the enzyme is reported here under.

A. Mathew (✉) · A. N. Eldo · A. G. Molly
Post Graduate and Research Department of Botany,
St. Peter's College, Kolenchery P.O., Kerala 682311, India
e-mail: abrahambotany@yahoo.com

Materials and methods

Microorganism: *Penicillium* sps. SPC-F 20 isolated from decayed citrus fruits was selected after preliminary screening [11]. The fungus was maintained on Potato Dextrose Agar (PDA) slants.

Inoculum: Spore inoculum was prepared by growing the culture on PDA slant at 30 °C for 7 days. The spores were dispersed in 10 ml of sterile 0.1% Tween 80 and 1 ml was taken as the inoculum.

Production media: Modified Mandel and Reese medium containing 1.4 g NH_4SO_4, 2.0 g KH_2PO_4, 0.005 g $FeSO_4{\cdot}7H_20$, 0.3 g $CaCl_2$, 0.3 g $MgSO_4{\cdot}7H_2O$, 0.0016 g $MnSO_4$, 0.0017 g $ZnCl_2$, 0.3 g Urea, 1.0 g Peptone, and 10 g Pectin in 1,000 ml distilled water was used.

Optimization of the production parameters

Temperature: Erlenmeyer flasks containing 50 ml of the medium was inoculated and incubated at a temperature

range of 10–50 °C at 150 rpm. Samples were analyzed for enzyme activity after 3 days of incubation.

pH: The impact of pH on enzyme production was determined by growing the fungus in various pH levels of range 3–10.

Nitrogen source: To determine the best nitrogen source, the fungus was grown in the medium containing different nitrogen sources at 0.5% level.

Sodium chloride concentration: The effect of sodium chloride on enzyme production by the fungus was carried out by incorporating sodium chloride at a range of 0–5%.

Substrate concentration: The substrate concentration for maximal enzyme synthesis was optimized by varying the pectin concentration from 0 to 5%.

Inoculum density and incubation period: Effect of inoculum density on enzyme production was determined with various levels of prepared inoculum. The optimum incubation time was determined by providing an incubation period of 24–120 h.

pH and temperature optima for enzyme activity

The optimal pH for enzyme activity was determined by conducting the enzyme assay in various pH ranging from 3 to 9, using Tris/HCl buffer and Citrate buffer. The optimum temperature for activity was measured by assaying the enzyme at different temperatures ranging from 30 to 90 °C.

Thermostability of polygalacturonase

For determining the thermostability, the enzyme was subjected to the temperature range of 50–90 °C for 1–6 h. The samples were taken at 1-h intervals and were assayed for activity.

All the experiments were conducted in triplicates.

Enzyme extraction: The culture broth was centrifuged at 10,000*g* for 20 min at 4 °C and the supernatant thus obtained was assayed for enzyme activity.

Enzyme assay: Polygalacturonase activity was determined by incubating 1 ml of the enzyme extract with 10 mg of pectin in 1 ml of 0.05 M Tris/HCl buffer containing 0.0001 M $CaCl_2$ at 50 °C for 20 min. The reaction was stopped by adding 3 ml of 3,5 dinitrosalicylic acid (DNS reagent). The tubes were held at 100 °C for 5 min, cooled immediately, and the absorbance of the reaction mixture was measured at 547 nm [12].

One unit of polygalacturonase was defined as the amount of enzyme that released 1 mM galacturonic acid min^{-1} and is expressed as IU ml^{-1}. The calibration curve was drawn with monogalacturonic acid as the standard. The protein content in the crude extract was estimated by the method of Lowry et al. [13] using bovine serum as the standard.

Results and discussion

Effect of temperature

Temperature is known to influence the metabolic rate of the organism involved in the process, which in turn determines the amount of end product [14]. Maximum polygalacturonase activity was noted at a temperature of 30 °C (Fig. 1). Increase in temperature thereafter caused a decrease in enzyme production, indicating a mesophilic nature of the fungus. High production temperature will reduce the contamination risk during fermentation [7]. However, a moderate optimal temperature for a longer incubation period is beneficial so as to reduce the cost of production.

pH

The fungus was found to produce polygalacturonases under both acidic and alkaline conditions. The maximum rate was obtained at a pH of 5 (Fig. 2). A change in pH beyond the

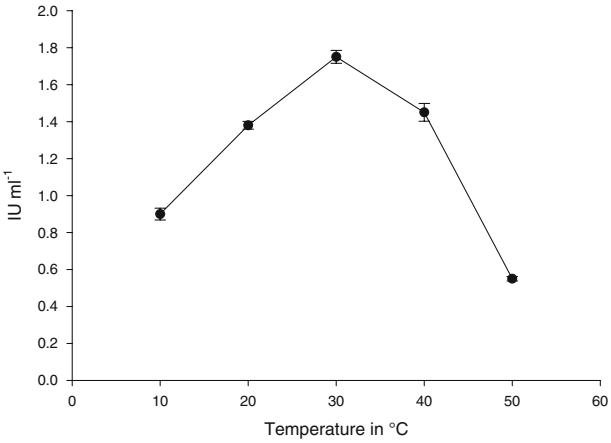

Fig. 1 Effect of temperature on enzyme production by *Penicillium* SPC-F 20 grown in basal medium containing 1% pectin

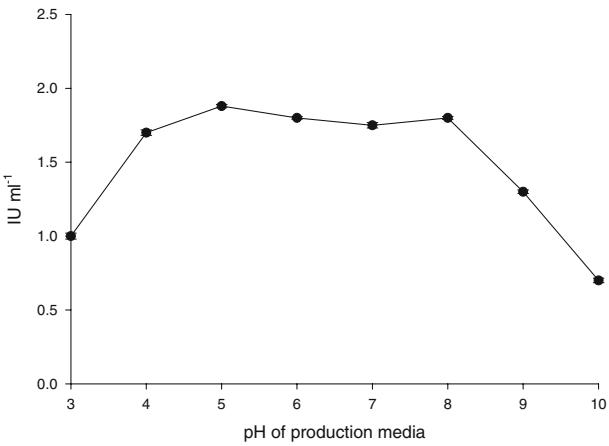

Fig. 2 Effect of pH on enzyme production by *Penicillium* SPC-F 20 grown in basal medium containing 1% pectin at 30 °C

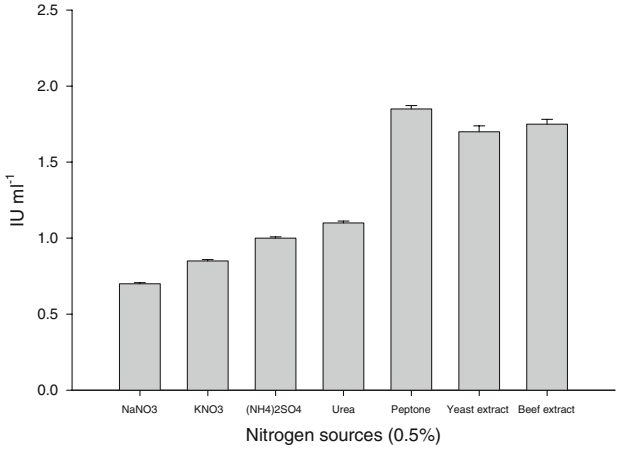

Fig. 3 Effect of various Nitrogen sources (0.5%) on enzyme production by *Penicillium* SPC-F 20 grown in basal medium (pH 5) containing 1% pectin at 30 °C

optimum may have a severe effect on enzyme production [15]. However, this particular strain showed considerable production at a pH range of 4–8. A wide range of initial pH of the medium during upstream and downstream bioprocess makes the end product either acidic or alkaline, which tend to have varied applications [4]. Even though a low pH range maintains asepsis [10], a wide range makes it possible for the use of diverse substrates for enzyme production.

Nitrogen source

Among the various nitrogen sources used, organic non-defined nitrogen sources were found to enhance the enzyme production (Fig. 3). This may be due to other nutrients and growth enhancers present in them. The enzyme production reached a maximum when peptone was used as the nitrogen source (1.85 IU ml^{-1}). Urea was found better than other defined nitrogen sources for maximal enzyme synthesis.

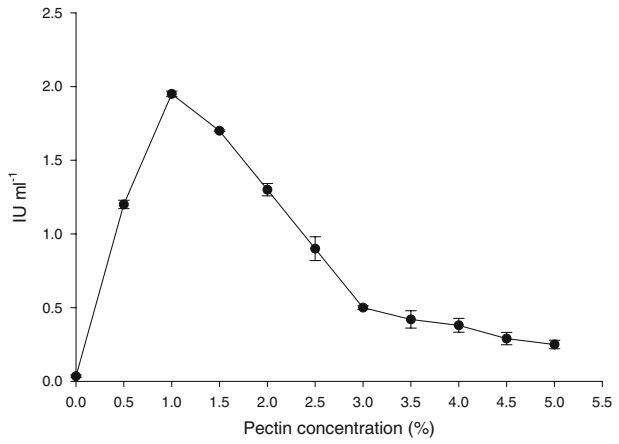

Fig. 4 Effect of pectin concentration on enzyme production by *Penicillium* SPC-F 20 grown in basal medium (pH 5) containing 1% pectin at 30 °C

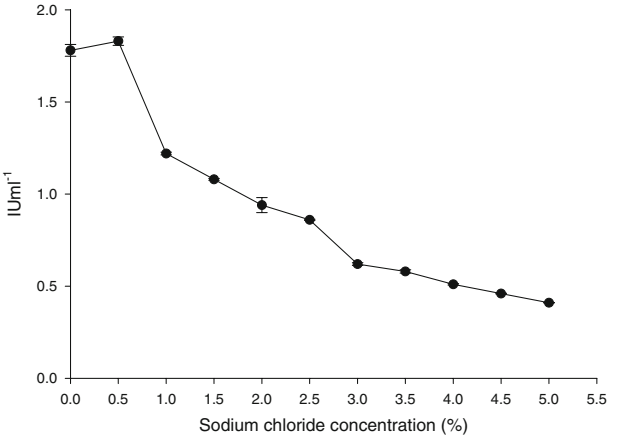

Fig. 5 Effect of sodium chloride concentration on enzyme production by *Penicillium* SPC-F 20 grown in basal medium (pH 5) containing 1% pectin at 30 °C

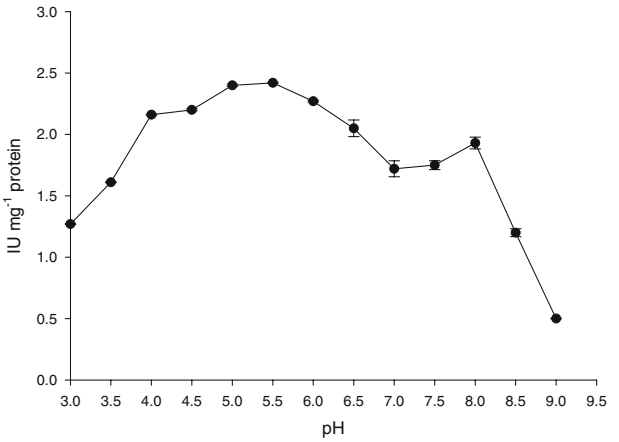

Fig. 6 Effect of pH on enzyme activity (IU mg^{-1} protein)

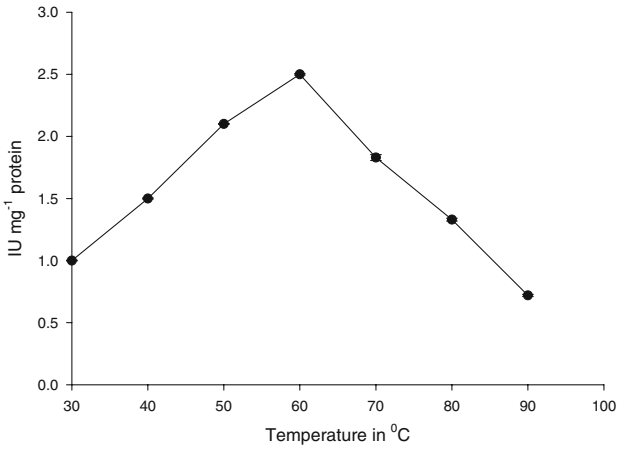

Fig. 7 Effect of temperature on enzyme activity (IU mg^{-1} protein)

Table 1 Thermal stability of extracellular polygalacturonase of *Penicillium* SPC-F 20 at different temperatures

Incubation time (h)	Specific activity (IU mg^{-1} protein)					Residual activity (%)				
	50 °C	60 °C	70 °C	80 °C	90 °C	50 °C	60 °C	70 °C	80 °C	90 °C
1	3.44 ± 0.05	3.10 ± 0.04	1.61 ± 0.05	0.81 ± 0.08	0.55 ± 0.07	75 ± 1.01	68 ± 0.09	35 ± 0.04	18 ± 0.06	12 ± 0.01
2	2.50 ± 0.08	2.20 ± 0.04	0.77 ± 0.05	0.72 ± 0.07	0.44 ± 0.07	54 ± 0.08	48 ± 0.15	17 ± 1.01	15 ± 0.02	9 ± 0.02
3	1.60 ± 0.04	1.40 ± 0.06	0.55 ± 0.04	0.50 ± 0.08	0.23 ± 0.09	35 ± 0.06	31 ± 0.28	12 ± 0.34	10 ± 0.02	5 ± 0.02
4	1.16 ± 0.05	1.11 ± 0.07	0.50 ± 0.03	0.39 ± 0.09	0.05 ± 0.01	26 ± 0.09	24 ± 0.04	11 ± 0.46	8 ± 0.03	2 ± 0.09
5	1.11 ± 0.03	1.03 ± 0.03	0.33 ± 0.01	0.28 ± 0.13	Nil	24 ± 1.03	22 ± 2.01	7 ± 0.05	6 ± 0.01	Nil
6	1.06 ± 0.04	1.00 ± 0.02	0.27 ± 0.07	0.26 ± 0.10	Nil	23 ± 2.01	21 ± 1.04	6 ± 0.01	5 ± 0.02	Nil

Substrate concentration and sodium chloride concentration

An increase in the pectin concentration resulted in an increase in enzyme production; the maximum level so attained was at 1% level (Fig. 4). Sodium chloride was not found to exert a significant effect on enzyme production. Even in its absence, considerable quantities of enzymes were produced (Fig. 5). A slight increase occurred as the concentration was raised to 0.5%; thereafter, an increase in the salt concentration resulted in drastic reduction in enzyme production.

Inoculum density and incubation time

An inoculum density of 4.5×10^7 spores ml^{-1} was found to be the best for maximal enzyme synthesis. An incubation period of 72 h resulted in a high level of enzyme production, which decreased with increase in incubation time. Phutela et al. [15] also reported a similar result in *Aspergillus fumigatus.*

pH and temperature optima for enzyme activity

The pH optima were determined using Tris/HCl buffer and Citrate buffer. Maximal enzyme activity was observed at a pH of 5.5 (Fig. 6). The acidic range of 4.0–6.5 showed considerable enzyme activity. In the alkaline pH also, enzyme activity was detected and a peak of activity was formed at 8.0. The optimal temperature for enzyme activity was found to be 60 °C, indicating its potential use in fruit clarification. Even at 90 °C, the enzyme was found to show catalytic activity (Fig. 7).

Thermostability of polygalacturonase

The effect of temperature on polygalacturonase activity was studied in the presence of substrate (Table 1). A residual activity of 5% was noted even after 6 h of incubation at 80 °C. Only an incubation for 5 h at 90 °C resulted in complete loss of activity. A high residual activity of 48 and 31% at 60 °C after 2 and 3 h of incubation indicate the potentiality of the thermostable enzyme. Further work like purification, characterization, and immobilization is in progress for enhancing the production and commercialization of the enzyme.

References

1. Favela-Torres E, Aguilar C, Contreras-Esquivel JC, Viniegra-Gonzalez G (2005) Pectinases. In: Pandey A, Webb C, Soccol CR, Larroche C (eds) Enzyme technology. Asiatech, New Delhi, pp 273–296
2. Reid I, Ricard M (2003) Pectinases in papermaking, solving retention problems in mechanical pulps bleached with hydrogen peroxide. Enzyme Microb Technol 26:115–123. doi:10.1016/S0141-0229(99)00131-3
3. Patil SR, Dayanand A (2006) Production of pectinases from deseeded sunflower head by *Aspergillus niger* in submerged and solid-state conditions. Bioresour Technol 97:2054–2058. doi:10.1016/j.biortech.2005.09.015
4. Hoondal GS, Tiwari RP, Tewari R, Dahiya N, Beg QK (2002) Microbial alkaline pectinases and their industrial applications: a review. Appl Microbiol Biotechnol 59:409–418. doi:10.1007/s00253-002-1061-1
5. Shubakov AA, El'kina EA (2002) Production of polygalacturonases by filamentous fungi *Aspergillus niger* ACMF-1119 and *Penicillium dierckxii* ACIMF-15. Chem Comput Simul Butlerov Commun 7:65–68
6. Madhaiyan M, Sundaram SP (2004) Occurrence of thermostable pectin degrading fungi on decayed fruits and vegetables. Asian J Microbiol Biotechnol Environ Sci 6:363–366
7. Brock TD (ed) (1986) Thermophilis. General molecular and applied microbiology. Wiley, New York, 1p
8. Janeck S (1993) Strategies for obtaining stable enzymes. Process Biochem 28:435–445. doi:10.1016/0032-9592(93)85026-C
9. Kaur A, Sharma D, Harchand RK, Singh P, Bhullar SS, Kaur A (1999) Production of a thermostable extra cellular inulinase by *Aspergillus fumigatus*. Indian J Microbiol 39:99–103
10. Pandey A, Selvakumar P, Carlos RS, Poonam N (1999) Solid state fermentation for the production of industrial enzymes. Curr Sci 77:149–162
11. Mathew A, Molly AG, Eldo AE (2007) Screening of thermostable polygalacturonase producing bacteria and fungi from decaying fruits and vegetables. J MicroWorld 9(1):169–171
12. Gomes I, Saha RK, Mohiuddin G, Hoq MM (1992) Isolation and characterization of a cellulase-free pectinolytic and hemicellulolytic thermophilic fungus. World J Microbiol Biotechnol 8:589–592. doi:10.1007/BF01238794
13. Lowry OH, Rosebrough NH, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

14. Patil SR, Dayanand A (2005) Optimization of process for the production of fungal pectinases from deseeded sunflower head in submerged and solid-state conditions. Bioresour Technol 97:2340–2344

15. Phutela U, Dhuna V, Sandhu S, Chadha BS (2005) Pectinase & polygalacturonase production by a thermophilic *Aspergillus fumigatus* isolated from decomposing orange peels. Braz J Microbiol 36:63–69. doi:10.1590/S1517-83822005000100013