Asian Journal of Chemistry Vol. 20, No. 6 (2008), 4273-4281

Enhanced Production of Exo-Polygalacturonase by *Fusarium solani* in Solid-State Fermentation

SAUNIA HAMID, HAQ NAWAZ BHATTI* and UZMA ABDUL QAYYUM
Industrial Biotechnology Laboratory, Department of Chemistry
University of Agriculture, Faisalabad-38040, Pakistan
Fax: (92)(41)9200764; E-mail: hnbhatti2005@yahoo.com

Fungal pectinases are among the most important enzymes with major applications in food and drinking industries. In the present investigation the feasibility of using orange peel for the production of exopectinase by *Fusarium solani* in solid-state fermentation has been evaluated. Optimization of process parameters was carried out for enhanced production of the enzyme. Maximum exopectinase activity of 102.21 ± 6.13 U/g of dry orange peel was achieved under optimum growth conditions. The optimum conditions were: initial moisture content of orange peel, 60 % (v/w); pH of the medium, 6.0; incubation temperature, 35 ± 1 ºC; amount of orange peel, 10 g and incubation period, 96 h. Addition of glucose (1 %) as carbon and energy additive and peptone (1 %) as nitrogen additive further enhanced the production of exopectinase.

Key Words: Exopectinase, *Fusarium solani*, Orange peel, Solid-state fermentation.

INTRODUCTION

Pectinases are a group of enzymes that break the pectin-containing substrates, which are the structural polysaccharides of plant cells and maintain the integrity of plant tissues. Pectin-containing substances are characterized by long chains of galacturonic acid residues. Carboxyl groups are present on these residues, which are sometimes replaced with methyl groups, forming methoxyl groups. Pectinases act by breaking glycosidic bonds of the long carbon chains (polygalacturonase, pectin lyase and pectate lyase) and by splitting off methoxyl groups (pectin esterase)[1,2].

Microbial pectinases have drawn a great deal of attention world wide because of their myriad applications. They are of great significance, especially, in food processing, textile and paper industries[3]. Over the years pectinases are being used in coffee and tea fermentations, oil extraction and treatment of industrial wastewaters containing pectinacious material[4]. The most upcoming application of pectinase is their use in the degumming of plant without any damage to the end products[5,6].

There has been an increasing trend towards proper utilization and value-addition of agro-industrial residues in recent years[7]. Various agro-industrial residues such as wheat bran[8], sugarcane bagasse[9], coffee pulp[10], lemon peel[11], apple pomace[12], grape pomace[7] and deseeded sunflower head[13] have been exploited for production of pectinases.

Solid-state fermentation (SSF) is receiving a renewed surge of interest, primarily because of increased productivity and prospectus of using a wide range of agro-industrial residues as substrates[14,15]. The selection of a particular strain, however, remains a tedious task, especially when commercially significant enzyme yields are to be obtained. A few of the potential fungal cultures exploited for production of pectinolytic enzymes were *Aspergillus niger*[1,8,9,13,16], *Aspergillus awamori*[7,17], *Aspergillus japonica*[18], *Aspergillus foetidus*[12,19], *Penicillium occitanis*[20] and *Penicillium viridicatum*[21]. However, these strains were used to produce pectinases but from different substrates. The present investigation involves the optimization of different process parameters to enhance pectinase production by *Fusarium solani* using orange peel as substrate in solid-state fermentation.

EXPERIMENTAL

All the chemicals used were of analytical grade and mainly purchased from Sigma Chemical Company, unless otherwise specified. Orange peel was obtained from a local fruit industry and stored at -10 °C until required. For any given series of experiments, sub-samples were removed and dried in an oven at 60-70 °C for 48 h. The solid mass was then milled and sieved to get particles having diameter in the range of 0.8-1.0 mm.

Microorganism and culture conditions: Pure culture of *F. solani* was obtained from National Fungal Culture Collection of Pakistan (NFCCP), Department of Plant Pathology, University of Agriculture, Faisalabad. It was maintained on potato dextrose agar (PDA) slants at 4 °C. The microorganism was grown in a basal medium, buffered to pH 5.5, containing (g/L): glucose, 20.0; trisodium citrate, 2.5; KH_2PO_4, 5.0; NH_4NO_3, 2.0; $(NH_4)_2SO_4$, 4.0; $MgSO_4 \cdot 7H_2O$, 0.2 and trace element solution (g/L $CoCl_2$, 2; $MnSO_4 \cdot H_2O$, 1.6; $ZnSO_4 \cdot H_2O$,1.4 and $FeSO_4 \cdot 7H_2O$, 0.5) was added at 1 mL/L. Inocula were prepared by transferring spores from slants to 500 mL Erlenmeyer flasks containing 150 mL of the basal medium as reported earlier[22].

Solid-state fermentation: *F. solani* was grown under solid-state fermentation using orange peel as the substrate and conditions were optimized for enhanced production of pectinase. The solid state fermentation was carried out considering different parameters like initial moisture levels (40-80 %), initial pH (4-8), initial temperature (25-45 °C), amount of substrate (5-20 g/250 mL flask), inoculum size (10 %, containing 1×10^7 spores/mL),

incubation period (24-144 h) and various carbon (maltose, fructose, glucose and sucrose) and nitrogen (urea, peptone, cotton seed meal and yeast extract) additives (1 % w/v) affecting the production of pectinase. The conditions were optimized by adopting search technique varying parameters one at a time as reported earlier[7,23]. The solid state fermentation studies were conducted in 250 mL Erlenmeyer flasks containing 10 g of orange peel moistened with mineral salt solution (g/L; trisodium citrate, 2.5; KH_2PO_4, 5.0; NH_4NO_3, 2.0; $(NH_4)_2SO_4$, 4.0; $MgSO_4 \cdot 7H_2O$, 0.2) to 50 % moisture content. Flasks were plugged with cotton and sterilized by autoclaving for 15 min at 121 °C and 1.1 kg cm^{-2}. After sterilization, the flasks were cooled and inoculated with 10 % inoculum and incubated at 30 ± 1 °C for 72 h under various experimental conditions. The optimum conditions achieved by each step were fixed for subsequent studies.

Enzyme isolation: Isolation of the enzyme was carried out as described by Bhatti *et al.*[22]. The clear supernatant was used as source of enzyme activity.

Enzyme activity assay: Exopectinase (exo-polygalacuturonase) activity was determined by measuring the reducing groups released from the pectin solution using 3,5-dinitrosalicylic acid method as described by Miller[24]. D-Galacturonic acid monohydrate was used as the standard. A suitably diluted enzyme (0.1 mL) was added to reaction mixture containing 1 % pectin solution and 50 mM acetate buffer (pH 5.5). The reaction mixture was incubated at 40 °C for 0.5 h and terminating by adding 1 mL of 3,5-dinitrosalicylic acid followed by heating at 100 °C for 5 min. The reducing sugars formed in the solution were measured at 535 nm. One unit (U) of exopec-tinase was defined as the quantity of enzyme that liberates one micromole of galacturonic acid per minute at 40 °C and pH 5.5. Enzyme production in solid state fermentation was expressed in units per gram of dry substrate (U/g). All the experiments were conducted in triplicate and the results were reported as mean ± SD.

RESULTS AND DISCUSSION

Production of extracellular exopectinase was studied under solid-state fermentation by *F. solani* using orange peel as agro-industrial waste. Results regarding the effect of initial moisture contents are shown in Fig. 1. High enzyme activity (48.88 ± 2.69 U/g) was attained when the initial moisture level was 60 % in comparison with that at low or high moisture levels at 30 ± 1 °C for 72 h. Fig. 2 shows the effect of pH on the production of exopectinase. The enzyme production peaked between a pH of 5 and 7, with maximum activity of 61.56 ± 3.69 U/gds at 6 after 72 h. Data regarding the effect of temperature on the production of exopectinase is presented in Fig. 3. High enzyme titers (79.49 ± 5.17 U/g) are obtained at temperature

of 35 ± 1 °C. The effect of different amounts of orange peel on the production of exopectinase is shown in Fig. 4. It is obvious from the data that maximum enzyme activity (82.16 ± 4.93 U/g) was observed with 10 g of the substrate as compared to low or high amount at 35 ± 1°C.

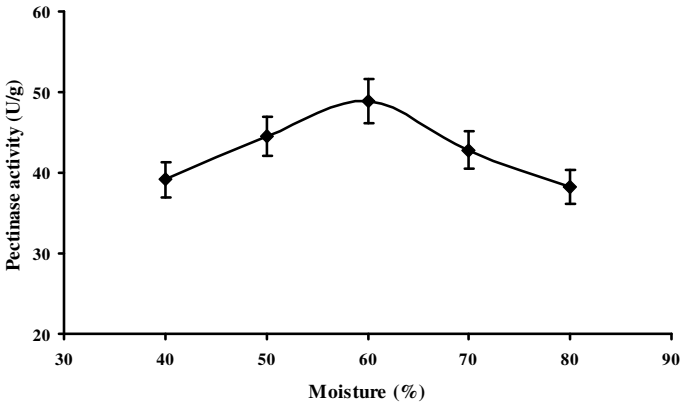

Fig. 1. Effect of moisture level on exopectinase production
Temperature, 30 ºC; pH, 5; incubation period, 72 h; amount of substrate, 10 g and inoculum size 10 %

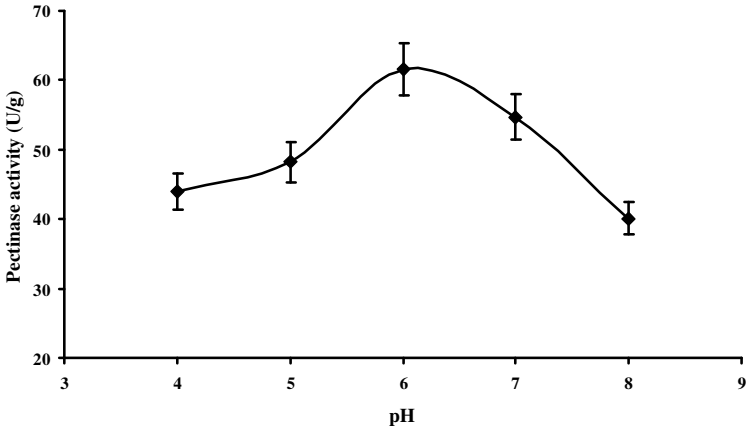

Fig. 2. Effect of pH on exopectinase production
Moisture, 60 %; pH, 5; incubation period, 72 h; amount of substrate, 10 g and inoculum size 10 %

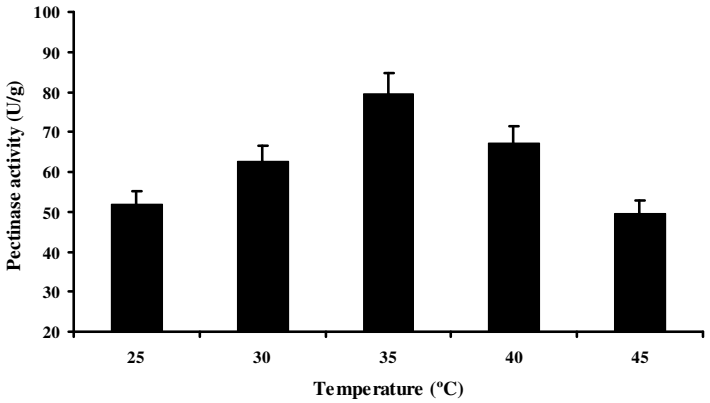

Fig. 3. Effect of incubation temperature on exopectinase production
Moisture, 60 %; pH, 6; incubation period, 72 h; amount of substrate, 10 g and inoculum size 10 %

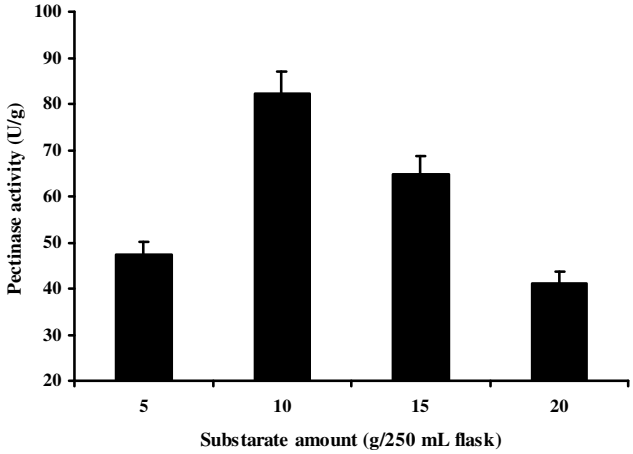

Fig. 4. Effect of amount of substrate on exopectinase production
Moisture, 60 %; pH, 6; incubation period, 72 h; incubation temperature, 35 °C and inoculum size 10 %

Production of pectinase was evaluated up to 144 h. A gradual increase in the production of enzyme over a period of 96 h was observed in solid state conditions (Fig. 5). After 96 h, it started to decrease with a value of 87.25 ± 5.24 U/g after 144 h. Orange peel was supplemented with different carbon sources such as glucose, maltose, sucrose and fructose separately to a final concentration of 1 % (w/v) in a solid media. Glucose and fructose were found to enhance pectinase production while maltose and sucrose inhibited yield of enzyme (Fig. 6). Finally various nitrogen sources were also employed to enhance the production of enzyme. It was observed that

all the sources tested *viz.*, urea, peptone, yeast extract and cotton seed meal, enhanced the production of exopectinase with maximum activity (120.23 ± 6.73 U/g) with peptone. The results are also shown in Fig. 6.

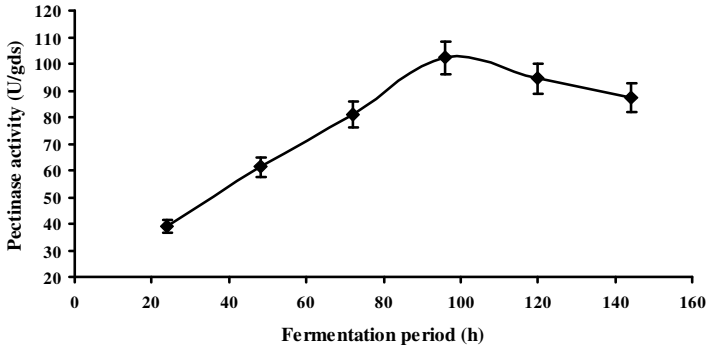

Fig. 5. Effect of incubation period on exopectinase production Moisture, 60 %; pH, 6; amount of substrate, 10 g; incubation temperature, 35 °C and inoculum size 10 %

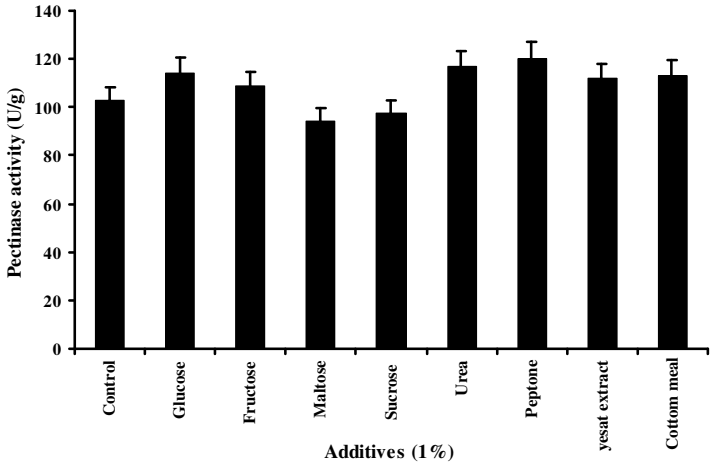

Fig.6. Effect of carbon and nitrogen additives on exopectinase production Moisture, 60 %; pH, 6; amount of substrate, 10 g; incubation temperature, 35 °C and inoculum size 10 %; additives (1 %)

The results reveal that variation of moisture content affects exopectinase production by *F. solani* using orange peel as the substrate. Moisture content of 50 and 70 % does not affect substantially the enzyme production (the difference was not statistically significant), whereas 60 % moisture was favourable to exopectinase production. According to Hours *et al.*[12], moisture

content of 85 % was ideal for the production of pectinase by *Aspergillus foetidus* using apple pomace as substrate. Highest pectinase activity was obtained with 66.7 and 80 % moisture using rice bran and apple pomace[25]. On the other hand, in the case of *Aspergillus niger*, better pectinase yield was obtained with 70 % moisture content[9] and an initial humidity level of 60 % was considered best for the production of pectinase by *Aspergillus awamori*[17]. Similarly a moisture levels of 65 and 70 % have been considered for better production of pectinases using grape pomace[7] and orange bagasse[21]. The present findings are in close agreement with those of Bladino *et al.*[17] and Botella *et al.*[7]. The significance of humidity level in solid state fermentation media and its influence on the biosynthesis and secretion of enzyme might be attributed to the interference of moisture in the physical properties of the substrate particles. High substrate moisture results in decreased substrate porosity, which in turn prevents oxygen penetration, alteration in particle structure, gummy texture and increased formation of aerial hyphae. At the same time, low moisture level lead to poor microbial growth and poor accessibility to nutrients, lower degree of swelling and high water retention[7,26].

The influence of pH on the production of exopectinase was significant. A wide range of pH from 2.3 to 7.2 affecting[19,27] the production of pectinases from different substrates by various microorganisms has been reported in the literature. Wide range of initial pH of the medium during the upstream bioprocess make the end product either acidic or alkaline, which tend to have varied applications[4]. Moreover, optimal pH is very important for growth of microorganism and its metabolic activities. As the metabolic activities of the microorganism are very sensitive to changes in pH, pectinase production by *F. solani* was affected if pH level was higher or lower compared to the optimum value. The results of incubation temperature on exopectinase production revealed that the variation in the temperature influenced the enzyme yield. Higher growth temperatures resulted in lower enzyme synthesis by mesophilic fungi. Incubation temperature is a critical process variable, which varies from organism to organism and slight changes in growth temperature may effect exopectinase production. At higher temperature, due to the production of large amount of metabolic heat, the fermenting substrate temperature shoots up thereby inhibiting microbial growth and enzyme formation[22,23].

Results regarding various amounts of orange peel, per unit volume, showed that there was statistically significant difference ($p < 0.05$) in the enzyme activity. The level of substrate per unit area of working volume of the flask influences the porosity and aeration of the substrate, which ultimately influences enzyme activities[23]. A gradual increase in the production of exopectinase was recorded up to 96 h in solid state fermentation. It is a

well-known fact that the incubation period depends on the nature of organism, nature of the fermenting material, concentration of the nutrients and physiological conditions. Generally the incubation time in synthetic media by pectinolytic fungi vary from 48-72 h. The optimized incubation period by several researches[13,16,21,28,29] indicate a wide range in solid-state fermentation (90-120 h). Results obtained in this study were similar to observe by Hours *et al.*[12]; Solis- Pereyra *et al.*[29]; Silva *et al.*[21] and Patil and Dayanand[13].

Addition of various carbon sources as additives on the production of exopectinase was examined. It was observed that the production of enzyme was influenced by glucose and fructose only while maltose and sucrose exhibited inhibitory effect. In case of nitrogen sources, all the additives enhanced the production of exopectinase using orange peel under solid state fermentation. The microorganisms require an adequate supply of carbon as energy source and nitrogen for various metabolic activities. The effect of various carbon and nitrogen sources are reported by several workers under solid state fermentation[7,8,13,18,25,28,29] indicating a wide range of concentrations.

Conclusion

Results of present investigation indicated that orange peel could be an attractive and promising substrate in solid-state fermentation for enhanced production of exopectinase by *F. solani*. Moisture content and addition of extra carbon and nitrogen sources were found to have exerted a marked influence on the yield of this industrial enzyme.

ACKNOWLEDGEMENTS

The authors thank the Chairperson, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan for providing necessary facilities to accomplish this work.

REFERENCES

1. L.R. Castilho, R.A. Medronho and T.L.M. Alves, *Bioresour. Technol.*, **71**, 45 (2000).
2. A. De-Gregorio, G. Mandalari, N. Arena, F. Nucita, M.M. Tripoda and R.B. Lo-Curto, *Bioresour. Technol.*, **83**, 89 (2002).
3. I. Reid and M. Ricard, *Enzyme Microb. Technol.*, **26**, 115 (2000).
4. G.S. Hoondal, R.P. Tiwari, R. Tewari, N. Dahiya and Q.K. Beg, *Appl. Microbiol. Biotechnol.*, **59**, 409 (2002).
5. G. Henriksson, D.E. Akin, R.J. Hanlin, C. Rodriguez, D. Archibald, L.L. Rigsby and K.E.L. Eriksson, *J. Appl. Environ. Microbiol.*, **63**, 3950 (1997).
6. M. Kapoor, Q.K. Beg, B. Bushan, K. Sing, K.S. Dadhich and G.S. Hoondal, *Process Biochem.*, **36**, 803 (2001).
7. C. Botella, A. Diaz, I. de-Ory, C. Webb and A. Blandino, *Process Biochem.*, **98**, 98 (2007).
8. V. Taragano, V.E. Sanchez and A.M.R. Pilosof, *Biotechnol. Lett.*, **19**, 233 (1997).

9. S. Solis-Pereyra, E. Favela-Torres, G. Viniegra-Gonzalez and M. Gutierrez-Rojas, *Appl. Microbiol. Biotechnol.*, **39**, 36 (1993).
10. F. Boccas, S. Roussos, M. Gutierrez, L. Serrano and G.G. Viniegra, *J. Food Sci. Technol.*, **31**, 22 (1994).
11. R.A. Larios, J.M. Garcia and C. Huitron, *Biotechnol. Lett.*, **11**, 729 (1989).
12. R.A. Hours, C.E. Voget and R.J. Ertola, *Biol. Waste*, **24**, 147 (1988).
13. S.R. Patil and A. Dayanand, *Bioresour. Technol.*, **97**, 2054 (2006).
14. A. Pandey, C.R. Soccol, P. Nigam, V.T. Soccol, L.P.S. Vandenberghe and R. Mohan *Bioresour. Technol.*, **74**, 81 (2000).
15. A. Pandey, *Biochem. Eng. J.*, **13**, 81 (2003).
16. A. Pandey, *Bioresour. Technol.*, **37**, 169 (1991).
17. A. Blandino, T. Iqbalsyah, S.S. Pandiella, D. Cantero and C. Webb, *Appl. Microbiol. Biotechnol.*, **58**, 164 (2002).
18. M.F.S. Teixeria, J.L.L. Filho and N. Duran, *Braz. J. Microbiol.*, **31**, 286 (2000).
19. F.C. Sebastian, A.A. Jorge and A.H. Roque, *Biotechnol. Lett.*, **18**, 251 (1996).
20. N. Hadj-Taieb, M. Ayadi, S. Trigui, F. Bouabdallah and A. Gargouri, *Enzyme Microb. Technol.*, **30**, 662 (2002).
21. D. Silva, K. Tokuioshi, E. Martins, R.D. Silva and E. Gomes, *Process Biochem.*, **40**, 2885 (2005).
22. H.N. Bhatti, M.H. Rashid, R. Nawaz, M. Asgher, R. Perveen and A. Jabbar, *Food Technol. Biotechnol.*, **45**, 51 (2007).
23. P. Ellaiah, K. Adinarayana, Y. Bharani, P. Padmaja and B. Srinivasulu, *Process Biochem.*, **38**, 615 (2002).
24. G.L. Miller, *Anal. Chem.*, **31**, 426 (1959).
25. D.R. Kashayap, S.K. Soni and R. Tewari, *Bioresour. Technol.*, **88**, 251 (2003).
26. A. Pandey, *Process Biochem.*, **27**, 109 (1992).
27. A.F. Parley and O.T. Page, *Can. J. Microbiol.*, **17**, 415 (1971).
28. P.D. Shivakumar and Krishnanand, *Lett. Appl. Microbiol.*, **20**, 117 (1995).
29. S. Solis-Pereyra, E. Favela-Torres, M. Gutierrez-Rojas, S. Roussos, G. Saucedo-Castaneda, P. Gunasekaran and G. Viniegra-Gonzalez, *World J. Microbiol. Biotechnol.*, **12**, 257 (1996).

(*Received*: 12 April 2007; *Accepted*: 25 February 2008) AJC-6380