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SHORT COMMUNICATION

UTILIZATION OF APPLE PEELS FOR THE PRODUCTION OF PLANT CELL-WALL DEGRADING ENZYMES BY *ASPERGILLUS FUMIGATUS* MS16

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ABSTRACT

Apple (*Malusdomesticaborkh*) is one of the popular fruit with an annual global production of several million tones. The use of apple in food production industry led to the generation of huge waste materials in the form of peels and pomace. Owing to the high amounts of lignocellulosics present in the wastes, these can be served as substrate for the production of plant cell-wall degrading enzymes (CWDE). The present study describes the potential of an indigenously isolated strain of *Aspergillusfumigatus*to produce plant CWDE using apple peels under submerged as well solid-state fermentation. It was observed that the strain was able to produce higher activity of pectinase and xylanase under both the types of fermentation at 30^{o} as well as at 40^{o}C. While less titers of filter paperase, endoglucanase, β-glucosidase was obtained. However, the production of plant CWDE was influenced when the apple peels were supplemented with carboxymethyl cellulose, xylan, pectin and lactose. Interestingly, a structurally irrelevant substrate, lactose, acted as inducer, particularly for the production of pectinase.

Key words: Apple peels, *Aspergillusfumigatus*, Pectinase, Xylanase.

INTRODUCTION

Plant cell-wall, comprised of lignin, cellulose and hemicelluloses is regarded as a renewable chemical feedstock (Kumar *et al.* 2008). However, the presence of diverse components renders it stable and its biodegradation requires activity of a number of enzymes. Consequently, the production of plant cell-wall degrading enzymes (CWDE) remains a subject of current research. Amongst plant CWDE much of the studies have been conducted on the production of cellulase, xylanase and pectinase because of the multitude of the applications of these enzymes (Ghorai*et al.* 2009). Cellulase (endoglucanase, exoglucanase, β-glucosidase, together) degrade the most abundant polymer found in plant cell-wall, cellulose to monomers; whereas, xylanase and pectinase act upon constituents of hemicelluloses, xylan and pectin, respectively. Although a number of bacterial species have been reported to produce plant CWDE but fungal enzymes are commercially employed. Because of hyphal mode of growth, fungi can penetrate crude substrates deeply and can elaborate an array of hydrolytic enzymes. Therefore, crude substrates including fruit and vegetable wastes are commonly used for the production of these enzymes (Ghorai*et al.* 2009).

Apple (*Malusdomesticaborkh.*) is one of the popular fruit with an annual global production of around 59.21 million tonnes (Mahawar*et al.* 2012). From this production, more than 80% is consumed as table fruit while rest is utilized for value added products including fruit juice production which lead to the generation of huge waste material in the form of peels and pomace. These wastes are rich in lignocellulosics and can serve as substrate for the production of plant CWDE. Whereas a number of literature reports describe potential utility of apple pomace (Mahawar*et al.* 2012), prospects of utilizing its peels, particularly for the production of fungal enzymes, have scarcely been reported (Berovic' and Ostrovers'nik 1997; Mahawar*et al.* 2012). Present study describes the utilization of apple peels (AP) for the production of plant CWDE from *Aspergillusfumigatus* MS16 strain under submerged and solid-state cultivation.

MATERIALS AND METHODS

*A. fumigatus*MS16 was retrieved from the culture collection of the department of Microbiology, University of Karachi, Pakistan. The strain was selected because of its ability to produce a number of hydrolytic enzymes on commercially purified substrates (Sohail*et al.* 2009b). Initially, the strain was cultivated in Mandel's medium (Mandelsand Weber 1969) containing different substrates (Table 1) to deduce the induction of a particular CWDE by the substrates. After removing fungal mass, cell-free culture supernatant (CFCS) was used as crude enzyme preparation. Crude enzyme preparation was analyzed for endoglucanase, β-glucosidase, filter paperase, xylanase and pectinase activity by adopting the methods described elsewhere (Miller, 1959, Sohail*et al.* 2009a, b, 2011). One IU of the enzyme was defined as the micromoles of reducing sugars (glucose, xylose or galacturonic acid) liberated from the suitable substrate in one min under standard assay conditions.

For the fermentation experiments, apple peels (AP) were collected from a local fruit juice seller, cut into small pieces, crushed and sun dried. The particle size of the peels was maintained by sieving through 100 mesh. The peels (1% w/v) were supplemented in MSM, for submerged fermentation (Smf). Spore suspension (5 x 10^6 spores/g of substrate) of *A. fumigates* MS16 was transferred to the flasks containing MSM supplemented with AP and incubated for 8 days at suitable temperature. CFCS was obtained after centrifugation at 5000 g for 20 min and used as crude enzyme preparation.

For solid state fermentation (SSF), 2 grams of the AP were placed in 250 ml Erlenmeyer flasks and autoclaved for 30 min. After cooling to room temperature it was moistened with MSM without carbon source to 65% moisture level. Fungal spore suspension was inoculated at 5 x 10^6 spores/g of substrate and incubated at suitable temperature for 8 days. This was followed by an addition of 50 ml of sterile sodium acetate buffer (50 mM; pH 4.8) containing 0.02% Tween 80 to each flask and agitated at 150 rpm for 2 hours. The slurry was filtered through four layers of muslin cloth and filter paper. The filtrate was centrifuged at 5000 g for 20 min and used as crude enzyme preparation.

In all fermentation experiments either, 0.5% w/v of carboxymethyl cellulose (CMC), pectin, xylanor lactose were supplemented separately in mineral salt medium to study the effect of additional carbon source on the production of plant CWDE.

RESULTS AND DISCUSSION

The data suggests that*A. fumigates* when grown in MSM supplemented with commercially purified carbon sources was able to produce cellulase (β-glucosidase, endoglucanase& filter-paperase), xylanase and pectinase (Table 1); however, the expression of these enzymes varied with the nature of substrates. For instance, significant titers of cellulases were observed when the medium was supplemented with Avicel, Sigmacell or carboxymethyl cellulose (CMC); pectin and xylan induced the production of pectinase and xylanase, respectively. However, a co-expression of xylanase was also observed in cellulose containing medium and induction of cellulase was noted in presence of xylan. Interestingly, lactose, a disaccharide that is not a constituent of plant cell-wall, was found as a better inducer of plant CWDE, particularly, forpectinase and xylanase. A previous report also described the role of lactose as inducer for endoglucanase and β-glucosidase (Seiboth *et al.* 2005), particularly when apple pomace was supplemented with lactose under SmF using *Trichoderma sp*. GIM 3. 0010 (Sun *et al.* 2010). Based on these results, lactose, cellulose, pectin and xylanwere used as inducers in fermentation of AP.

The most important factor in fermentation is the choice of the substrate (Pandey *et al.* 2000). Since, AP has not been widely used as a substrate for the production of fungal enzymes, so the main objective of the study was to explore the utility of this substrate for the production of enzymes under submerged as well solid-state conditions. Results indicate that submerged fermentation (Smf) of AP at 30^{o}C yielded higher titers of β-glucosidase, pectinase and xylanase (Table 2) which indicatethat AP contains sufficient nutrients for the growth of fungi and production of industrially important enzymes. Furthermore, since the preparation is rich in xylanase and pectinase activities, it can be supplemented in animal-feed to improve utilization of high-forage diet (Ghorai*et al.* 2009, Jayani*et al.* 2005). It was also noted that the production of pectinase was enhanced when AP was supplemented with pectin or lactose.

The submerged fermentation of AP at 40^{o}C gave more amounts of β-glucosidase compared to at 30^{o}C and the activity of this enzyme significantly increased when AP was supplemented with CMC. It may be due to the fact that the higher temperature facilitates hydrolysis of biological waste (Veeken and Hamelers, 1999) and hence the higher production of enzyme.

An earlier report suggests that the production of pectinase by *A. niger* under SSF fermentation of apple pomace is advantageous as it yields concentrated product with less effluent (Berovic' and Ostrovers'nik, 1997). The results of the present study under SSF revealed that AP is a good substrate for the production of pectinase at 30^o and 40^{o}C (Table 3). The production of pectinase at 40^{o}C is of some significance as there are very few reports suggesting that pectinases show optimum activity at temperatures more than 50^{o}C (Gummadi and Panda 2003). It was noted that thexylanase production was decreased when the fermentation temperature was increased from 30^o to 40^{o}C, while the production of β-glucosidase remained unaffected.

The supplementation of AP with CMC resulted in an up-regulation of β-glucosidase, pectinase and xylanase production when the SSF was carried out at 30^{o}C. Similar finding was also made by Sun *et al.* (2010) where supplementation of CMC to apple pomace enhanced the production of endoglucanase and β-glucosidase by *Trichodermasp.* GIM 3.0010 under SSF at 30°C. Mamma *et al.* (2008) reported about the production of more amounts of pectinase compared to xylanase or cellulase while studying the multienzyme preparation of *A. niger*during SSF of orange-peels. Similar finding was made by Botella *et al.* (2005) in SSF of grape pomace by *A. awamori,* higher titers of pectinase and xylanase were obtained while cellulase production was inhibited.

Table 1. Production of plant cell wall degrading enzymes in presence of commercially purified substrates supplemented to Mineral salt medium.

Substrates	Enzymes (IU/ml)				
	β-glucosidase	**Endoglucanase**	**Filterpaperase**	**Pectinase**	**Xylanases**
Glucose	0	0	0.016 ± 0.008	0.0638 ± 0.008	0.051 ± 0.089
Lactose	0.057 ± 0.039	0.028 ± 0.009	0.003 ± 0.006	0.664 ± 0.183	0.142 ± 0.135
Maltose	0.057 ± 0.039	0.007 ± 0.012	0.010 ± 0.011	0.372 ± 0.322	0.109 ± 0.095
Sucrose	0	0.128 ± 0.031	0.010 ± 0.010	0.403 ± 0.032	0.342 ± 0.069
Avicel	0.054 ± 0.047	0.030 ± 0.004	0.025 ± 0.006	0.424 ± 0.071	3.179 ± 1.888
Starch	0	0.074 ± 0.074	0.009 ± 0.007	0.471 ± 0.122	0.149 ± 0.133
Pectin	0.107 ± 0.014	0.039 ± 0.010	0.006 ± 0.002	11.095± 0.522	0.114 ± 0.099
Sigma cell	0.023 ± 0.027	0.041 ± 0.020	0.016 ± 0.006	0.827 ± 0.167	1.349 ± 1.599
Carboxy Methyl cellulose	0.043 ± 0.018	0.180 ± 0.006	0	0.680 ± 0.047	0.114 ± 0.066
Cellulose	0	0.013 ± 0.022	0.018 ± 0.016	0.119 ± 0.190	0.141 ± 0.122
Xylan from oat spelt	0.102 ± 0.060	0.096 ± 0.081	0.027 ± 0.016	0.236 ± 0.407	0.266 ± 0.091

(Values followed by + indicates standard deviation)

Table 2.Production of -glucosidase (BGL), endoglucanase (EG), filterpaperase (FP, pectinase and xylanase by *A. fumigatus*MS16 through submerged fermentation of apple peels (AP) with and without supplementation of pectin, lactose, carboxymethyl cellulose (CMC) and xylan.

MSM supplemented with	Enzyme production									
	30°C					40°C				
	BGL	EG	FP	Pectinase	Xylanase	BGL	EG	FP	Pectinase	Xylanase
AP	0.95± 0.01	0.05± 0.003	0.04±0	1.12± 0.05	0.62± 0.02	0.1± 0.055	0.005	0.05± 0	1.0± 0.03	0.005±0
AP±CMC	0.05±0	0.08±0	0.01±0	0.6± 0.08	0.2± 0.07	0.35±0.066	0.05± 0	0.005±0 0	0.42± 0.066	1.5± 0.18
AP±Pectin	0.05±0	0.001±0	0.008±0	5.5± 0.47	1.25± 0.095	0.01±0	0.02± 0.08	0.005± 0	5.0± 0.14	0.5± 0.07
AP±Lactose	0.005± 0	0.01 ±0.02	0.04±0	3.0± 0.15	0.15± 0	0.17±0.05	0.005± 00	0.01±0	2.8± 0.06	0.43± 0.02
AP±Xylan	0.1± 0.02	0.07± 0.04	0.02±0	0.75± 0.08	1.75± 0.21	0.15±0.08	0.02± 0	0.01±0	0.65± 0.3	1.7± 0.05

(All the experiments were conducted in triplicates and mean values are represented)

Table 3.Production of -glucosidase (BGL), endoglucanase (EG), filterpaperase (FP, pectinase and xylanase by *A. fumigatus*MS16 through SSF of apple peels (AP) with and without supplementation of pectin, lactose, carboxymethyl cellulose (CMC) and xylan.

MSM supplemented with	Enzyme production at									
	30°C					40°C				
	BGL	EG	FP	Pectinase	Xylanase	BGL	EG	FP	Pectinase	Xylanase
AP	0.1± 0.05	0.08±0	0.005±0	0.65± 0.04	0.1± 0.08	0.1± 0.01	0.005±0	0.05±0	1.0± 0.07	0.005±0
AP±CMC	0.21± 0.01	0.09± 0.006	0.01±0	0.72± 0.1	0.12±0	0.04±0	0.06±0	0.1±0	0.38± 0.06	0.005±0
AP±Pectin	0.001±0	0.001±0	0.001±0	0.3± 0.02	0.05±0	0.005±0	0.005±00	0.005±0	0.005±0	0.005±0
AP±Lactose	0.05± 0	0.08±0	0.005±0	0.8± 0.06	0.05±0	0.01±0	0.012±00	0.012±0	0.005±0	0.025± 0
AP±Xylan	0.15± 0.02	0.09±0	0.01±0	0.51± 0.08	0.25± 0.01	0.03±0	0.05±0	0.08±0	0.45± 0.05	0.31±.08

(All the experiments were conducted in triplicates and mean values are represented)

Conclusion: Apple peel can be a good source of substrate for the production of cell-wall degrading enzymes.

REFERENCES

Berovic', M. and H. Ostrovers' nik (1997). Production of *Aspergillusniger*pectolytic enzymes by solid state bioprocessing of apple pomace. J. Biotechnol. 53: 47–53.

Botella, C. I. de Orya, C. Webb, D. Cantero and A. Blandino (2005). Hydrolytic enzyme production by *Aspergillusawamori*on grape pomace. Biochem. Engg. J. 26: 100–106.

Ghorai, S., S.P. Banik, D. Verma, S. Chowdhury, S. Mukherjee and S. Khowala (2009). Fungal biotechnology in food and feed processing. Food Res. Int. 42: 577–587.

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

Jayani, R.S., S. Saxena and R. Gupta (2005). Microbial pectinolytic enzymes: A review. Process Biochem. 40(99): 2931-2944.

Kumar, R. S. Singh and O.V. Singh (2008). Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. J. Ind. Microbiol. Biotechnol. 35:377–391.

Mahawar, M., A. Singh and K. Jalgaonkar (2012). Utility of apple pomace as a substrate for various products: A review. Food Bioproducts Process. 90:597–605 .

Mamma, D., E. Kourtoglou and P. Christakopoulos (2008). Fungal multienzyme production on industrial by-products of the citrus-processing industry. Bioresour. Technol. 99: 2373–2383.

Mandels, M. and J. Weber (1969). The production of cellulases and their applications. Adv. Chem. Ser., 95: 391-414.

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

Pandey, A., C. R. Soccol and D. Mitchell (2000). New developments in solid state fermentation: I-bioprocesses and products. Process Biochem. 35: 1153–1169.

Seiboth, B., H. Lukas, N. Salovuori, L. Karin, D.G. Robson, J. Vehmaanperä, M.E. Penttilä and C.P. Kubicek (2005). Role of the bga1-encoded extracellular β-galactosidase of *Hypocreajecorina* in cellulase induction by lactose. Appl. Environ. Microbiol. 71:851–857.

Sohail, M., A. Ahmad and S.A. Khan (2011). Production of cellulases from *Alternaria* sp. MS28 and their partial characterization. Pak J Bot. 43:3001-3006.

Sohail, M., R. Siddiqi, A. Ahmad and S.A. Khan (2009b). Cellulase production from *Aspergillusniger*MS82: Effect of temperature and pH. New Biotechnol. 25:437-441.

Sohail, M., S. Naseeb, S. Sherwani, S. Aftab, S. Sultana, S. Shehzad, A. Ahmad and S.A. Khan (2009a). Distribution of hydrolytic enzymes in native fungi: *Aspergillus*the predominant genus of hydrolase producers. Pak J. Bot. 41: 2567-2582.

Sun, H., X. Ge, Z. Hao and M. Peng (2010). Cellulase production by *Trichoderma*sp. on apple pomace under solid state fermentation. Afr. J. Biotechnol. 9: 163-166.

Veeken, A. and B. Hamelers (1999). Effect of temperature on hydrolysis rates of selected biowaste components. Bioresour. Technol. 69: 249-254.