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Solid State Production of Polygalacturonase and Xylanase by *Trichoderma* Species Using Cantaloupe and Watermelon Rinds

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Different solid state fermentation (SSF) sources were tested such as cantaloupe and watermelon rinds, orange and banana peels, for the production of polygalacturonase (PG) and xylanase (Xyl) by *Trichoderma harzianum* and *Trichoderma virens*. The maximum production of both PG and Xyl were obtained by *T. harzianum* and *T. virnes* grown on cantaloupe and watermelon rinds, respectively. Time course, moisture content, temperature, pH, supplementation with carbon and nitrogen sources were optimized to achieve the maximum production of both PG and Xyl of *T. harzianum* and *T. virens* using cantaloupe and watermelon rinds, respectively. The maximum production of PG and Xyl of *T. harzianum* and *T. virens* was recorded at 4–5 days of incubation, 50–66% moisture, temperature 28–35°C and pH 6–7. The influence of supplementary carbon and nitrogen sources was studied. For *T. harzianum*, lactose enhanced PG activity from 87 to 120 units/g solid, where starch and maltose enhanced Xyl activity from 40 to 55–60 units/g solid for *T. virnes*. Among the nitrogen sources, ammonium sulphate, ammonium nitrate, yeast extract and urea increased PG activity from 90 to 110–113 units/g solid for *T. harzianum*. Similarly, ammonium chloride, ammonium sulphate and yeast extract increased Xyl activity from 45 to 55–70 units/g solid for *T. virens*.

Keywords: cantaloupe, watermelon, rind, *Trichoderma*, solid fermentation

Introduction

Recently, a significant interest in using solid state fermentation (SSF) instead of submerged fermentation (SmF). The advantages of SSF in comparison to traditional SmF are better yields, easier recovery of products, the absence of foam formation and smaller reactor volumes. Moreover, contamination risks are significantly reduced due to the low water contents and, consequently, the volume of effluents decreases (Raimbault, 1998). Another very important advantage is that, it permits the use of agricultural and agro-industrial residues as substrates which are converted into products with high commercial value like secondary metabolites, organic acids, pecticides, aromatic compounds, fuels, and enzymes (Martins *et al*., 2002). Furthermore, the utilization of these compounds helps in solving pollution, which otherwise cause their disposal (Couto and Sanroman, 2005). For enzyme production, the costs of these techniques are lower and the production higher than submerged cultures (Pandey, 1991; Sukumaran *et al*., 2009).

The polygalacturonases catalyze the hydrolytic cleavage of the O-glycosyl bond of α-D-(1-4)polygalacturonan. The pattern of degradation proceeds in either a random (endo-polygalacturonase, EC3.2.1.15) or terminal fashion (exo-polygalacturonase, EC 3.2.1.67). Xylanases (EC.3.2.1.8) are responsible for hydrolysis of xylan; they first attack the internal main-chain linkages and subsequently releasing xylosyl residues by endwise attack of xylooligosaccharides. These enzymes are commonly used in textile, paper, and pulp industries, production of juice and fruit extracts (Silva *et al*., 2002; Gonzales *et al*., 2003; Couto and Sanroman, 2005). Polygalacturonase (PG) and xylanase (Xyl) production by SSF has been studied extensively using several agricultural and agro-industrial residues such as wheat, corn, rice, sugar cane and beet, banana waste, potato, tea, apple, and citrus fruits, wheat flours and corn (Botella *et al*., 2007; Mamma *et al*., 2008; Sun *et al*., 2008; Rodriguez-Fernandez *et al*., 2011; Delabona *et al*., 2013). However, watermelon and cantaloupe rinds have never been used as solid support for enzyme production. Watermelon biomass can be categorized as three main components which are the flesh, seed, and rind. The flesh constitutes approximately 68% of the total weight, the rind approximately 30%, and the seeds approximately 2% (Kumar, 1985). Singh *et al*. (1975) determined the rind of fully ripened watermelon to contain approximately 20% cellulose, 23% hemicellulose, 10% lignin, 13% pectin. Several possibilities exist for the use of watermelon rind to produce value-added products. The USDA ARS is currently processing a patent to utilize extracted rind citrulline, an amino acid that helps to remove nitrogen from the blood for conversion to urine (Pons, 2003). Other research has been conducted on the utilization of the rind as an ingredient in products including pickle, candy, vadiyam, and cheese (Simonne *et al*., 2002; Madhuri and Devi, 2003).

Trichoderma harzianum is an efficient biocontrol agent

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that is commercially produced to prevent development of several soil pathogenic fungi. The active ingredient is a beneficial microbe that does not cause disease to humans and is not likely to harm the environment (Roco and Pérez, 2001). In addition, *T. harzianum* and *T. reesi* widely used in the production of Xyl and PG with SSF on several solid supports (Seyis and Aksoz, 2005; Azin *et al.*, 2007).

The main purpose of this study is using watermelon and cantaloupe rinds, with high hemicellulose and pectin contents, as new agricultural wastes for production of PG and Xyl from *T. harzianum* (CECT 2413) and *T. virens* (ATCC 52571) with SSF. However, very little information has been reported on production of Xyl and PG from *T. virnes*. The optimization of the physiological conditions of production of PG and Xyl from *T. harzianum* and *T. virens* using cantaloupe and watermelon rinds is the last goal.

Materials and Methods

Microorganism

T. herzianum and *T. virnes* were obtained from National Research Centre, Cairo, Egypt and maintained on potato dextrose agar. The slants were grown at 28°C for seven days and stored at 4°C.

Agriculture wastes

Watermelon and cantaloupe rinds, orange and banana peels were chosen as the sole nutrient source for solid-state fermentation (SSF). They dried in an oven at 60°C for 48 h. The solid was then milled in a commercial mill and sieved. The mean diameter of the solid was 0.7 mm.

Optimization methodology of solid-state fermentation (SSF)

SSF was performed to study the effect of various physico-chemical parameters required for the optimum production of PG and Xyl by *T. herzianum* and *T. virnes*. Prior to inoculation, the agriculture waste was sterilized in an autoclave for 15 min at 120°C and 1.2 atmospheres. To each 100 ml Erlenmeyer flask, 5 g of sterilized agriculture waste, the required volume of spore suspension to obtain a final spore concentration of 5×10^5 spores/g, and appropriate amount of water need to adjust the moisture of dried substrate, which contained 10% moisture after dried, to the desired level were added. Incubation time (1 to 7 day), incubation temperature (20, 30, 35, 40, 45°C), moisture content of the substrate (30, 40, 50, 66, 80%) and incubation pH (4 to 8) are the physico-chemical parameters optimized. The pH was adjusted using 0.1 M NaOH or HCl. Studies were also performed to evaluate the influence of different carbon sources (glucose, maltose, starch, sucrose, lactose at 1% w/v) and nitrogen sources (peptone, urea, sodium nitrate, ammonium sulphate, ammonium nitrate at 1% w/v) when add to the fermentation medium contained agriculture waste. Each experiment is done in three sets.

Enzyme extraction

Crude enzyme was extracted by mixing a 5 g of fermented matter with 50 ml distilled water on a rotary shaker (180 rpm/min) overnight. The suspension is then centrifuged at 7,000 rpm for 10 min and the supernatant is designated as a crude extract.

Enzyme assays

PG and Xyl activities were assayed by determining the liberated reducing end products using galacturonic acid and xylose as standards, respectively (Miller, 1959). The reaction mixture (0.5 ml) contained 1% substrate, 0.05 M sodium acetate buffer pH 5.5 and a suitable amount of crude extract. Assays were carried out at 37°C for 1 h. Then 0.5 ml dinitrosalicylic acid reagent was added to each tube. The tubes were heated in a boiling water bath for 10 min. After cooling to room temperature, the absorbance was measured at 560 nm. Substrates used are polygalacturonic acid and birchwood xylan for PG and Xyl, respectively. One unit of enzyme activity is defined as the amount of enzyme which liberated one µmol of reducing sugar per min under standard assay conditions.

Results and Discussion

The effect of different substrates on PG and Xyl production

Different SSF sources such as orange and banana peels, cantaloupe and watermelon rinds are tested in SSF for the production of PG and Xyl by *T. harzianum* and *T. virens*. Figure 1 showed that the maximum production of both PG

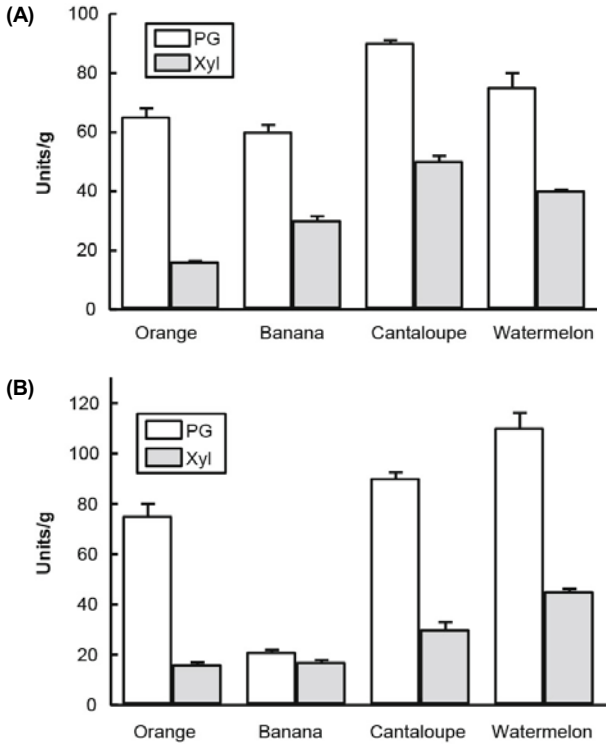

Fig. 1. The effect of different fruit peels and rinds on production of PG and Xyl by *T. harzianum* (A) and *T. virnes* (B) in SSF.

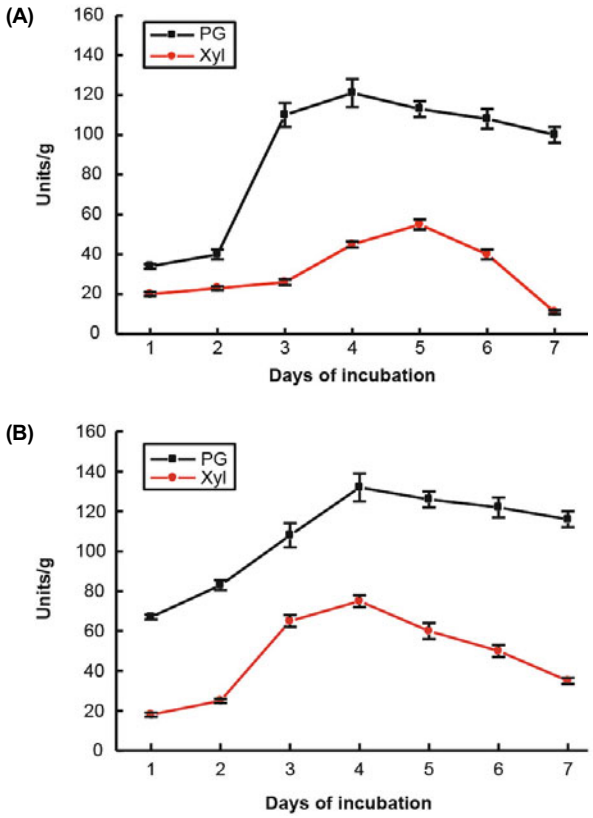

Fig. 2. The effect of incubation time on production of both PG and Xyl in SSF by *T. harzianum* using cantaloupe (A) and *T. virnes* using watermelon (B) rinds as substrates, respectively.

and Xyl were obtained by *T. harzianum* (90 and 50 units/g solid, respectively) and *T. virnes* (110 and 45 units/g solid, respectively) in SSF containing cantaloupe and watermelon rinds, respectively. Very little information has been reported on the use of fruit rinds in SSF for enzyme production. Therefore, the optimization of the production of both PG and Xyl of *T. harzianum* and *T. virens* using cantaloupe and watermelon rinds, respectively, was performed in the following studies.

The effect of incubation time on PG and Xyl production

Figure 2 shows the time course experiments of both PG and Xyl production by *T. harzianum* and *T. virens* grown on cantaloupe and watermelon rinds in SSF, respectively. For *T. harzianum*, a gradual increase was detected in PG and Xyl production from day 1 to day 4 and 5 (121 and 55 units/g solid, respectively), after which a reduction of its activity was observed. For *T. virnes*, the two enzymes exhibited their maximum activity at day 4 (132 and 75 units/g solid, respectively). Similarly, *Penicillium decumbens* was grown on a mixture of corn straw (90%) and wheat bran (10%) the maximum activity of xylanase was measured after 4 days of fermentation (Yang *et al.*, 2001). Couri *et al.* (2000) also studied the production of xylanase by *A. niger* using different agroindustrial residues – mango peel and wheat bran – as the solid substrate. The maximum xylanase activity was reached after 72 h and 24 h of fermentation using wheat bran and mango peel, respectively. In the same line, the production of xylan-degrading enzymes by a koji mold, *Aspergillus oryzae* RIB 128, has been tested on dried wheat bran, rice bran and orange peel (Yamane *et al.*, 2002). The highest productivity was reached when wheat bran was used as the substrate after 4 days of fermentation. Similar results were reported for pectinase from *A. sojae* in SSF, where the maximum activity was reached at day 3 and day 5 of cultivation (Heerd *et al.*, 2012). On contrast, the xylanase and polygalacturonase activities of *A. awamori* grown on grape pomace in SSF reached a maximum value after 24 h of fermentation after which a reduction of its activity was observed (Botella *et al.*, 2007).

The effect of initial moisture content of substrate on PG and Xyl production

Moisture content is a crucial factor in SSF that influences the growth of the microorganism and thereby enzyme production. Higher moisture content decreases porosity, changes particle structure, reduces gas volume and decreases diffusion, which results in lowered oxygen transfer (Sivaramakrishnan *et al.*, 2006). On the contrary, lower moisture content causes reduction in solubility of the nutrients of the substrate, low degree of swelling and high water tension. Figure 3 shows the effect of moisture content on both PG

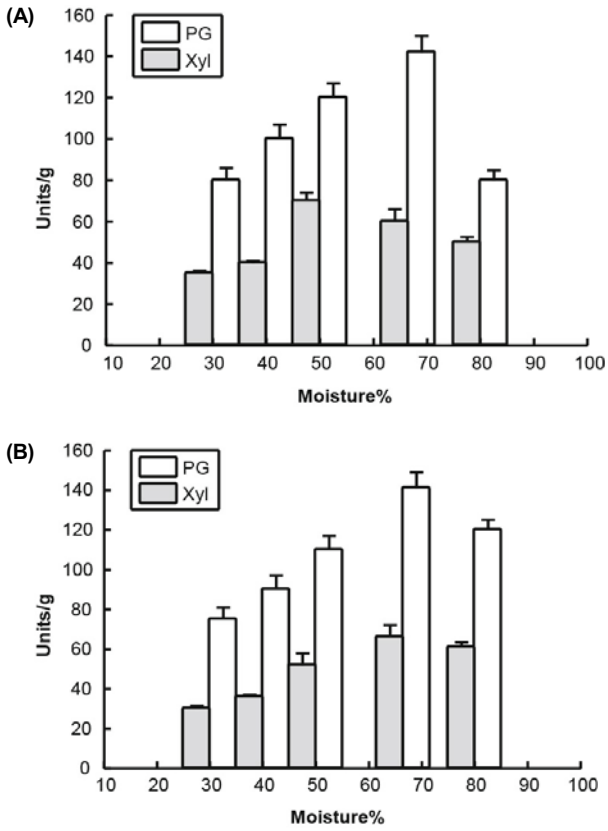

Fig. 3. The effect of moisture% of cantaloupe (A) and watermelon (B) rinds on production of both PG and Xyl by *T. harzianum* and *T. virnes* in SSF, respectively.

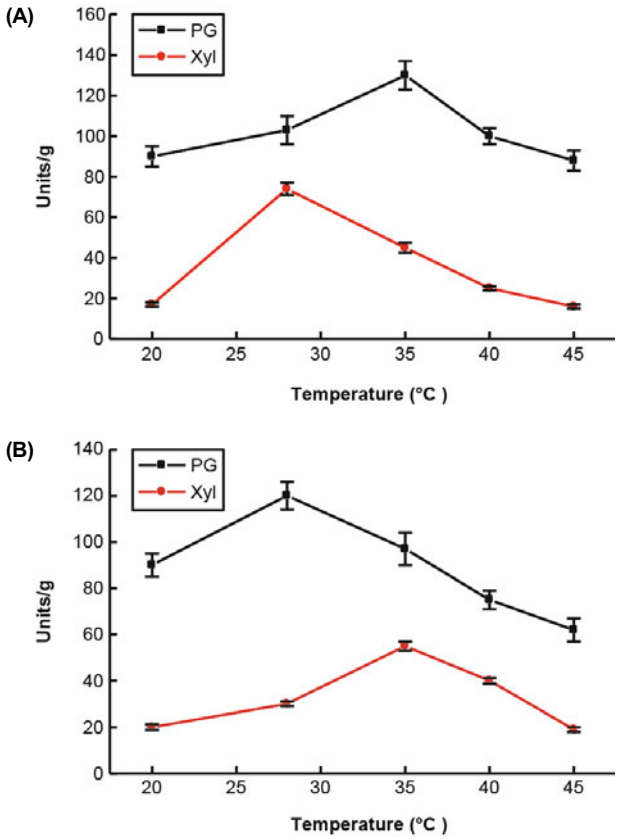

Fig. 4. The effect of incubation temperature on production of both PG and Xyl by *T. harzianum* (A) and *T. virnes* (B) in SSF using cantaloupe and watermelon rinds as substrates, respectively.

and Xyl production by *T. harzianum* and *T. virens* grown on cantaloupe and watermelon rinds in SSF, respectively. Optimal PG and Xyl production was obtained at 66 and 50% moisture content of cantaloupe (142, 70 units/g solid, respectively) (Fig. 3A) and 66% moisture content of watermelon (141, 52 units/g solid, respectively) (Fig. 3B). Similarly, both enzyme activities of *Aspergillus awamori* cultured on grape pomace were less low when the moisture content was higher or lower than 65% (Botella *et al.*, 2007). The optimum moisture contents for xylanase production by *T. longibrachiatum* and *A. tereus* were 55 and 75%, respectively (Gervais and Melon, 2003). A high production of xylanase of *Aspergillus* species was detected at 40–50% moisture using dry koji as substrate (Lu *et al.*, 2003). An initial moisture content of 40% provided better conditions for production of pectinases from *A. niger* than those of 25, 55, and 70% (Castilho *et al.*, 2000).

The effect of temperature on PG and Xyl production

Temperature is known to influence the metabolic rate of the organism involved in the fermentation processes. The effect of temperature on both PG and Xyl production by *T. harzianum* and *T. virens* grown on cantaloupe and watermelon rinds in SSF, respectively, is shown in Fig. 4. Optimal PG and Xyl production (130 and 74 units/g solid, respectively) was obtained at 35°C and 28°C for *T. harzianum*, respectively. On the contrary, the maximum activity of PG and Xyl (120 and 55 units/g solid, respectively) of *T. virens* was detected at 28°C and 35°C, respectively. Similar optimal temperatures of production of PG and Xyl from *Penicillium decumbens* (Yang *et al.*, 2001), *A. niger* (Couri *et al.*, 2000), *A. oryzae* (Yamane *et al.*, 2002), and *A. awamori* (Botella *et al.*, 2007) were ranged from 28°C to 32°C. In the steady-state operation for production of xylanase by *A. niger* the optimum temperature is 28°C. Results show that higher incubation temperature favors biomass growth and lower temperature favors the biosynthesis of xylanase (Yuan *et al.*, 2005). The maximal polygalacturonase production by mixed culture of *A. niger* and *Saccharomyces cerevisiae* was detected at 37°C (Zhou *et al.*, 2011).

The effect of pH on PG and Xyl production

The effect of initial pH on PG and Xyl production by *T. harzianum* and *T. virens* grown on cantaloupe and watermelon rinds in SSF, respectively, is shown in Fig. 5. Optimal PG and Xyl production of *T. harzianum* was obtained at pH 7 and 6 with 120 and 70 units/g solid, respectively. On the contrary, the maximum activity of PG and Xyl of *T. virens* was detected at pH 6.0 and 7.0 with 140 and 60 units/g solid, respectively. At the medium pH (6.0), the maximal xylanase production by *A. terreus* under SSF using palm as substrate was reported (Lakshmi *et al.*, 2009). Patil and Dayanand (2006) reported that pH 5.0 was optimum for the maximum production of pectinases of *A.*

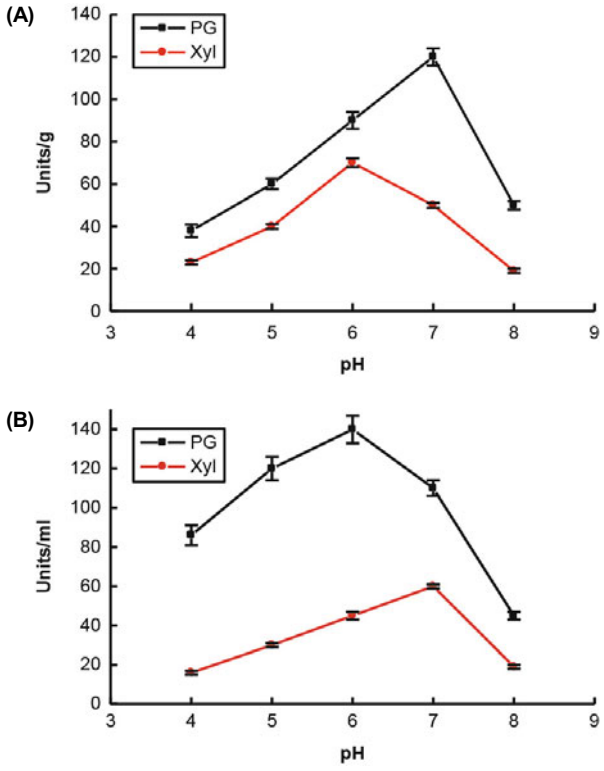

Fig. 5. The effect of pH on production of both PG and Xyl by *T. harzianum* (A) and *T. virnes* (B) in SSF using cantaloupe and watermelon rinds as substrates, respectively.

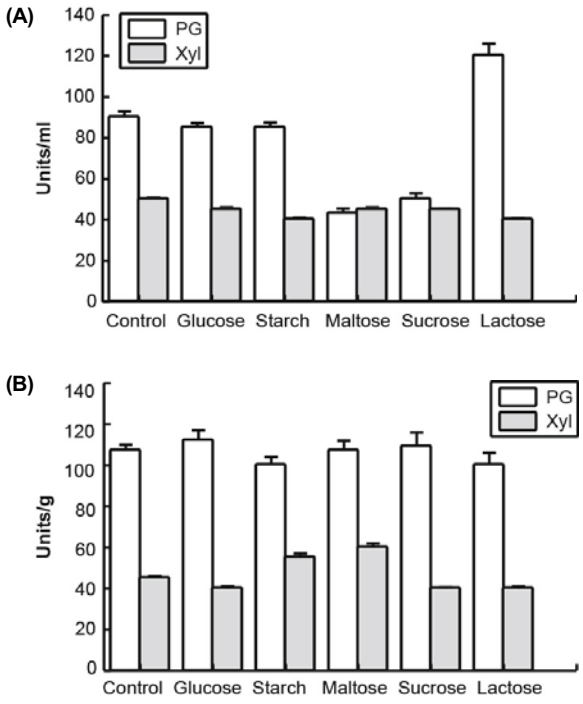

Fig. 6. The effect of carbon source (1%) supplementation on production of both PG and Xyl by *T. harzianum* (A) and *T. virnes* (B) in SSF using cantaloupe and watermelon rinds as substrates, respectively.

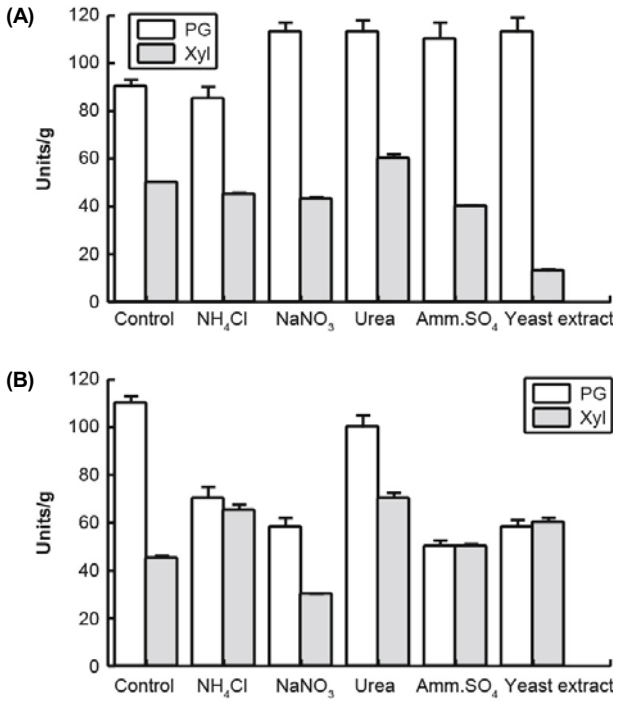

Fig. 7. The effect of nitrogen source (1%) supplementation on production of PG and Xyl by *T. harzianum* (A) and *T. virnes* (B) in SSF using cantaloupe and watermelon rinds as substrates, respectively.

niger using deseeded sunflower head in both submerged and solid state fermentation.

The effect of carbon source supplementation on PG and Xyl production

The influence of supplementary carbon sources such as starch, sucrose, maltose, lactose or glucose at 1% (by mass) on production of PG and Xyl was studied. For *T. harzianum*, lactose enhanced PG activities from 87 to120 units/g solid, while maltose and sucrose inhibited the activity. However, all carbon sources had slightly effect on Xyl (Fig. 6). Starch and sucrose enhanced the Xyl activities from 40 to 55–60 units/g solid, while all carbon sources exhibited slightly effect on PG activities for *T. virnes*. Botella *et al.* (2007) reported that when 6% glucose was added as extra carbon source, the production of xylanase and polyglacturonase increased significantly. However, at 8% both enzyme activities declined. In contrast, when wheat bran was used as the solid substrate, xylanase was resistant to catabolic repression even at 10% glucose (Farani de Souza *et al.*, 2001).

The effect of nitrogen source supplementation on PG and Xyl production

Studies on supplementation of nitrogen sources such as ammonium sulphate, ammonium nitrate, ammonium chloride, yeast extract or urea at 1% concentration to the solid substrates showed various effects on PG and Xyl production by *T. harzianum* and *T. virens*. Among the nitrogen sources, ammonium sulphate, ammonium nitrate, yeast extract and urea increased PG activities of *T. harzianum* from 90 to 110–113 units/g solid and decreased PG activities of *T. virens* (Fig. 7). However, urea increased Xyl activities of *T. harzianum* and *T. virens.* Similarly, ammonium chloride, ammonium sulphate and yeast extract increased Xyl activities of *T. virens.* In *A. fischeri* grown on wheat bran as substrate in SSF, the addition of $NaNO_2$ enhanced xylanase production, whereas yeast extract had no effect (Senthikumar *et al.*, 2005). $NaNO_3$ as nitrogen source improved the production

Table 1. Comparison of PG and Xyl production from other microorganisms grown on agriculture wastes

Microorganism	Substrate	PG U/g[a]	Xyl U/g	References
T. harzianum and *T. virens*	cantaloupe and watermelon	140	80	Present study
Aspergillus soja	Crushed maize	30	-	Ustok *et al.* (2007)
A. awamori	Grape pomace	25	40	Botella *et al.* (2005)
A. niger	Citrus peel	118	65	Rodriguez-fernandez *et al.* (2011)
A. niger	Deseeded sunflower	34	-	Patil and Dayanand (2006)
A. niger	Ordos Plateau	36	-	Debing *et al.* (2006)

[a] U/g, units/g solid

of xylanase by *A. terreus* under SSF using plam as substrate was reported (Lakshmi *et al*., 2009). Debing *et al*. (2006) reported that an optimal prescription of dextrose 8.5%, wheat bran 24.5%, ammonium sulphate 5.7%, and water 61.3% was found to be ideal for pectinavse production by *A. niger* in solid state condition.

Comparison of PG and Xyl production pattern by *T. harzianum* and *T. virens* used in this study and other microorganisms established the potential of *T. harzianum* and *T. virens* for economic PG and Xyl production (Table 1). In fact, the present microorganisms are better producers of PG and Xyl (approximately 140 and 80 units/g solid, respectively) compared to other microorganisms (118 and 65 units/g solid, respectively) (Botella *et al*., 2005; Debing *et al*., 2006; Patil and Dayanand, 2006; Ustok *et al*., 2007; Rodriguez-fernandez *et al*., 2011)

Conclusion

The present study reveals that watermelon and cantaloupe rinds can be used as optional substrates to other agricultural/agro-industrial wastes, wheat, corn, rice, sugar cane and beet, banana waste, potato, tea, apple, and citrus fruits, which are used for production of xylanase and polygalacturonase. This study established the potential of *T. harzianum* and *T. virens* for economic PG and Xyl production comparing to other microorganisms. This work gives an insight into the exploitation of a new agriculture wastes for the production of some industrial enzymes in appreciable levels.

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References

Azin, M., Morevej, R., and Zareh, D. 2007. Production of xylanase by *Trichoderma longibrachiatum* on a mixture of wheat bran and wheat straw: optimization of culture condition by Taguchi method. *Enzyme Microb. Technol.* **40**, 801–805.

Botella, C., de Ory, I., Webb, C., Cantero, D., and Blandino, A. 2005. Hydrolytic enzyme production by *Aspergillus awamori* on grape pomace. *Biochem. Eng. J.* **26**, 100–106.

Botella, C., Diaz, A., de Ory, I., Webb, C., and Blandino, A. 2007. Xylanase and pectinase production by *Aspergillus awamori* on grape pomace in solid state fermentation. *Process Biochem.* **42**, 98–101.

Castilho, L.R., Medronho, R.A., and Alves, T.L.M. 2000. Production and extraction of pectinases obtained by solid state fermentation of agroindustrial residues with *Aspergillus niger*. *Bioresour. Technol.* **71**, 45–50.

Couri, S., Terzi, S., Pinto, G.S., Freitas, S.P., and da Costa, A.C.A. 2000. Hydrolytic enzyme production in solidstate fermentation by *Aspergillus niger* 3T5B8. *Process Biochem.* **36**, 255–261.

Couto, S.R. and Sanroman, M.A. 2005. Application of solid-state fermentation to food industry – a review. *J. Food Eng.* **22**, 211–219.

Debing, J., Peijun, L., Stagnitti, F., Xianzhe, X., and Li, L. 2006. Pectinase production by solid fermentation from *Aspergillus niger* by a new prescription experiment. *Ecotoxicol. Environ. Safety* **64**, 244–250.

Delabona, P. da-S., Pirota, R.D.P.B., Codima, C.A., Tremacoldi, C.G., Rodrigues, A., and Farinas, C.S. 2013. Effect of initial moisture content on two Amazon rainforest *Aspergillus* strains cultivated on agro-industrial residues: Biomass-degrading enzymes production and characterization. *Ind. Crops Prod.* **42**, 236–242.

Farani de Souza, D., Marques de Souza, C.G., and Peralta, R.M. 2001. Effect of easily metabolizable sugars in the production of xylanase by *Aspergillus tamarii* in solid state fermentation. *Process Biochem*. **36**, 835–838.

Gervais, P. and Molin, P. 2003. The role of the water in solid state fermentation. *Biochem. Eng. J.* **13**, 85–101.

Gonzales, G.V., Torres, E.F., Aguilar, C.N., Gomez, S.J.R., Godinez, G.D., and Augur, C. 2003. Advantages of fungal enzyme production in solid-state over liquid fermentation systems. *Biochem. Eng. J.* **13**, 157–167.

Heerd, D., Yegin, S., Tari, C., and Fernandez-Lahore, M. 2012. Pectinase enzyme-complex production by *Aspergillus* spp. In solid-state fermentation: A comparative study. *Food Bioproduct Processing* **90**, 102–110.

Kumar, P. 1985. Watermelon-utilization of peel waste for pickle processing. *Indian Food Packer*. **39**, 49–52.

Lakshmi, G.S., Rao, C.S., Rao, R.S., Hobbs, P.J., and Prakasham, R.S. 2009. Enhanced production of xylanase by a newly isolated *Aspergillus terreus* under solid state fermentation using palm industrial waste: A statistical optimization. *Biochem. Eng. J.* **48**, 51–57.

Lu, W., Li, D., and Wu, Y. 2003. Influence of water activity and temperature on xylanase biosynthesis in pilot-scale solid state fermentation by *Aspergillus sulphurous*. *Enzyme Microb. Technol.* **32**, 305–311.

Madhuri, P. and Devi, K. 2003. Value addition to watermelon fruit waste. *J. Food Sci. Technol.* **40**, 222–224.

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

Martins, E.S., Silva, D., Da Silva, R., and Gomes, E. 2002. Solid-state production of thermostable pectinases from thermophilic *Thermoascus urantiacus*. *Process Biochem.* **37**, 949–954.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for the determination of reducing sugar. *Anal. Chem.* **31**, 426–429.

Pandey, A. 1991. Aspects of fermenter design for solid-state fermentations. *Process Biochem*. **26**, 355–361.

Patil, S.R. and Dayanand, A. 2006. Optimization of process for the production of fungal pectinases from deseeded sunflower head in submerged and solid-state conditions. *Bioresour. Technol*. **97**, 2340–2344.

Pons, L. 2003. Exploring important medicinal uses for watermelon rinds. Available online at URL http://www.ars.usda.gov/is/pr/2003/030221.htm. (accessed 2006).

Raimbault, M. 1998. General and microbiological aspects of solid substrate fermentation. *Elec. J. Biotechnol.* **1**, 1–15.

Roco, A. and Pérez, L.M. 2001. *In vitro* biocontrol activity of *Trichoderma harzianum* on *Alternaria alternate* in the presence of growth regulators. *J. Biotechnol.* **4**, 68–73.

Rodriguez-Fernández, D.E., Rodriguez-León, J.A., de Carvalho, J.C., Sturm, W., and Soccol C.R. 2011. The behavior of kinetic parameters in production of pectinaseand xylanase by solid-state fermentation. *Bioresour. Technol.* **102**, 10657–10662.

Senthilkumar, S.R., Ashokkumar, B., Raj, K.C., and Gunasekaran, P. 2005. Optimization of medium composition for alkali-stable xylanase production by *Aspergillus fischeri* Fxn 1 in solid-state fermentation using central composite rotary design. *Bioresour.*

Technol. **96**, 1380–1386.

Seyis, I. and Aksoz, N. 2005. Xylanase production from *Trichoderm harzianum* 1073 D3 with alternative carbon and nitrogen sources. *Food Technol. Biotechnol.* **43**, 37–40.

Silva, D., Martins, E.S., Da Silva, R., and Gomes, E. 2002. Pectinase production by *Penicillium viridicatum* RFC3 by solid-state fermentation using agricultural wastes and agro-industrial by-products. *Braz. J. Microbiol.* **33**, 318–324.

Simonne, A., Carter, M., Fellers, R., Weese, J., Wei, C.I., Simonne, E., and Miller, M. 2002. Chemical, physical, and sensory characterization of watermelon rind pickles. *J. Food Process Preserv.* **26**, 415–431.

Singh, R., Kumar, J.C., and Nandpuri, K.S. 1975. A study on the influence of the structural chemical constituents of the skin of water melon (*Citrullus lanatus* Sch.) fruit on the incidence of its blossom-end-rot and cracking. *Indian J. Horticult.* **32**, 98–101.

Sivaramakrishnan, S., Gangadharan, D., Nampoothiri, K.M., Soccol, C.R., and Pandey, A. 2006. α-Amylases from microbial sources – An overview on recent developments. *Food Technol. Biotechnol.* **44**, 173–184.

Sukumaran, R.K., Singhania, R.R., Mathew, G.M., and Pandey, A. 2009. Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. *Renew. Energ.* **34**, 421–424.

Sun, X., Liu, Z., Qu, Y., and Li, X. 2008. The effect of wheat bran composition on the production of biomass-hydrolyzing enzymes by *Penicillium decumbens*. *Appl. Biochem. Biotechnol.* **146**, 119–128.

Ustok, F.I., Canan Tari, C., and Gogus, N. 2007. Solid-state production of polygalacturonase by *Aspergillus sojae* ATCC 20235. *J. Biotechnol.* **127**, 322–334.

Yamane, Y., Fujita, J., Shimizu, R., Hiyoshi, A., Fukuda, H., Kizaki, Y., and Wakabayashi, S. 2002. Production of cellulose- and xylan-degrading enzymes by a koji mold, *Aspergillus oryzae*, and their contribution to the maceration of rice endosperm cell wall. *J. Biosci. Bioeng.* **93**, 9–14.

Yang, X., Chen, H., Gao, H., and Li, Z. 2001. Bioconversion of corn straw by coupling ensiling and solid-state fermentation. *Bioresour. Technol.* **78**, 277–280.

Yuan, Q.P., Wang, J.D., Zhang, H., and Qian, Z.M. 2005. Effect of temperature shift on production of xylanase by *Aspergillus niger*. *Process Biochem.* **40**, 3255–3257.

Zhou, J.M., Ge, X.Y., and Zhang, W.G. 2011. Improvement of polygalacturonase production at high temperature by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisiae*. *Bioresour. Technol.* **102**, 10085–10088.