Malaysian Journal of Microbiology, Vol 2(1) 2006, pp.7-14

Xylanase Production by a Local Isolate, *Trichoderma* spp. FETL c3-2 *via* Solid State Fermentation Using Agricultural Wastes as Substrates

Pang, P. K.[1], Darah. I.[1], Poppe, L.[2], Szakacs, G.[3] and Ibrahim, C.O. [1]*

[1]Fermentation and Enzyme Technology Laboratory, School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
[2]Institute of Organic Chemistry, [3]Department of Agricultural Chemical Technology, Budapest University of Technical and Economics, Gellert ter 4, Budapest H-1111 Hungary
Email: oibrahim@usm.my

ABSTRACT

The production of xylanase by a local isolate *Trichoderma* spp. FETL c3-2 via solid state fermentation system using sugar cane baggase:palm kernel cake as substrates was investigated. The optimized solid state fermentation (SSF) system consists of 5 g of sugar cane baggase: palm kernel cake of ratio 90:10 (%, w/w), moisture content of 75% (v/w), pH of moistening agent of pH 7.0, at 30°C and inoculum size of $1x10^8$ spores/ml. The SSF system was also supplemented with 4% (w/w) dextrin and 6% (w/w) of tryptone as additional carbon and nitrogen sources, respectively. Cellulose at the concentration of 0.2% (w/w) was found to be a significant inducer for xylanase production. Using the optimized SSF system, a maximum xylanase productivity of 75.0 U per mg glucosamine after 4 days of fermentation time at 30°C was obtained. The modifications of the SSF system resulted an increase in xylanase productivity by 180% and growth by 40% compared to the basal SSF system.

Keywords: Xylanase production, solid state fermentation, *Trichoderma* spp., agricultural wastes

INTRODUCTION

Malaysia generates abundant agricultural wastes with the volume of approximately 5 million tones annually and is expected to double by the year 2010. Some of these wastes include, oil palm trunks and fronds, palm kernel cake, sugar cane baggase, rice husk, rice straws, coconut fibers and meal, cocoa pods, rubber wood dusts and many other wastes materials. The management of these wastes effectively and economically must be given utmost priority in the country in ensuring not only in reducing the detrimental impact of the wastes to the environment, but most importantly in the transformation of these wastes into useful raw materials for the production of added value commodities of industrially commercial potentials. Currently most of these agricultural wastes mainly palm kernel cake are exported to European countries as animal feeds while some of the palm oil and coconut fibres are used as insulators in cushions and mattresses. Others may be disposed or burned. However, based on the solid state fermentation (SSF) processes, agricultural wastes can readily be used as substrates for the cultivation of numerous microorganisms for the production of various metabolites which are important for industrial applications. Some of these products include, enzymes, flavoring compounds, pigments, pharmaceutical products and industrial chemicals. Enzymes remain the most frequently reported metabolites produced *via* SSF, some of which include cellulases, xylanases, lipases, β-glucosidases, mannanases, phytases, proteases, lignin degrading enzymes and pectinases.

In our laboratory, we are particularly interested in the production of xylanase for the application in the deinking process coupled with the cellulase which was also produced via SSF. Pulp and paper industries form the largest consumer of cellulases and hemicellulases (Bajpai, 1999). Xylanase can either be produced *via* submerged system or SSF using bacteria, yeast and filamentous fungi. Some of these microorganisms including protozoa are present in the rumen of animals. The production of xylanase by the SSF method offers several advantages over the submerged system simply because of its low operational and production cost. The reduction in the production cost is attributed to the simple and low cost production facilities. At the same time, SSF uses inexpensive agrowaste materials as substrates. The use of agrowastes not only helps to overcome the problem of solid waste management but also allows the development of biotechnological processes from cheap natural resources.

This paper describes the production of xylanase from a local isolate, *Trichoderma* spp. FETL c3-2 via SSF using sugar cane baggase and palm kernel cake as substrates. Some of the governing physico-chemical parameters on the production of the enzyme in the SSF system were examined.

***Corresponding author**

MATERIALS AND METHODS

Source of microorganisms for screening of xylanase producer

The soil samples obtained from the Northern Region of Peninsular Malaysia was used to isolate *Trichoderma* spp. FETL c3-2 using the cellulose agar medium containing (%, w/v): cellulose 3.00, $NaNO_3$ 0.30, $(NH_4)_2SO_4$ 0.10, KH_2PO_4 0.10, $(NH_4)_2HPO_4$ 0.05, $MgSO_4$ 0.05, KCl 0.05, $FeSO_4.7H_2O$ 0.01, Bacto agar 2.00, yeast extract (Difco) 0.03 and trace amount of trace elements; $FeSO_4.7H_2O$; 5.00, $MnSO_4.H_2O$; 1.60, $ZnSO_4.7H_2O$; 3.45 and $CoCl_2.6H_2O$; 2.00. The pH was adjusted to pH 7.0. Isolates of *Trichoderma* species obtained were purified for use in the screening of xylanase production in the SSF system. *Trichoderma* species were identified based on the morphological characteristics observed under light microscope. The fungus was maintained on potato dextrose agar and kept at 4°C prior to use. Subculturing was carried out every 3 months.

Substrate for solid state fermentation (SSF)

The substrates used in the SSF system consist of sugar cane baggase (SC), palm kernel cake (PKC) and rice husks (RH) which were obtained locally. All the substrates were dried under sunlight until constant weight. Proximate analysis was carried out for all the substrates based on the methods described by AOAC (1997).

Cultivation system of SSF

The cultivation of the fungus in the SSF system was performed in a 500-ml Erlenmeyer flask containing 5 g substrate with the addition of known volume of the moistening agent to give the desired moisture content in the SSF system. The moistening agent solution consists of (g/l) NH_4NO_3; 5.00, corn steep liquor; 2.00, NaCl; 1.00, and $MgSO_4.7H_2O$; 1.00. The moistening agent solution was also supplemented with trace elements consisting of (mg/l): $FeSO_4.7H_2O$; 5.00, $MnSO_4.4H_2O$; 1.60, $ZnSO_4.7H_2O$; 3.45 and $CoCl_2.6H_2O$ 2.00 of pH 7.0. Cultivation was carried out at known incubation period at 30°C with the inoculum concentration of $1x10^6$ spores/ml. The inoculum was prepared by growing the isolates on malt extract agar at 37°C until sporulation. The spores were harvested using 0.1% (v/v) Tween 80 (Smith *et al* 1996). The number of spores was estimated by direct microscopic counting using haemocytometer.

Optimization of SSF system for xylanase production

The optimization of SSF system for xylanase production was performed based on the modification of the physical parameters and the supplementation of additional nutrients. The effect of physical parameters was determined based on the modification of moisture content in the range of 65 -85% (v/w), pH of the moistening agent in the range of pH 6 -10, cultivation temperature in the range of 25 - 40°C and inoculum sizes in the range of $1x10^6$ - $1x10^8$ spores/ml.

The effect of supplementation of additional carbon and nitrogen sources and inducers on xylanase production was examined. The carbon sources examined consist of maltose, starch, sorbitol, lactose, dextrin and sucrose, while the nitrogen sources consist of peptone, urea, tryptone, yeast extract and sodium nitrate. The concentration at 4.0% (w/w) was used for both the carbon and nitrogen sources. The effect of different concentration of carbon and nitrogen sources was also determined in the range of 2 - 10% (w/w) on the xylanase production. The effect of inducers on xylanase production was examined using cellulose, xylan, carboxymethyl cellulose and xylose at the concentration of 0.8% (w/w). Similarly, the effect of concentration of inducers was determined in the range of 0.2 - 1.0% (w/w).

All experiments were carried out in triplicates and the results were presented as mean of the triplicates experiments.

Analysis

Growth of the fungus was determined based on the glucosamine method as described by Swift (1972). Growth was expressed as mg glucosamine per g of substrate. Xylanase activity was determined by the method of Gassesse and Gashe (1997) at 50°C using oat spelt xylan as substrate, which was dissolved in 50 mM citrate buffer, pH 4.5. Xylanase activity was expressed as U per g substrate. One unit (U) of xylanase activity is defined as the amount of enzyme that releases 1 µmol of reducing sugar equivalent to xylose per min. All data were presented as xylanase productivity and expressed as units (U) per mg of glucosamine of fungal growth.

RESULTS AND DISCUSSION

Isolation of *Trichoderma* spp. for xylanase production

A total of 29 *Trichoderma* isolates were obtained using cellulose agar from the Malaysian soils and isolate FETL c3-2 was selected for further studies based on the high xylanase production and growth on sugar cane baggase. The isolate was reconfirmed to be placed under the genus of *Trichoderma* sp. based on the colony and structural morphologies observed under light microscope (Figure. 1). The fungus grew extremely well in the cellulose agar giving a greenish colouration of the mycelia. As shown in Figure 1, *Trichoderma* sp. FETL c3-2 is characterized by the presence of septate mycelia. The conidiophores are branched consisting of a group 3 or 4 philiades which is swollen in the middle and narrow at the ends. The philiades bear conidia which are ellipsoidal in shape. Currently, the isolate has not been identified to the species level, thus it is designated as *Trichoderma* spp. FETL c3-2.

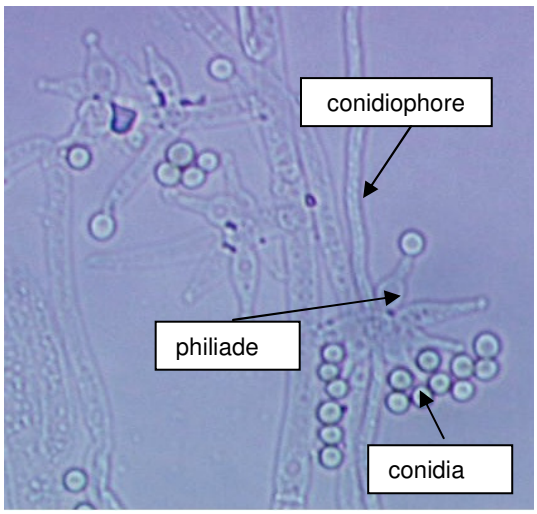

Figure 1: *Trichoderma* sp. FETL c3-2 under light microscope showing the condiophores and the conidia (400 x magnification)

Production of xylanase on various agrowaste as substrates via SSF system by *Trichoderma* spp. FETL c3-2

Table 1 shows the level of xylanase production by *Trichoderma* spp. FETL c3-2 on various substrates at different moisture content. The use of sugar cane baggase revealed the highest xylanase activity of 61.11 U/g substrate after 3 days of fermentation with the enzyme productivity of 37.04 U per mg glucosamine. Palm kernel cake did not improve the enzyme production although good growth was observed compared to other substrates. However, the use of sugar cane baggase and palm kernel cake at the ratio of 1:1 resulted a production level of 55.18 U/g substrate after 4 days with a lower productivity of 32.27 U per mg glucosamine. Pang and Ibrahim (2005) have shown that *Aspergillus niger* USM AI 1 preferred palm kernel cake as substrate for the production of xylanase. The results, thus suggested that the substrate requirement may vary with the types of microorganism used and subsequently the enzyme productivity. Kang *et al*. (2004) have shown that the production of cellulase and hemicellulase are inducible and were affected by the nature of the substrate used in the fermentation. The high production using sugar cane baggase corresponded to the nutritional content of sugar cane baggase. Sugar cane baggase contains 47.34% of cellulose and 29.3% hemicellulose. Other components present include protein of 0.86%, crude fibre 49.66%, lipid 1.20% and sugar 6.22%. Palm kernel cake contains about 16% protein content which provides the requirement for fungal growth. The physical characteristic of sugar cane baggase are seen as loose, less compact texture than the palm kernel cake, giving ease in oxygen diffusion, nutrients absorption and assimilation by the fungal mycelia.

Table 1 also shows that the mixture of substrates improved the xylanase productivity. The addition of palm kernel cake significantly enhanced the growth of the fungus, although the xylanase activity dropped. Palm kernel cake contains protein content of about 16% has been used as animal feeds for a number of years (Panigrahi and Powell, 1991). It has also been shown that the mixture of palm kernel cake and rice husk as substrates helped to improve the growth of *Aspergillus flavus* USM A10 and therefore enhanced the lipase production in the SSF system (How and Ibrahim, 2004). The substrates mixture of rice husk and palm kernel cake although resulted higher xylanase productivity required a longer time of 7 days to achieve maximum activity.

Effect of substrate ratio on xylanase production

The effect of the substrate ratio of sugar cane baggase and palm kernel cake was examined as a comparison to the use of only sugar cane baggase. As indicated in table 2, it was observed that increasing the amount of sugar cane baggase improved the xylanase activity significantly. A maximum production of xylanase of 64.37 U/g was obtained with the growth of 2.08 mg glucosamine per g substrate. Increase amount of PKC did not improve the enzyme production, however, the growth of the fungus dropped with the decrease in the amount of palm kernel cake. Based on the high xylanase activity and growth in the sugar cane baggase and palm kernel cake at the ratio of 90:10%, the substrate mixture was selected to be studied in the subsequent experiments.

Although xylanase productivity was higher using 100% sugar cane baggase as substrate, the good growth in the presence of palm kernel cake could be further exploited for enhancing enzyme production. Based on that reasoning, the substrates sugar cane baggase:palm kernel cake were selected for further investigation.

Effect of moisture content on xylanase production

Moisture content is an important parameter in solid state fermentation processes. Water affects the physical properties of the substrate mainly by causing swelling of the substrates and facilitates effective absorption of the nutrients from the substrates for growth and metabolic activities (Pandey, 1992). The moisture content was adjusted by adding the moistening agent to give the moisture content ranging from 65 - 85% (v/w). As shown in Figure 2, the moisture content of 75 - 80% resulted significant xylanase production with the productivity of 32 - 35 U per mg glucosamine. It was also observed that the growth of the fungus at the moisture content of 75% was higher than 80% although xylanase activity remains unchanged. Thus, the moisture content of 75% was selected to be used in subsequent experiments. Lower moisture content of less than 75% or higher than 75% did not improve the enzyme production or the growth of the

Table 1: The production of xylanase and growth of *Trichoderma* sp. on various substrates in SSF cultivation system.

Substrate [moisture content,%]	Maximum xylanase activity (U per g substrate)	Maximum growth (mg glucosamine per g substrate)	Time taken to achieve maximum xylanase (days)	Xylanase productivity (U per mg glucosamine)
Single substrate				
Sugar cane baggase (SC)[80]	61.11	1.65	3	37.04
Palm kernel cake (PKC) [50]	8.61	3.63	4	2.37
Rice husk- 2 mm (RH) [50]	26.06	1.36	5	19.16
RH - 1 mm [50]	22.39	1.16	5	19.30
*Mixed substrates**				
SC/PKC [65]	55.18	1.71	4	32.27
RH/PKC [80]	34.39	2.62	4	13.13
RH/PKC [50]	68.17	1.99	7	34.26
RH/SC [65]	56.38	1.87	7	30.15

* substrate used at the ratio of 50:50. Data were presented as mean of triplicate experiments
# The moisture content represents the maximum moisture content used to give the adequate wetting of the substrate

Table 2: Effect of ratio of the SC:PKC on xylanase production by *Trichoderma* spp. FETL c3-2

Substrate ratio (%)	Maximum xylanase activity (U per g substrate))	Maximum growth (mg glucosamine per g substrate)	Xylanase productivity (U per mg glucosamine)
Control (50:50)	55.18	2.19	25.19
70:30	56.38	2.15	26.22
80:20	59.44	2.15	27.65
90:10	64.38	2.08	30.95
100:0	61.11	1.65	37.04

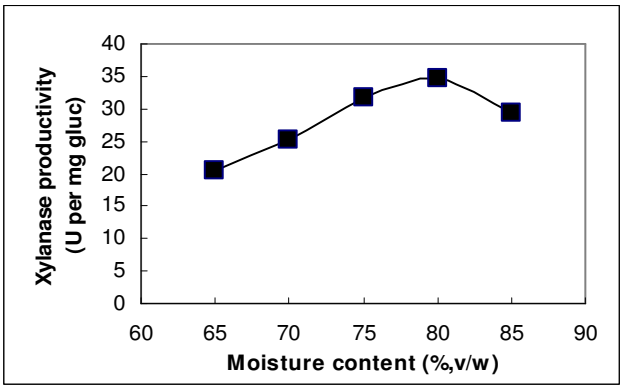

Figure 2: Effect of moisture content on the productivity of xylanase by *Trichoderma* spp. FETL c3-2

fungus. Low water content is related to insufficient substrate swelling which prevented the nutrient absorption from the substrates. Furthermore, low moisture content not only lessened substrate swelling, but also reduced nutrient solubility and caused higher water tension which also resulted poor growth of the fungus (Ikasari and Mitchell, 1994). On the other hand, higher water content resulted reduction in substrate porosity and caused oxygen limitation within the substrates which subsequently affected the oxygen transfer within the biomass, thus resulting poor growth (Raimbault and Alazard, 1980).

Effect of temperature on xylanase production

Temperature plays a more prominent role in solid state fermentation than in the submerged fermentation. The net temperature in SSF system is influenced not only by the environmental temperature, but also by the increase in temperature generated from the metabolic activities of the fungi growing on the solid substrates. The production of xylanase by *Trichoderma* spp. FETL c3-2 in the SSF system of sugar cane baggase:palm kernel cake shows the maximum productivity of 30 - 32 U per mg glucosamine in the temperature range of 25 - 30^{o}C (Figure 3). The fungal growth and enzyme production dropped at temperature above 30^{o}C and *Trichoderma* spp. FETL c3-2 exhibited no growth and no enzyme production at the temperature of 40^{o}C. The optimum temperature for enzyme production is similar to the optimum temperature for growth of *Trichoderma* spp. FETL c3-2 in its natural habitat. Similar observation was also reported by Sudgen and Bhat (1994) in *Sporotrichum thermophile* and by Biswas *et al* (1990) in *Aspergillus orchraceus*.

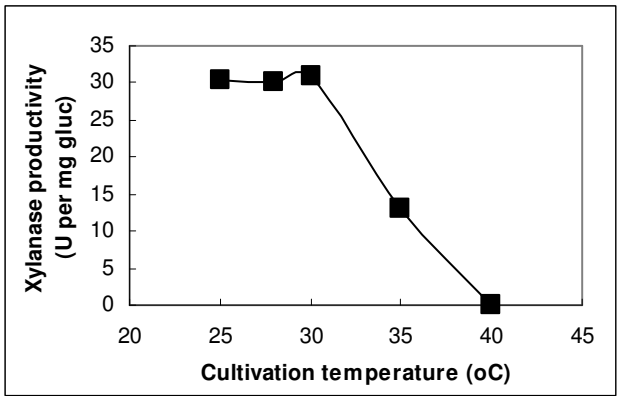

Figure 3: Effect of cultivation temperature on the productivity of xylanase by *Trichoderma* spp. FETL c3-2

Effect of pH of the moistening agent on xylanase production

Although pH change is normally monitored in submerged fermentation processes, SSF processes was also reported to be influenced by the environmental pH of the process (Jecu, 2000, Panagiotou *et al*. 2003). Therefore, the initial pH of the substrate was adjusted by adding the moistening agent at different pH. The results shown in Figure 4 clearly indicated that pH is an important parameter for optimal growth and xylanase production by *Trichoderma* spp. FETL c3-2 in SSF system. The optimum pH was pH 7 which gave the xylanase productivity in the range of about 32.0 – 34.0 U per mg glucosamine. The growth and enzyme production dropped significantly above pH 7. Optimum pH not only provides suitable condition for growth and enzyme production, but will also determine the enzymatic action on the substrates and enhance the enzyme stability.

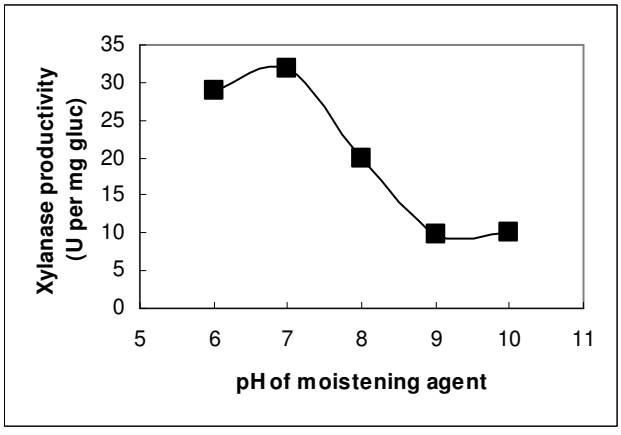

Figure 4: Effect of pH of the moistening agent on the productivity of xylanase by *Trichoderma* spp. FETL c3-2. The composition of moistening agent used is described in materials and methods

Effect of inoculum size on xylanase production

The effect of inoculum size was examined using the spore suspension of concentration from $1x10^6$ - $1x10^8$ spores/ml for a fermentation period of up to 7 days. The results of the enzyme productivity profiles at different inoculum sizes are shown in Figure 5. As shown in the figure, higher inoculum size of $1x10^8$ spores/ml resulted a higher xylanase productivity compared to other inoculum sizes with the maximum productivity of 27.0 – 30.0 U per mg glucosamine obtained after 4 days. Lower inoculum sizes resulted a lag phase on the first day of the cultivation where no fungal growth and enzyme production by the fungus was obtained. At lower inoculum sizes, it was observed that the time taken to achieve maximum growth or enzyme productivity was much longer. This is clearly shown that with the inoculum size of $1x10^6$ spores/ml, the enzyme has not achieved maximum productivity even after 7 days of fermentation. Higher enzyme production at higher inoculum is related to the rapid growth of the fungus which resulted higher degradation of the substrates and increase availability of the nutrients.

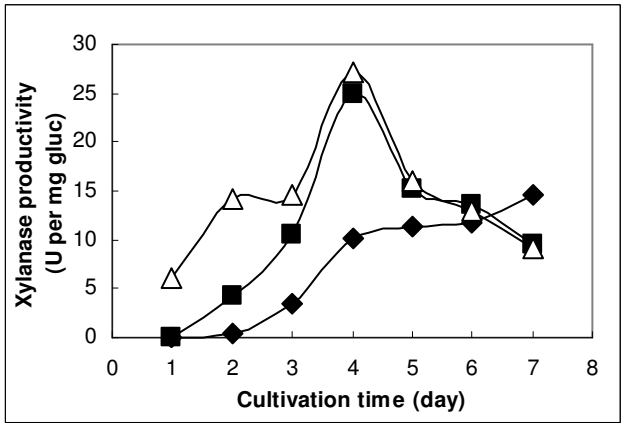

Figure 5: Effect of inoculum sizes on the productivity of xylanase by *Trichoderma* spp. FETL c3-2
Symbols: 1 x 10^6 (♦), 1 x 10^7 (■) and 1 x 10^8 (▲) spores/ml of moistening agent.

Effect of supplementation of carbon sources to the SSF system

Carbon sources in media formulation are used to enhance growth and subsequently resulted in higher enzyme production, which is normally observed in the synthesis of primary metabolites, such as enzymes. Poor growth in SSF system is associated with poor nutritional level in solid substrates. Therefore, the effect of additional carbon sources to the solid substrate (sugar cane baggase:palm kernel cake) was examined at 4% (w/w) and the results obtained are shown in Figure 6. When compared to the control system without the addition of any carbon sources (xylanase productivity of 30.0 – 32.0 U mg glucosamine),

it was observed that maltose and dextrin were found to enhance or maintain the enzyme production. The maximum productivity of xylanase of about 35.0 U per mg glucosamine was obtained with dextrin, while slightly lower productivity of about 28.80 U per mg glucosamine was obtained with maltose (Figure 6a). Other carbon sources were found to be less effective in enhancing the enzyme productivity. The effect of dextrin concentration was determined in the range of 2 - 10% (w/w) The enzyme production and growth were maximum up to 4% (w/w) with the xylanase productivity in the range of 36.0 – 38.0 U per mg glucosamine and the production dropped significantly at higher concentration (Figure 6b). The phenomenon of catabolite repression by high concentration of additional carbon sources in the production of enzymes *via* SSF system has been reported previously (Gessesse and Mamo, 1999, Solis Pereira *et al*. 1993).

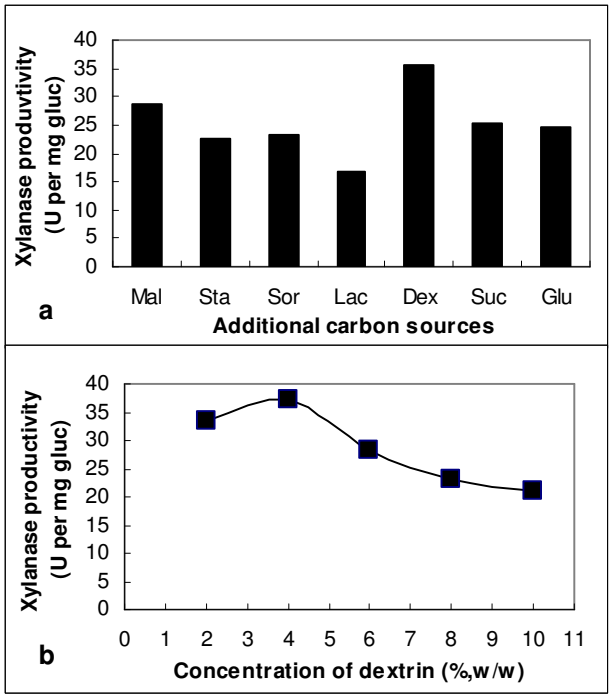

Figure 6: Effect of additional carbon sources in the SSF system on the productivity of xylanase by *Trichoderma* spp. FETL c3-2

a) Types of carbon sources: Mal; maltose, Sta; starch, Sor; sorbitol, Lac; lactose, Dex; dextrin, Suc; sucrose and Glu; glucose.
b) Effect of dextrin concentration on xylanase productivity

Figure 7a. As shown in the figure, the maximum xylanase productivity of 44.5 U per mg glucosamine was obtained with the addition of tryptone, while yeast extract, sodium nitrate and urea showed a productivity in the range of 40.0 – 42.0 U per mg glucosamine. However, with all the nitrogen sources examined, the growth of the fungus were not significantly affected in the range 2.6 - 2.8 mg glucosamine per g substrate. Pang and Ibrahim (2005) also reported an increment of only 40% in growth of *Aspergillus niger* USM AI 1, although the xylanase production increased by about 157%. The effect of concentration of tryptone in the range of up to 6% (w/w) did not affect the enzyme production and growth rate of the fungus with the enzyme productivity in the range of 50.0 – 52.0 U per mg glucosamine (Figure 7b). Higher tryptone concentration of 8% or more resulted in a drop of the enzyme productivity.

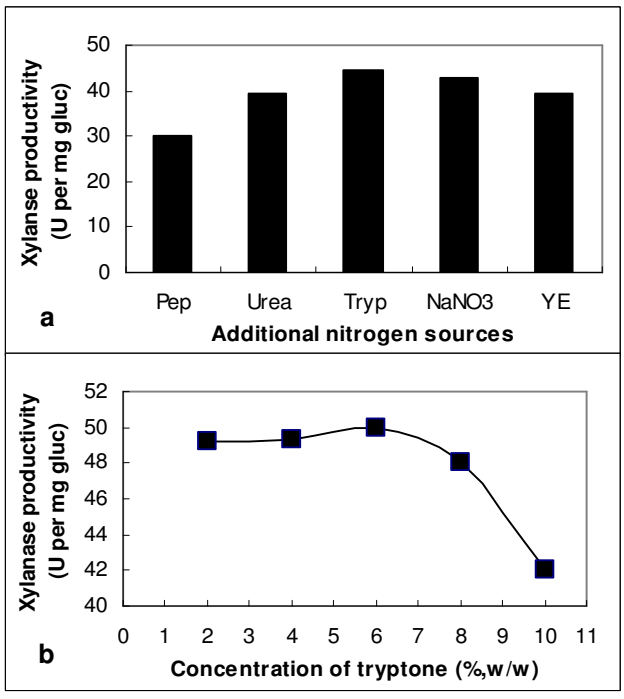

Figure 7: Effect of additional nitrogen sources in the SSF system on the productivity of xylanase by *Trichoderma* spp. FETL c3-2

a) Types of nitrogen sources: Pep; peptone, Tryp; tryptone, NaNO3; sodium nitrate, and YE; yeast extract.
b) Effect of tryptone concentration on xylanase productivity

Effect of supplementation of nitrogen sources to the SSF system

The effect of supplementation of nitrogen sources in SSF system on the growth and enzyme production is shown in

Effect of the supplementation of inducers to the SSF system

Most industrial enzymes are highly inducible. Therefore, the production of xylanase was examined in the presence

of several related compounds which act as inducers. As shown in Figure 8, the presence of cellulose enhanced the productivity achieving about 65 U per mg glucosamine, while other inducers such as xylan, carboxymethyl cellulose and xylose resulted on the productivity in the range of about 40 - 47 U per mg glucosamine (Figure 8a). The growth of the fungus was not affected in the presence or absence of the inducers with the growth of about 2.3 mg per g substrate. The concentration of cellulose in the range of 0.2 - 1.0% (w/w) was examined and it was found that the cellulose concentration of 0.2% resulted in the maximum xylanase productivity of 75.0 U per mg glucosamine, while increasing cellulose concentration resulted in a rapid drop in the enzyme productivity (Figure 8b). Many microorganisms have shown that xylose was a better inducer for xylanase production than cellulose as observed in the SSF system of *Aureobasidium pullulans* (Priem *et al*. 1991), *Fusarium oxysporum* (Singh *et al*. 1992) and *Thermomyces lanuginosus* (Purkarthofer *et al*. 1993). However, cellulose has also been reported to be a significant inducer in *Penicillium janthinellum* NCIM 1171 and *Trichoderma viride* NCIM 1051 (Adsul *et al*. 2004).

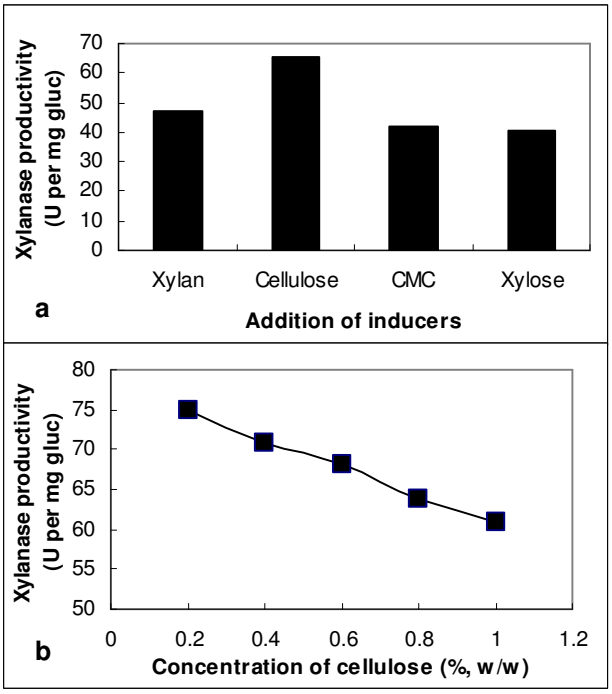

Figure 8: Effect of inducers in the SSF system on the productivity of xylanase by *Trichoderma* spp. FETL c3-2
a) Types of inducers,
b) Effect of cellulose concentration on xylanase productivity

Based on the results obtained so far, the SSF system consisting of 5 g of sugar cane baggase:palm kernel cake of ratio 90:10% (w.w), moisture content of 75% (v/w), pH of moistening agent of pH 7.0, at 30^{o}C, inoculum size of 1x10^{8} spores/ml containing 4% (w/w) of dextrin, 6% (w/w) of tryptone and 0.2% (w/w) of cellulose, a maximum xylanase productivity of 75.0 U per mg glucosamine was obtained after 4 days of fermentation. The results show an increment of about 180% in the enzyme productivity compared to the basal SSF system. However, the increase in growth was only about 40%. Comparison on the productivity by other microorganisms is relatively difficult considering the differences in the cultivation systems, types of microorganism and substrates. Nevertheless, the amount of xylanase productivity by *Trichoderma* sp. FETL c3-2 (160 U/g substrate) can be considered significant and comparable to many other microorganisms ranging from as low as 14 U/g to 5000 U/g depending on the microorganisms and substrates used (Haltrich *et al*. 1996, Gawande and Kamat, 1999, Jecu; 2000, Kang *et al*. 2004, Pang and Ibrahim, 2005).

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Science, Technology and Innovation for the financial support provided under the Malaysian-Hungary Co-operation Programme in Science and Technology and EA IRPA Research Grant 09-02-05-2166 EA 004. This work forms a part of Ph.D thesis of Pang Pei Kheng.

REFERENCES

Adsul, M.G., Ghule, J.E., Singh, K., Shaikh, H., Bastawde, K.B., Gokhale, D.V. and Varma, A.J. (2004). Polyssacharides from baggase: applications in cellulase and xylanase production. *Carbohydrates Polymers*, **57: 67 – 72**.

AOAC (1997). Official Methods of Analysis of AOAC International, 16th edition (Cunniff, P.A. Ed). **pp. 25-28, 65-72, 11-116**, Virginia AOAC International.

Bajpai, P. (1999). Applications of enzymes in the pulp and paper industry. *Biotechnology Progress*, **15: 147 – 157.**

Biswas, S.R., Jana, S.C., Mishra, A.K. and Nanda, G. (1990). Production, purification and characterization of xylanase from a hyperxylanolytic mutant of *Aspergillus ochraceus*. *Biotechnology and Bioengineering*, **35: 244 - 251**.

Gawande, P.V. and Kamat, M.Y. (1999). Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. *Journal of Applied Microbiology*, **29: 109 - 117**.

Gessesse, A. and Gashe, B.A. (1997). Production of alkaline xylanase by alkaliphilic *Bacillus* sp. isolated from an alkaline soda lake. *Journal of Applied Microbiology*, **83: 402-406**.

Gessesse, A. and Mamo, G. (1999). High level xylanase by an alkalophilic *Bacillus* sp. by using solid state fermentation. *Enzyme Microbe and Technology*, **25: 68-72.**

Haltrich, D., Nidetzky, B., Kulke, K.D., Steiner, W. and Zupanie, S. (1996). Production of fungal xylanases. *Bioresource Technology*, **58: 137 -161**.

How, S.P. and Ibrahim, C.O. (2004). Selection and optimization of lipase production from *Aspergillus flavus* USM A10 via solid state fermentation (SSF) on rice husk and wood dust as substrates. *Pakistan Journal of Biological Sciences*, **7: 1249 – 1256**.

Ikasari, L. and Mitchell, D.A. (1994). Protease production by *Rhizopus oligosporus* in solid state fermentation. *Applied Microbiology and Biotechnology*, **10: 320 - 324**.

Jecu, L. (2000). Solid state fermentation of agricultural wastes for endoglucanase production. *Industrial Crops and Products*. **11: 1 – 5**.

Kang, S.W., Park, Y.S., Lee, J.S., Hong, S.I. and Kim, S.W. (2004). Production of cellulose and hemicellulase by *Aspergillus niger* KK2 for lignocellulosic biomass. *Bioresource Technology*, **91: 153 – 156**.

Panagiotou, G., Kekos, D., Macris, B.J. and Christakopoulous, P. (2003). Production of cellulolytic and xylanolytic enzymes by *Fusarium oxysporum* grown on corn stover in solid state fermentation. *Industrial Crops and Products* **18: 37 – 45.**

Pandey, A. (1992). Recent process developments in solid state fermentation. *Process Biochemistry*, **27: 12 - 17**.

Panigrahi, S. and Powell, C.J. (1991). Effects of high rate of inclusion of palm kernel meal in broiler chicks diet. *Animal Feed Science and Technology*, **34: 37 – 47**.

Pang P.K. and Ibrahim C.O. (2005). Xylanase production by a local fungal isolate, *Aspergillus niger* USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate. *Songklanakarin Journal of Science and Technology*, **27**: in press

Priem, B., Dobberstein, J. and Emeis, C.C. (1991). Production of β-1,4-xylanase in continuous culture by *Aureobasidium pullulans* CBS 58475. *Biotechnology Letters.* **13:149-154**.

Purkarthofer, H., Sinner. M. and Steiner, W. (1993). Cellulase free xylanase from *Thermomyces lanuginosus*: optimization of production in submerged and solid state cultures. *Enzyme Microbial and Technology*, **15: 677 - 682**.

Raimbault, M. and Alazard, D. (1980). Culture method to study fungal growth in solid fermentation. *European Journal of Applied Microbiology and Biotechnology*, **9: 199 - 209.**

Singh, A., Kumar, P.K.R. and Schugerl, K. (1992). Bioconversion of cellulosic materials to ethanol by filamentous fungi. *Advances in Biochemical Engineering and Biotechnology,* **45**: **29 - 55.**

Smith, P.J., Rinzema, A. Tramper, J., Schlosser, E.E. and Knol, W. (1996). Accurate determination of process variables in a solid state fermentation system. *Process Biochemistry*, **31**: **669 - 678**.

Solis-Pereira, S., Favela-Torres, E., Biniegra-Gonzalez and Gudenez-Rojas, M. (1993). Effect of different carbon sources on the synthesis of pectinase by *Aspergillus niger* in submerged and solid state fermentation. *Applied Microbiology and Biotechnology*, **39**: **36 – 41**.

Sudgen, C and Bhat, M.K. (1994). Cereal straw and pure cellulose as carbon sources for growth and production of plant cell wall degradation enzymes by *Sporotrichum thermophile. World Journal of Microbiol and Biotechnology*, **10**: **444 - 251**.

Swift, J. (1972). The estimation of mycelial biomass by determination of the hexosamine content of wood tissues decayed by fungi. *Soil Biological and Biochemistry*, **5: 321 - 322**.