SCREENING OF MICROORGANISMS FOR BETA-MANNANASE PRODUCTION IN SOLID STATE FERMENTATION

Saw, H. Y. [1], Wong, Y. P. [1], Janaun, J. [1,] *, Prabhakar, A. [1], Subbarao, D. [1], Anton, A. [2], Wong, C. M. V. L. [2], Kim, C. H. [3], & Krishnaiah, K. [1]

[1]School of Engineering and Information Technology,
Universiti Malaysia Sabah, Locked Bag No. 2073, 88999 Kota Kinabalu, Sabah, Malaysia.
[2] Biotechnology Research Institute,
Universiti Malaysia Sabah, Locked Bag No. 2073, 88999 Kota Kinabalu, Sabah, Malaysia.
[3] Korea Research Institute of Bioscience and Biotechnology (KRIBB),
52 Eoeun-dong Yuseong-gu, Daejon, 305-333, Korea.

ABSTRACT. *A total of 15 strains were isolated and screened for beta-mannanase production at temperatures of 30, 45, and 65 ^{o}C in solid state fermentation using palm kernel cake (PKC) as substrate. The operating conditions were 20 % of inoculum, 110 % moisture content, pH 7.0, and PKC particle size 0.85 mm. At different temperatures, the highest beta-mannanase producers were identified: at 30 ^{o}C, isolate TW1 with 339.96 U/g dry PKC at 120 h; at 45 ^{o}C, isolate S51 with 73.03 U/g dry PKC at 12 h; at 65 ^{o}C, isolate GJ1-2 with 7.63 U/g dry PKC at 72 h. Isolate TW1 appeared to produce the highest enzyme activity when compared relatively with the other strains, and was selected for further investigation.*

KEYWORDS: Beta-mannanase, palm kernel cake, screening, solid state fermentation

INTRODUCTION

Palm kernel cake (PKC), an oil extraction residue from the palm oil industry, is a feed component in animal feed formulation (Hishamuddin, 2001). While PKC is a suitable and valuable feed source for ruminants like beef and dairy cattle, its inclusion in poultry feed formulation is only up to about 20 %, due to its high shell content and unfavourable fiber composition that cause indigestion and give low metabolizable energy, causing adverse effects to poultry (Abu and Yeong, 1999; Chin, 2002). The unfavourable fiber composition of PKC comes from its cell wall, mainly in the form of beta-mannan type hemicellulose with some cellulose (Jaafar Daud *et al*., 2004). Sundu and Dingle (2003) reported that PKC contains up to 78 % hemicellulose and 12 % cellulose. As the solution to high shell content in PKC is applying enhanced sieving technique to reduce the shell content, the problem of unfavorable fiber composition requires biotechnological improvement to PKC (Chin, 2002). This biotechnological improvement includes microbial and/or commercial enzymatic degradation of PKC to release digestible sugars, with mannose as the intended product, which can be fully absorbed and metabolized by poultry. The choice of enzyme is beta-mannanase (Sundu and Dingle, 2003; Jaafar Daud *et al*., 2004), and this enzyme has been useful in bioconversion of biomass wastes to fermentable sugars and upgrading of animal feedstuff (Lin and Chen, 2004).

Beta-mannanase can be isolated from bacterium, fungus, and plant sources (Hossain *et al*., 1996). Its production by microorganisms is promising due to low cost, high production rate, and readily controlled conditions (Feng *et al*., 2003). In view of this, it is desirable to perform microbial degradation of PKC through solid state fermentation (SSF). SSF is defined as the fermentation involving solids in the absence or near absence of free water, where the

substrate must possess enough moisture to support the growth and metabolism of microorganism (Pandey, 2003). From the literature, a wide selection of microorganisms has been identified as beta-mannanase producers. Among them are *Bacillus* sp. (Hossain *et al*., 1996), *Sclerotium* sp. (Gübitz *et al*., 1996), *Aspergillus* sp. (Lin and Chen, 2004), and *Trichoderma* sp. (Ferreira and Filho, 2004). However, reported microorganisms that can produce beta-mannanase through SSF with PKC as substrate are limited to *Bacillus* sp. and *Aspergillus* sp. (Chin, 2002; Ong *et al*., 2004; Noraini *et al*., 2004). Screening for more potential beta-mannanase producers had been performed by Saw *et al*. (2005), but the types of enzymes produced had not been characterized and the work was limited to submerged fermentation.

This paper reports the preliminary screening for microorganisms isolated in Sabah, Malaysia, and the determination of their beta-mannanase activities in solid state fermentation using PKC as substrate, and at different temperatures.

MATERIALS AND METHODS

Source of microorganisms

14 strains of microorganism were obtained from the Biotechnology Research Institute (BRI), Universiti Malaysia Sabah (UMS). They were isolated at two different temperatures. At 45 ^{o}C, the strains were isolates S51, S52, S72, S76, S86, S87, S811, S812, G1.1, and G1.2. At 65 ^{o}C, the strains were isolates GJ1-2, GJ2-1, GJ4B, and GJ8a. These strains were grown at their respective temperatures and maintained on nutrient agar medium, stored at 4 ^{o}C, and sub-cultured fortnightly. Isolate TW1 was isolated from unsterilized PKC at 30 ^{o}C in the Chemical Engineering Programme Laboratory, School of Engineering and IT, UMS. It was grown and maintained on Potato-Dextrose-Agar (PDA) medium, stored at 4 ^{o}C, and sub-cultured fortnightly.

Seed culture preparation

The 14 isolates from BRI were each inoculated into 25 ml of sterilized fermentation medium containing 1.5 g/l locust bean gum, 1.0 g/l yeast extract, 1.0 g/l polypepton, 1.0 g/l NH_4NO_3, 1.4 g/l KH_2PO_4, 0.2 g/l $MgCl_2$, and 10 ml tap water in 150 ml conical flasks. The initial pH was adjusted to 7.0 and the isolates were cultured at 45 and 65 ^{o}C accordingly for 24 hours at 180 rpm (Abe *et al*., 1994; Chin *et al*., 2005; Chin, 2007). For isolate TW1, spores were harvested by adding 10 ml of sterilized distilled water containing 0.1 % Tween 80 to a sporulated (7-days-old) PDA slant culture.

Solid state fermentation

PKC was obtained from IOI Edible Oils Sdn. Bhd., Sandakan, Sabah, Malaysia. It was sieved with BSS 410 screens to obtain a constant particle size of 0.85 mm, oven-dried at 90 – 100 ^{o}C for 24 h, and cooled to room temperature in desiccators filled with silica gels prior to use. In the SSF for each isolate, 5 g of PKC was mixed with a certain amount of mineral medium and 1 M NaOH in a 200 ml conical flask to obtain a constant 110 % moisture content (dry weight basis) and initial pH of 7.0. The mineral medium contained 2.0 g/l KH_2PO_4, 5.0 g/l NH_4NO_3, 1.0 g/l NaCl, and 1.0 g/l $MgSO_4.7H_2O$. The sample was autoclaved at 121 ^{o}C for 20 minutes, cooled to room temperature and inoculated with a constant 20 % (dry weight basis) of isolate. The flask was incubated at its isolation temperature (30, 45 or 65 ^{o}C) in an incubator, with the relative humidity maintained at 80 – 90 % by a tray of sterilized distilled water. The flask was removed from the incubator at each sampling time and subjected to

immediate enzyme assay. All experiments were done in triplicate (Chin *et al*., 2005; Chin, 2007).

Beta-mannanase extraction and assay

1 g of fermented sample was extracted using 9 ml of sterilized 0.2 mM sodium phosphate buffer pH 7.0 by vortexing the mixture for 5 min. 2.5 ml of the supernatant was mixed with 2.5 ml of 1.0 % locust bean gum solution and 2.5 ml of 50 mM phosphate buffer pH 5.0. After 15 min of incubation at 55 ^{o}C, the liberated reducing sugars (mannose equivalents) were estimated by dinitrosalicylic acid (DNS). The colour developed was read at 575 nm using a spectrophotometer with mannose as standard. A blank contained 2.5 ml of 50 mM phosphate buffer pH 5.0, 2.5 ml of 1 % locust bean gum solution and 2.5 ml of distilled water was prepared. One unit (IU) of beta-mannanase was defined as the amount of enzyme releasing 1 µmol mannose equivalent per minute under the assay condition (Miller, 1959; Chin *et al*., 2005; Chin, 2007).

RESULTS AND DISCUSSION

This work is a preliminary screening for beta-mannanase producers in SSF with PKC as substrate. The parameters for SSF are defined, namely the substrate particle size, inoculum size, moisture content, initial pH, and temperature (Barrios-González *et al*., 2005). For preliminary work, these parameters are set constant. The SSF temperature is set according to the isolation temperature for each isolate.

Table 1 shows the maximum beta-mannanase activity for each isolate in SSF at each isolation temperature. Beta-mannanase activity is detected with each strain. At 30 ^{o}C, there is only 1 strain, namely TW1 that produces 399.96 IU/g dry PKC at 120 h. At 45 ^{o}C, there are 10 strains with the maximum enzyme activity ranging from 8.24 – 73.03 IU/g dry PKC at a time frame ranging from 12 – 144 h. Isolate S51 produces the highest beta-mannanase among them at 73.03 IU/g dry PKC at 144 h. At 65 ^{o}C, there are 4 strains with the maximum enzyme activity ranging from 6.79 – 7.63 IU/g dry PKC at a time frame ranging from 72 – 144 h. Isolate GJ1-2 produces the highest beta-mannanase among them at 7.63 IU/g dry PKC at 72 h.

Table 1. Maximum beta-mannanase activity of isolates in SSF at different isolation temperatures.

Temperature (^{o}C)	Isolate	Time (h)	Maximum Enzyme Activity (IU/g dry PKC)
30	TW1	120	339.96
45	S51	144	73.03
	S52	48	55.66
	S72	120	25.64
	S76	36	29.79
	S811	48	20.87
	S812	72	22.45
	S86	36	26.11
	S87	12	29.19
	G1.1	24	15.91
	G1.2	72	8.24
65	GJ1-2	72	7.63
	GJ2-1	144	7.15
	GJ4B	144	7.01
	GJ8a	144	6.79

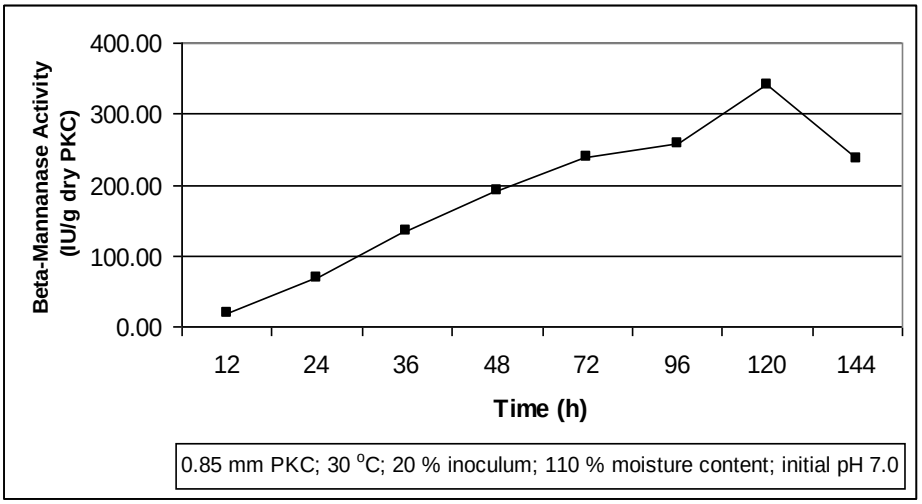

Figure 1. Beta-mannanase activity profile for isolate TW1.

From the 15 strains, it is observed that the isolates at a lower isolation temperature produce higher beta-mannanase activity in SSF. The top beta-mannanase producer from this work is isolate TW1 at 30 ^{o}C. The enzyme activity profile of isolate TW1 is shown in Figure 1. Table 2 shows the beta-mannanase production by isolate TW1 with other beta-mannanase producers in SSF of PKC reported in the literature. All the strains are beta-mannanase producers at 30 ^{o}C. The enzyme activity of isolate TW1 is relatively higher when compared to the works by Chin (2007) and Noraini *et al*. (2004), where *Bacillus subtilis* and *Aspergillus niger* were used respectively and each operating parameters had been optimized. Comparing with the work by Ong *et al*. (2004), isolate TW1 produces about 6.6 fold less enzyme activity, where the operating parameters of each study vary. In spite of the lack of the optimization of operating parameters in the SSF of isolate TW1, its beta-mannanase activity remains high comparatively. The optimization of its operating parameters could probably lead to higher enzyme activity. Isolate TW1 is a potentialy good beta-mannanase producer.

Table 2. Comparison of isolate TW1 with other beta-mannanase producers in SSF of PKC.

Strain	Temp. (^{o}C)	Incubation time (h)	Inoculum size (%)	Moisture Content (%)	Initial pH	Beta-mannanase activity (IU/g)	Author
A. niger	30	192	10	60	NS[a]	2252.00	Ong *et al*. (2004)
Isolate TW1	**30**	**120**	**20**	**110**	**7.0**	**339.96**	**This work**
B. subtilis	30	36	20	150	7.0	334.00	Chin (2007)
A. niger	30	72	10	60	6.0	297.48	Noraini *et al*. (2004)

[a] Not stated

CONCLUSION

Screening for beta-mannanase producing microorganisms for 15 strains isolated at 30, 45 and 65 ^{o}C respectively was performed via SSF with 20 % of inoculum, 110 % moisture content, initial pH 7.0, and PKC particle size 0.85 mm. Each strain is selected to give beta-mannanase activity, ranging from 6.79 – 339.96 IU/g dry PKC at a time frame of 12 – 144 h. At 30 ^{o}C, isolate TW1 gives 339.96 IU/g dry PKC at 120 h. At 45 ^{o}C, isolate S51 gives the highest enzyme activity at 73.03 U/g dry PKC at 12 h, and at 65 ^{o}C, isolate GJ1-2 gives the highest enzyme activity at 7.63 U/g dry PKC at 72 h. Among all the strains, isolate TW1 produces the highest enzyme activity, and its enzyme activity is relatively high when compared to other beta-mannanase producers in the SSF of PKC in the literature. Isolate TW1 is a potential good beta-mannanase producer and is selected for further investigation.

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