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Studies on Extraction of Mannanase Enzyme by *Aspergillus terreus* SUK-1 from Fermented Palm Kernel Cake

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Abstract: Microbial mannanases have become biotechnologically important in industry but their application is limited due to high production cost. In presents study, the extraction of mannanase from fermented Palm Kernel Cake (PKC) in the Solid State Fermentation (SSF) was optimized. Local isolate of *Aspergillus terreus* SUK-1 was grown on PKC in (SSF) using column bioreactor. The optimum condition were achieved after two washes of fermented PKC by adding of 10% glycerol (v/v) soaked for 10 h at the room temperature with solvent to ratio, 1:5 (w/v).

Key words: Mannanase, *Aspergillus terreus* SUK-1, extraction, solid state fermentation, palm kernel cake

INTRODUCTION

Solid state fermentation is a fermentation process that involved the growth of microorganism on moist solid materials in absence or near the free flowing water (Vyas and Deepak, 2005; Sabu *et al*., 2006). As it was reported that SSF technique is yet to be explored for mannanase production (Noraini *et al*., 2004; Ong *et al*., 2004; Regulapati *et al*., 2007; Abd-Aziz *et al*., 2008; Norita *et al*., 2010) despite it has many advantages such as lower capital investment, improved product recovery and easy downstream processing etc. over the conventional submerged process (Mitchell and Lonsane, 1993; Pandey *et al*., 2001; Naveena *et al*., 2003).

Mannanase enzyme is important in paper industry including bioleaching pulp (Gubitz *et al*., 1996), waste bioconversion of biomass to fermentable sugars (Chandrakant and Bisaria, 1998), Increasing the quality of feed quality (Marini *et al*., 2006) and reduce viscosity of coffee extracts (Hagglund *et al*., 2003). Furthermore, the mano-oligosaccharides which derived from the hydrolysis of mannanase and mannan have been report to use as no nutritional food additives selective growth of human-beneficial intestinal micro flora, *Bififobacterium* species (Mitsuoka, 1990).

Various sources of mannanase enzymes that have been reported in previous studies either derived from animal sources, plants and microorganisms. According to (Ibrahim, 2007), the production of enzymes from microorganisms is much higher and cost effective. Filamentous fungi are commonly used for enzyme production in SSF since have ability to secrete large amount of protein into the growth medium (Van Zyl *et al*., 2009). Many previous studies using PKC as a cheap medium for mannanase production in SSF by filamentous fungi, *Aspergilus* have been report previously (Abdeshahiann *et al*., 2010; Rashid *et al*., 2012). This is because that *Aspergillus* species have been known as potential fungi in the production of a wide range of microbial enzyme (Gao *et al*., 2008). Therefore, in this study we use our locally strain, *Aspergillus terreus* SUK-1, which was isolated from palm oil mill sludge and capable to enhanced mannanase production (Rashid *et al*., 2011).

A few studies on enzyme extraction in SSF have been reported in the literature; mostly studies were reported to be more focused on enzyme production. To the best of author's knowledge, no reports on mannanase recovery from the fermented PKC in SSF have been published yet. In the present study, the optimization of the various factors affecting the extraction of mannanase enzyme from fermented PKC under SSF in column reactor was reported.

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MATERIALS AND METHODS

Microorganism: A local isolate *Aspergillus terreus* SUK-1 from School of Biosciences and Biotechnology, Universiti Kebangsaan Malaysia (UKM) were grown and maintained on Potato Dextrose Agar (PDA). Spore suspension of 10^7 spore mL^{-1} was prepared by harvesting from 7-days old cultures of both molds with 15 mL sterile distilled water.

Substrate: Palm Kernel Cake (PKC) was supplied by Malaysian Agriculture Research and Development (MARDI) and used as a solid substrate. PKC was ground to a particle size of 2 mm.

Mannanase production by *A. terreus* SUK-1 in SSF bioreactor: The mannanase production in SSF was performed in a stainless steel horizontal bioreactor with dimension of 70 cm height and 22 in internal diameter (Working volume 10 L). It consists of three layers of trays where each of them can be loaded with 0.3 kg of PKC and medium optimized (0.5% (g/g PKC) proteose peptone and 1% (g/g PKC) urea) with ratio initial moisture 1:0.75 (w/v) (Rashid *et al*., 2012). Each of the trays (0.3 kg PKC) was inoculated 5.5% (w/v) spore suspension of *A. terreus* SUK-1. The mixtures were performed aseptically outside the column of bioreactor. The airflow rate in the bioreactor was adjusted to 3 L min^{-1}. Meanwhile, the temperature was maintained at 30°C by the circulation of temperature-controlled water. All the trays in the SSF bioreactor were incubated for 4 days.

Mannanase extraction in column bioreactor: After 4 days of fermentation process, all the trays containing of fermented PKC (0.9 kg) were removed out from the column bioreactor. The fermented PKC were loading out from trays and mixing together in the column bioreactor for mannanase extraction process. In this study, column bioreactor acts as the chamber of enzyme extraction. Mannanase enzyme was extracted from fermented PKC by adding 900 mL (substrate to the solvent ratio; 1:10 (w/v)) in column bioreactor. The aerator pump with aeration rate of 4 L min^{-1} is switched on for agitation fermented PKC's mixture for 24 h at the room temperature. After 24 h of soaking time, the mixture is discharged out to the outlet of column bioreactor and filtered using cheese cotton to collect the extract of crude mannanase. The extract was centrifuged at 10,000x g for 10 min and the supernatant was stored at 4°C. This supernatant was used as the source of mannanase enzyme. The parameters selected for this study were soaking time, type of solvents, volume of solvent, physical state of extraction condition and number of washes. Data presented are the averages of three replicates Duncan's Multiple Range (DMR) test for all data.

Mannanase enzyme assay: Mannanase activity was carried out according to the method described by McCleary (1978) using Azo carob Galactomannan as substrate. One unit (U) of mannanase activity was defines as mannose realesed/min/g of substrate.

Statistical analysis: The data were analyzed statistically by ANOVA and Duncan's Multiple Range Test (DMR) using software packages SPSS version 19.0. All measured values are the averages of three replicates and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Effect of soaking time for mannanase extraction: The soaking time was optimized for optimum mannanase enzyme recovery from fermented PKC. As shown in Fig. 1, the soaking time period was varying from 1 to 24 h. Overall, the recovery of mannanase has been improved when the soaking time period was prolonged. The Fig. 1 indicated that 10 h of soaking time was sufficient for maximum recovery of mannanase enzyme (18.63±1.2 U g^{-1}) from fermented PKC. After 10 h of soaking time, the recovery of mannanase enzyme remained constant. It was postulated that the minimum time for the total penetration of solvent through the fermented PKC was achievable (Palit and Banerjee, 2001). Prolonging the soaking time of the extraction process resulted in loss of enzyme activity (Singh *et al*., 1999; Chandra *et al*., 2008). Different observation can be seen which showed no inhibition of enzyme activity when soaking time is prolonged. However, the 10 h soaking time obtained in this study is too long when compared with

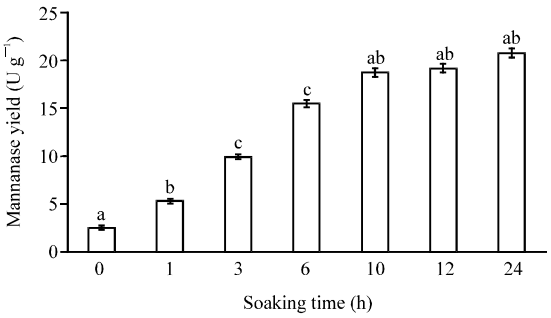

Fig. 1: Effect of soaking time on mannanase extraction. Means, on each bar, followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to Duncan Multiple Range test

another studies of enzyme extraction in SSF using the shaking flask (Palit and Banerjee, 2001) reported that 2.5 h of soaking time is sufficient for the recovery of amylase from fermented rice bran. There is also a previous report concluded that 1 h soaking time is the optimum time for galactosidase cellulase recovery (Singh and Garg, 1995; Smiley *et al*., 1976; Fadel, 2000). Generally, enzyme extraction studies using shaking flask showed good recovery of the enzyme in a short time. This is because that shaking flask condition for enzyme extraction is more efficient in providing a large surface area for solvent to leaching out of enzymes from fermented substrate in SSF (Chandra *et al*., 2008).

Effect of solvent on mannanase extraction: Different solvents were used in this study included distilled water, 0.2 M acetate buffer pH 5.5, of 10% (v/v) methanol, 10% (v/v) glycerol, 10% (v/v) ethanol and 10% (v/v) acetone. From Figure 2 showed that the organic solvent, 10% (v/v) glycerol gave the best result yielding about of 24.38±1.49 U g^{-1}. This result suggested that organic solvent is more efficient for enzyme recovery (Ikasari and Mitchell, 1996; Tunga *et al*., 1999; Palit and Banerjee, 2001; Negi and Banerjee, 2009). This explanation can be described by the Debye-Huckel theory (Maron and Prutton, 1965). According to above theory, the lower dielectric constant, the stronger will be the interaction between the enzymes and solvent. Organic solvents possess a lower dielectric constant compared with other inorganic solvent, i.e., distilled water thus may able to explain in this study that the higher recovery of crude mannanase using glycerol solvent. It was attributed that the organic solvents easily form a hydrogen bond with the protein molecule, granting a good stability of enzyme molecules during enzyme extraction process (Stryer, 1975). Comparable results were obtained that glycerol solvents was found to be the best extraction solvent in recovery of amylase and glucoamylase, respectively (Palit and Banerjee, 2001; Negi and Banerjee, 2009). Tunga *et al*. (1999) reported that among organic solvents i.e., glycerol, acetone, ethanol and methanol, the ethanol solvent enhanced significantly in protease recovery from fermented wheat bran. In contrast, inorganic solvent, i.e., distilled water served as the good solvent in enzyme extraction due to readily available, save and low cost (Ahmad, 2008; Chandra *et al*., 2008). In the present study, an increase of 25.14% for mannanase recovery can be obtained using glycerol solvent than distilled water. For this reason, subsequent experiments were carried out with glycerol solvent for further optimization.

Effect of solid to solvent ratio on mannanase extraction: In Solid State Fermentation (SSF) system free flowing

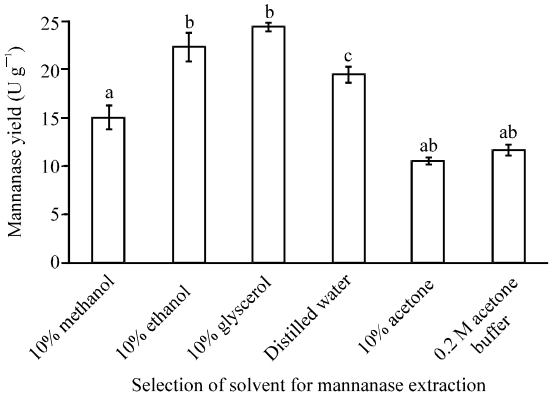

Fig. 2: Selection of solvents on mannanase extraction. Means, on each bar, followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to Duncan Multiple Range test

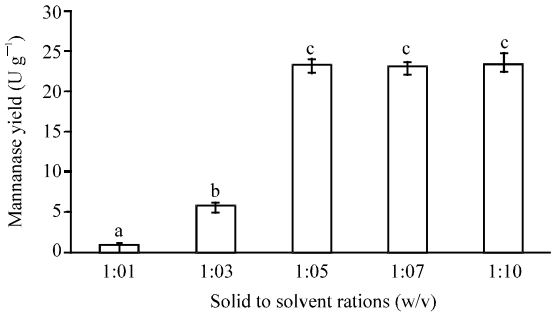

Fig. 3: Effect of solvent volume on mannanase extraction. Means, on each bar, followed by the same letter are not signifi cantly different ($P \leq 0.05$) from each other according to DMR test

solvent is very much limited. Thus adequate amount of solvent is required to leaching out of mannanase enzyme from fermented PKC. Different solid to solvent ratio (w/v) selected for this study were 1:1 (w/v), 1:3 (w/v), 1:5 (w/v), 1:7 (w/v) and 1:10 (w/v) using 10% glycerol (v/v) for 10 h soaking time. Figure 3 shows the yield of mannanase achieved by 0.896±0.23 U g^{-1} using solid to solvent ratio of 1:1 (w/v) and increased slightly to 5.806±0.43 U g^{-1} when the volume of solvent was increased to the ratio of 1:3 (w/v). This observation can be explained that lower volume of solvent resulted to the insufficient solvent volume to penetrate the solid fermented mass (Ramesh and Lonsane, 1988; Palit and Banerjee, 2001; Chandra *et al*., 2008). It was found that the solid to solvent ratio of 1:5 (w/v) i.e., 5 L solvent in 1 kg of fermented PKC was sufficient for the extraction of mannanase enzyme yielding about of 23.24±0.83 U g^{-1}. Hence, the yield mannanase remains constant even

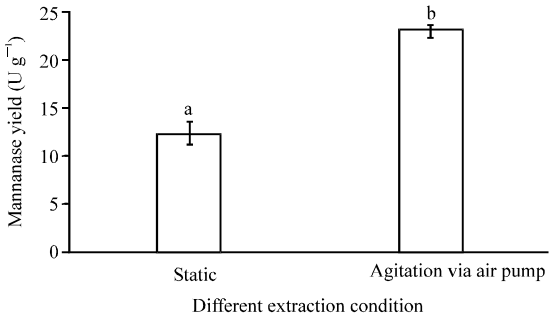

Fig. 4: Effect of different extraction condition on mannanase extraction. Means, on each bar, followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to DMR test

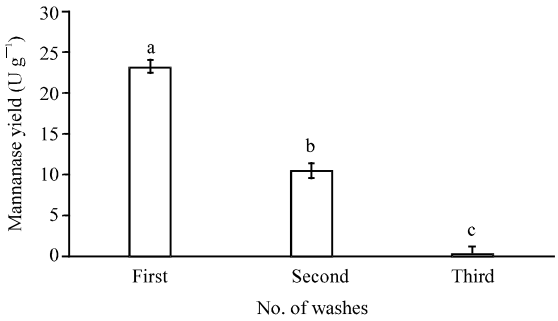

Fig. 5: Effect of number of washes on mannanase extraction. Means, on each bar, followed by the same letter are not signifi cantly different ($p \leq 0.05$) from each other according to DMR test

though the volume of solvent was increased by many folds. Higher solvent to solid ratios caused dilution of the enzyme product thus reducing enzyme activity (Lonsane and Krishnaiah, 1992). Different observation obtained in this study which the yield of mannanase did not show significantly reduction when the volume of solvents was increased from 5-10 L in 1 kg of fermented PKC. However, it is necessary to use of low PKC substrate to the solvent ratio to maintain the product in a concentrated form to achieve the highest recovery of the enzyme. Therefore, the ratio of 1:5 (w/v) is selected for further experiments.

Effect of physical state of mannanase extraction: Two different extraction condition i.e., stationary and agitation via air pump were employed during extraction of mannanase enzyme from fermented PKC in column biorector. The results show that the agitation via air pump was more effective for mannanase extraction than static condition which each of them gave mannanase yield, 23.2±0.83 and 12.17±0.5 U g^{-1}, respectively (Fig. 4). The agitation via air pump gave 47.5% more mannanase yield compared to the statistic condition. This probably because there was a drag force was added by air pump which facilitated the extraction of mannanase than static condition.. Similar observation obtained that agitation and shaking condition was most efficient ways in enzyme extraction from fermented substrate in SSF, thus supporting the result in this study (Tunga *et al*., 1999; Palit and Banerjee, 2001; Chandra *et al*., 2008).

Effect of number of washes on mannanase extraction: In earlier experiment, single washing with the solvent was applied to extract mannanase enzyme from fermented PKC. A few number of washing (i.e., single second and third) with solvents in repeated manner for mannanase extraction from fermented PKC were studied (Fig. 5). The results show that the first two washings were sufficient to recover in large amounts of mannanase enzyme which were the first and second washings recovered 23.2±0.83 and 10.54 ±0.93 U g^{-1}, respectively. Meanwhile, the third washings achieved mannanase yield of 0.32±0.93 U g^{-1} suggesting that the first two washings were enough to extract out completely of mannanase enzyme from fermented PKC. Similar observation has been made by previous reports that the first two washings were optimum on the enzyme extraction from fermented substrate in SSF (Ghildyal *et al*., 1992; Singh *et al*., 1999; Castilho *et al*., 2000; Chandra *et al*., 2008).

CONCLUSION

It can be concluded that 10% glycerol solution (v/v) with two washes for 10 h soaking time under agitation via air pump at solvent to ratio of 1:5 (w/v) resulted in the optimum mannanase extraction from fermented PKC.

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