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Palm Kernel Cake as Substrate for β-Mannanase production by *Bacillus subtilis* ATCC3366 under Submerged and Solid State Fermentations

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Abstract

Beta-Mannanase activity and growth profile of Bacillus subtilis ATCC3366 during submerged fermentation (SmF) and solid state fermentation (SSF) using palm kernel cake as substrate were examined in this study. After 36 h of fermentation, β-Mannanase produced by Bacillus subtilis in SmF system was recorded at 8 U/ml, whereas in SSF system was 230 U/g dry PKC. In SmF system, a long lag phase of 28 h were observed before the number of viable cells reached the maximum of 2.08×10^8 CFU/ml at 36 h; whereas in SSF system was 3.75×10^9 CFU/ml at 24 h, with no lag phase observed. Optimum conditions of enzyme activity were also examined. The optimum pH was 7.0, and optimum temperature was 55°C.

Keywords: Palm kernel cake (PKC); Bacillus subtilis ATCC3366; Solid-state Fermentation (SSF); Submerged Fermentation (SmF); β-mannanase

1.0 Introduction

Palm Kernel Cake (PKC) is a byproduct from the extraction of palm kernels [1] and is available in large quantities averaging 50% of palm kernels utilized [2]. Malaysia currently produces an annual quantity of 1.4 million tones of PKC as solid residue left behind after the extraction of oil from the kernels of the palm fruits [3]. Being a good source of mannan (25 to 32 %) [4] PKC is expected to be a suitable carbon source for β-Mannanase enzyme production.

Mannan based carbon sources commonly used to cultivate β-Mannanase producing microorganism included copra meal, locust bean gum, and konjac mannan [5]. Although locust bean gum represents the most common carbon source; however, no data have demonstrated the best carbon source to cultivate microorganism [6].

β-Mannanses are useful in many fields including biobleaching of pulp in paper industry; bioconversion of biomass wastes to fermentable sugars; upgrading of animal feed stuff; and manno-oligosacchrides production [6, 7, 8, 9].

Utilization of *Bacillus subtilis* in fermentation for enzyme production is not new. *Bacillus subtilis* has been reported in production of cellulases [10] and α-amylase [11] from banana waste. On top of that, many strains of β-Mannanase producing microorganisms screened reported to be *Bacillus*, including *Bacillus* sp. KK01 [8]; *Bacillus licheniformis* [7]; and

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Bacillus sp. [5]. In view of this, ability of *Bacillus subtilis* ATCC 3366 to growth and produce β-Mannanse in PKC liquid media was investigated in this present study.

2.0 Approach and Methods

2.1 Microorganism and Inoculum Preparation

Bacillus subtillis ATCC3366 was maintained on nutrient agar (Pepton from meat, 5g/L; meat extract, 3g/L; agar-agar 12g/L; pH 7.0), stored at 4°C, and subcultutred every 4 weeks.

2.2 Substrate

Palm kernel cake (PKC) was obtained from *IOI Edible Oils Sdn. Bhd*. It was sieved through standard mesh sieves to obtain particles size of 850 μm, and stored in container at 4°C until use.

2.3 Submerged Fermentation Operation

Submerged fermentation (SmF) was carried out in 150 mL conical flasks containing 25 ml of inoculated PKC filtrate or locust bean gum liquid medium.

PKC filtrate liquid medium was prepared from 20 g of PKC boiled for 20 minutes in 500 ml distilled water; 1g/L yeast extract; 1g/L polypepton; 1g/L NH_4NO_3; 1.4g/L KH_2PO_4; 0.2g/L $MgCl_2$; 10mL tap water; and pH 7.0.

Locust bean gum liquid medium consisted of locust bean gum.

2.4 Solid State Fermentation Operation

A loop full of cells from nutrient agar were transferred into 150 mL conical flaks containing 30 mL locust bean gum liquid medium Solid state fermentation (SSF) was carried out in 200 mL conical flasks containing 5 g (dry weight) of PKC. filtrate liquid medium, which prepared from 20 g of PKC boiled for 20 minutes in 500 ml distilled water; 1g/L yeast extract; 1g/L polypepton; 1g/L NH_4NO_3; 1.4g/L KH_2PO_4; 0.2g/L $MgCl_2$; 10mL tap water; and pH 7.0.

2.5 Cell Concentration Determination

In submerged fermentation, at every 12 hrs internal, 1 ml of fermentation medium was aseptically diluted to suitable concentration by decuple dilution. One ml of diluted mixture was transferred to agar plates in quintuplicate to perform plate count of bacteria using drop plate method.

2.6 β-Mannanase Enzyme Assay

The reaction mixture consisted of 2.5 ml of 1% locust bean gum solution, 0.5 ml of 50 mM sodium acetate buffer (pH 7.0), and 0.5 ml crude enzyme extract. After 15 min of incubation at 55°C, the liberated reducing sugars (mannose equivalents) were estimated by dinitrosalicylic acid (DNS) method of Miller (1959) [12]. The colour developed was read at 575 nm using spectrophotometer (Novespec II). Mannose was used as the standard. The

blank contained 0.5 ml of 50 mM sodium acetate buffer (pH 5.0), 2.5 ml of 1 % locust bean gum solution and 0.5 ml of distilled water. One unit (IU) of β-Mannanase is defined as the amount of enzyme releasing 1 µmol mannose equivalent per minute under the assay condition.

3.0 Results and Discussion

3.1 Growth Profile of Bacillus subtilis on PKC medium

During the fermentation, *Bacillus subtilis* ATCC3366 was inoculated in PKC liquid medium, where PKC filtrate was the sole carbon source. Referring to Figure 1, the bacteria were able to take up the dissolved nutrients from PKC medium and convert them into biomass. The growth was a typical batch growth curve, which included lag phase and exponential phase.

The cells undergone its first lag phase immediately after inoculation and lasted for the first 24 hrs of fermentation in order to adapt to new environment. The lag phase followed by an exponential phase or log phase. After the adaptation period, the cells were now able to multiply rapidly, and the cells number increased exponentially between 24 hrs to 36 hrs of fermentation.

The exponential phase ended at 36 hrs of fermentation, followed by a second lag phase of the growth cycle. A second lag phase was possible in PKC medium since the PKC filtrate was a complex substrate, which contained more then one carbon source. After one carbon was exhausted, the cells probably were in the process of adapting their metabolic activities to utilize the second carbon source. Since the first carbon source was more readily utilize than the second, the lag phase appeared to be longer then the first.

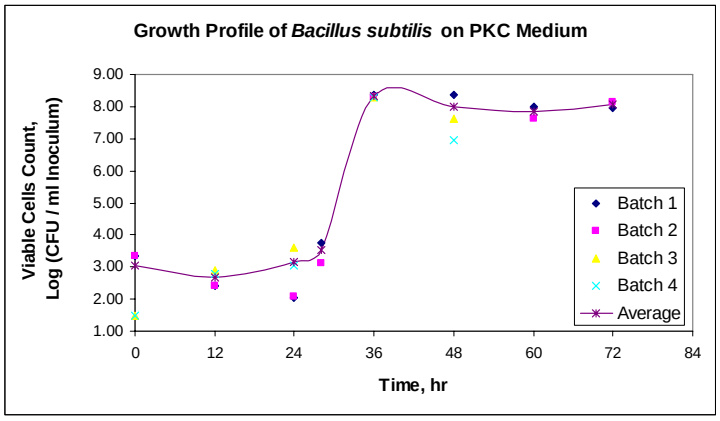

Figure 1: Growth profile of *Bacillus subtilis* ATCC3366 in PKC medium. Fermentation conditions: 30°C; 150 rpm of aeration; and initial pH 7.0.

3.2 Production of β-Mannanase of Bacillus subtilis

β-Mannanase enzyme activity for *Bacillus subtilis* ATCC 3366 after 36 hrs of fermentation at 30°C was 21.39 U or 8.56 Uml^{-1}**.** One unit of enzyme is defined as the amount of enzyme which liberates 1 µmol reducing sugar per minute under the experimental conditions. Table 1 below shows the comparison of β-Mannanase enzyme activity for different strains of microbes reported during submerge fermentation. Higher enzyme activity may be achieved if medium optimization would be done.

Table 1: β-Mannanase enzyme activity of different strains microbes.

Strain	β-Mannanase enzyme activity (U ml^{-1})	Carbon Source	Fermentation Condition	Researcher
Bacillus licheniformis NK-27	212.00	LB	Aeration 0.75vvm; agitation 600 rpm; 30°C; 36 hrs	Feng *et al*. (2003)
Aspergillus niger NCH-189	28.00	Defatted copra	30°C; aeration 120 rpm; 3 days	Lin &Chen (2004)
Bacillus subtilis ATCC3366	8.56	PKC	30 °C; aeration 150 rpm 36 hrs	Current study (2005)
Bacillus sp. KK01	2.00	Copra meal	30°C; aeration 180 strokes min^{-1}; 24 hrs	Hossain *et al*. (1996)
Bacillus sp.	1.60	Copra meal	30°C; 48 hrs	Abe *et al*. (1994)

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