Growth and Survival of *Lactobacillus casei* in Rice Bran and Banana Peel Medium

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Abstract

The aim of this study was to evaluate the effect of different types of lignocellulose biomass as carbon source on the growth and survival of *Lactobacillus casei* ATCC 393. The microbe was grown as a pure culture in three types of media, such as rice bran, banana peel and MRS medium. Rice bran and banana peel were kept at 121°C for 15 minutes and the extraction was conducted to collect the turbid liquid to be used as a medium for the fermentation. The medium was inoculated with 22 hours old inoculum culture at 37°C. The viable cell counts of bacteria (CFU/mL), cell dry weight measurement (g/L), pH and sugar content (g/L) were monitored and recorded during the fermentation. The performances of the fermentations were analyzed and compared in term of biomass produced and cell viability. The finding indicates that banana peel substrate produced higher sugar content compared to rice bran. However, the growth and the survival of *Lactobacillus casei* are better in rice bran medium.

Keywords. Rice bran; Banana peel; Digestive bio-regulator; *Lactobacillus casei*

1 Introduction

Production of digestive bio-regulator is gaining interest in the community due to the health benefits offered by the microbe. Digestive bio-regulator also known as probiotics; are live microorganisms that capable to confer positive effects to the host as defined by Fuller [1]. The application is not limited to human only, it expand not only in aquatic treatment but also in the animal rearing industry [2]. Common probiotics bacteria are from lactic acid bacteria such as *Lactobacillus casei, Lactobacillus plantarum,* and *Bifidobacterium* spp. [3-8]. Numerous studies have shown that the addition of digestive bio-regulator in the ruminant's feed provides several benefits on the ruminal fermentation such as supplying lactic acid constantly, preventing excess accumulation of lactic acid, reducing production of methane and increase ruminal digestibility [9-11].

Agricultural residue or crop residue is abundantly available due to the increasing plantation in order to satisfy the demands of the increase population. In Malaysia itself, in 2009 the annual grain production is about 2.2 million tons, whereby 0.44 million tons of these amount are waste [12]. This abundance of availability has been seen as a worthy raw material in industry such as production of enzyme [13], biogas production [14-15] and in production of animal feeds and probiotics [2-3], [9]. Aside from the improvement management of vast residues, the recycle of agricultural residue in other biotechnology process offer a side source of cash to the farmers.

Rice bran and banana peel are available throughout the year in Malaysia and are reported to have high carbohydrates composition [16-17]. The application of rice bran and banana peel as new carbon sources in the fermentation are reported in many studies [17], [18-23]. However, information regarding the utilization of those raw materials for the production of digestive bio-regulator is still limited. The objective of this study was to evaluate the effect of different types of lignocellulose biomass on the growth and survival of *Lactobacillus casei.* In this study, rice bran and banana peel were chosen as the lignocellulose biomass.

2 Methods

Rice bran was procured from Kilang Beras BERNAS Sungai Besar, Selangor and banana peel was collected from the stall around Universiti Teknologi Malaysia. Forty grams of rice bran or banana peel was carefully weighted into 400 mL of distilled water, kept at 121°C for 15 minutes, centrifuged and strained through two layers of cheesecloth. The turbid liquids of rice bran and banana peel were then autoclaved at 121°C for 15 minutes. The extracts of rice bran (RB) and banana peel (BP) were used as the medium in the submerged fermentation of *Lactobacillus casei.*

2.1 Digestive Bio-regulator Culture

Lactobacillus casei ATCC 393 was obtained from American Type Cell Culture (ATCC, Virginia, USA). The culture was grown statically for 22 hours at 37°C in MRS broth with Tween 80 (peptone, 10 g/L; beef extract, yeast extract, 5 g/L; glucose, 20 g/L; dipotassium hydrogen phosphate (K2HPO4) 2 g/L; sodium acetate, 5 g/L; ammonium citrate 2 g/L; magnesium sulphate (MgSO4), 0.2 g/L; manganous sulphate (MnSO4); 0.05 g/L and Tween 80, 1 mL).

2.2 Production of Digestive Bio-regulator Biomass

The biomass production was conducted in 100 mL conical flask with 100 mL of working volume to create a microaerophilic conditions. Each medium RB, BP and MRS medium was inoculated with 1% (v/v) inoculum culture and incubated statically at 37°C for 33 hours. The pH for each medium was not controlled and sample was taken every 3 hours for chemical and microbiological analysis. Under similar growth conditions, *L. casei* was inoculated in MRS broth with Tween 80.

2.3 Chemical and Microbiological Analysis

The culture was subjected to the measurement of microbial growth, substrate consumption and the production of organic acid. The viability study was conducted using standard plate count method on MRS agar. Meanwhile, the strength of the culture was estimated at 590 nm. Cell mass concentration was quantified with dry weight measurement. The medium pH was measured using pH meter before and after the fermentation.

3 Results and Discussion

Both RB and BP medium were found to have different initial pH value, where BP medium was found to be more acidic compared to RB medium. The sugar solubility in BP medium is higher compared to RB medium. The comparison of pH value and sugar content before and after fermentation are presented in Table 1. Meanwhile, the effect of different types of medium on the growth and survival of *Lactobacillus casei* are shown in Table 2. Figure 1 and 2 show the growth and viability of *Lactobacillus casei* in RB medium and BP medium, respectively.

Table 1. Comparison of initial pH and sugar content of RB, BP and MRS medium

Medium	Initial pH	pH after 33 hour incubation	Initial Sugar Content (g/L)
Rice Bran Extract	6.11	4.52	3.42
Banana Peel Medium	5.39	5.35	8.78
MRS with Tween 80	6.50	4.14	20.00

Table 2. Comparison of fermentation of *Lactobacillus casei* in banana peel and rice bran medium at incubation time of 24 and 27 hours

	Banana peel medium		Rice bran medium	
	24 hours	27 hours	24 hours	27 hours
OD at 590 nm	0.081	0.086	0.262	0.297
Cell dry weight (g/L)	2.8	2.9	3.8	3.5
Cell viability (CFU/mL)	2.00×10^6	2.00×10^5	3.70×10^6	1.09×10^6

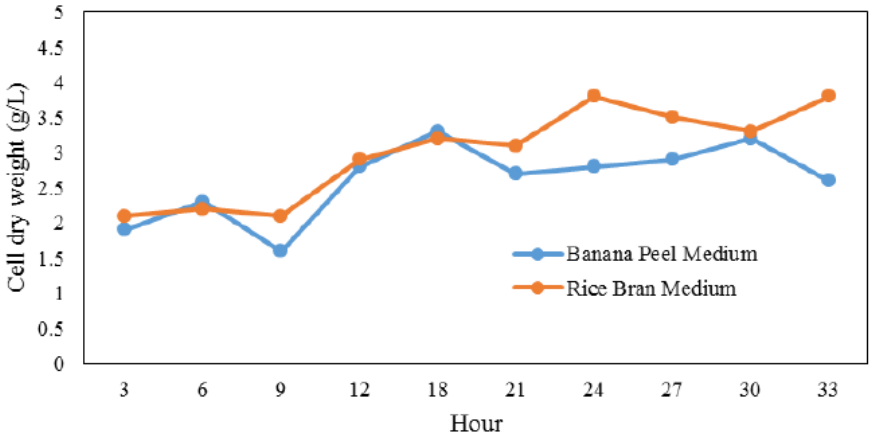

Figure 1. Cell dry weight of *Lactobacillus casei* ATCC 393 produced in rice bran (RB) and banana peel (BP) medium.

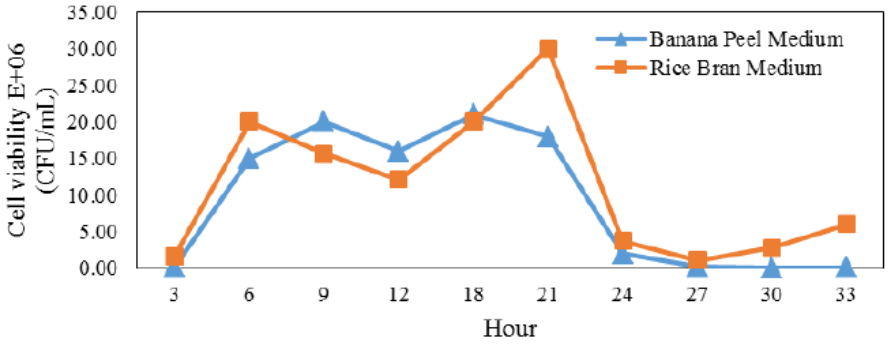

Figure 2. Cell viability of *Lactobacillus casei* ATCC 393 in rice bran (RB) medium and banana peel (BP) medium.

Sugar content in different medium is presented in Table 1. From the results depicted in Table 1, it was observed that the sugar content in BP medium was higher compared to the sugar content in RB medium. Sugar content in MRS medium is far too high when compared to the two media. Initial pH for BP medium is lower and more acidic compared to RB medium and MRS medium. Table 2 indicates that *Lactobacillus casei* was capable to grow in banana peel and rice bran. However, it was found that due to the acidic condition of BP medium (initial pH of 5.39 as shown in Table 1), the growth of *Lactobacillus casei* was not as good as in the RB medium even though the concentration of sugar in BP medium was higher compared to in RB medium. As reported by Yoon *et al.,* [24] *L. casei* was unable to survive in the acidic conditions. This finding shows that, initial pH plays an important role in the growth and survival of *L casei*. Meanwhile, fermentation of *L. casei* in RB medium seems to have a favorable response although the concentration of sugar in the RB is lower compared to BP medium. The highest biomass produced for banana peel and rice bran was 3.3g/L and 3.8g/L, respectively. The viability of *Lactobacillus casei* ATCC 393 reached 2.1 x 10^7 CFU/mL and 3.0 x 10^7 CFU/mL for both BP medium and RB medium, respectively.

4 Conclusion

The purpose of this study was to evaluate the effect of rice bran and banana peel extract as medium fermentation on the production of *Lactobacillus casei*. From the findings, BP has high sugar concentration compared to RB medium, however initial pH value of BP medium was lower and the acidity was higher compared to RB medium. The growth of *L. casei* in BP medium was not satisfying due to high acidity. Further study to determine the optimum conditions of the initial pH and solid loadings of RB and BP will be conducted to gain more viable and higher cell biomass of *L. casei*.

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