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Full paper

Effects of the Initial Rice Bran Concentration on the Production of *Lactobacillus casei* as Digestive Bio-regulator

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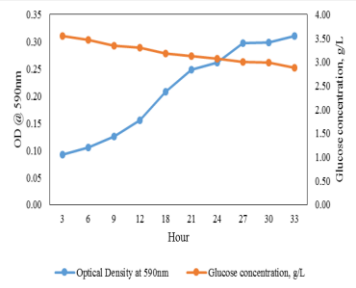
Graphical abstract



Abstract

Digestive bio-regulator or probiotic is defined as live bacteria with clinically documented health effects in humans and animals. Nowadays, there is increasing interest in probiotics from all over the world. However, the cost of producing probiotics products is still high. To reduce the cost, there is a need to study the usage of agro waste as an inexpensive substrate. The viability of the cell was counted in colony unit per mL (CFU/mL) and the growth was measured using dry weight measurement (g/mL). The sugar concentration was measured using glucose analyzer. At initial substrate concentration of 20% (w/v) and at incubation time of 10hr, the viability cell was 3 x 10^8 CFU/mL and cell dry weight was 0.0076 g/mL. From the results of this study, it is found that when the initial substrate concentration increased, the viability and growth of *Lactobacillus casei* increased.

Keywords: *Lactobacillus casei;* viability; probiotics; rice bran; agro waste

1.0 INTRODUCTION

People believed that all microorganisms were harmful and not to be consumed and the origins of all the diseases. However, the existence of non-pathogenic microorganisms has been proven directly responsible in the health regulatory of human and animals. As an example the suppression of non-pathogenic microorganisms that the pathogenic microorganisms are not causing any disturbances to the host health [1-2]. Through symbiosis, the interactions between the host and gut microorganisms resulted in mutual benefits for both side [3]. The microorganisms that capable of conferring beneficial effects to the host upon consumption are known as probiotics [4]. In animal husbandry, feed efficiency is one of the quality indicator for the health of the animal. It is also an indicator on the economical side of animal husbandry [5]. In animal husbandry, live microbials supplement is commonly known as "direct-fed microbial", which according to Seo *et al*., [6] defined as "dietary supplements that inhibit gastrointestinal infection and provide optimally regulated microbial environments in the digestive tract". It can also be known as digestive bio-regulator, where this term fittingly visualizes the roles of probiotics where they assist in re-harmonizing if there are any disturbances of the gastrointestinal tract and as first line defense in mitigating the colonization of unwanted microorganism.

The increasing interest in the production of digestive bio-regulator is correlated with the increasing awareness on food safety [7-9]. In animal husbandry, it was common to use antibiotics as an important approach in ensuring the quality in animal production and to avoid any health disability of the animal. Even though the performance of antibiotics growth promoters are going well, the adverse effects that put fear of development antibiotic-resistant pathogen leads to the prohibition of antibiotics usage in animal feeds starting with Sweden in 1986 and followed by European Union (EU) member nations in 2006 [7, 10]. In animal health, digestive bio-regulator has been reported gives significant effect in increasing feed efficiency [11]; increase the ruminal digestibility [6, 12]. The improvement in weight gain of calves during pre-weaning and weaning period [11], production of high concentration of propionate and energy efficiency and in the meantime lowering the acetate concentration [13] and many more are reported. Consequently, the industrial production of digestive bio-regulator is gaining more attention [14].

Among the digestive bio-regulator bacteria, *Lactobacillus* spp. are one of the commonly used [6]. Studies have reporting the beneficial effects of *Lactobacillus* spp. consumption in

ruminal husbandry [5, 15-16]. *Lactobacillus casei* is recognized as safe probiotic bacteria and there is no reports regarding on the safety issue found regarding *Lactobacillus casei* in animal [17]. *L. casei* has high tolerance of acid and bile, high adhesion ability to colonize the intestinal tract. These traits are valuable traits as probiotics [5]. In addition, *L. casei* able to inhibits the growth of pathogenic bacteria by pH reduction through the production of organic acids such as lactic acid, aside competing for nutrients and adhesion site against pathogens [18]. However, the production cost can be very high [19]. Therefore, many efforts to optimize the process in engineering and biological scope have been done, such as medium optimization [20], using low cost culture media [21-22] and process fermentation [23-24]. For some applications, the substrate for growth of probiotics can be produced from waste substrates such as rice bran [25], and thus, decreasing the production cost.

Inexpensive waste such as rice bran can be used as substrate. Rice bran is a by-product during milling process in the production of white rice from brown rice. Rice bran is a part between hull and white rice, and this part is reported to be the most nutritious part [26-27]. Table 1 shows the chemical composition of rice bran. Rice bran is recognized as a good source of vitamins and other nutrients, albeit still underutilized in many countries including Malaysia [28].

Table 1 Chemical composition of rice bran [27]

Chemical Composition	Compound (g/100g)
Crude protein	16.61
Crude fat	17.87
Crude fiber	24.15
Carbohydrate	33.24
Moisture	8.41

Some studies reported the successful usage of rice bran as carbon sources in fermentation process, and some of the studies is depicted in Table 2. In the study by Elok Zubaidah *et al*., [29] the concentration of rice bran used in the preparation of rice bran medium was at 12% w/v. Meanwhile, in the production of D-lactic acid, studies conducted by Tanaka *et al*., [30] rice bran used was at 100 kg/m^3 and in the studies reported by Gao *et al*., [25] the concentration of rice bran was used at 3:10 (w/w) rice bran/water mix ratio. Hence, it was hypothesized that different initial rice bran concentration has certain effects pertaining to the biomass production of *L. casei*. In addition, the application of rice bran as carbon source is still limited in the production of digestive bio-regulator. Therefore, different initial rice bran concentrations were used in this study and the effects of initial rice bran concentration to the growth and survival of *Lactobacillus casei* was evaluated. Rice bran medium in this study is used with no addition nutrients.

Table 2 The application of rice bran as carbon sources

Microorganisms	Production	Ref.
Lactobacillus casei *Lactobacillus plantarum* B_2	Synbiotic product (Probiotic-Prebiotic)	[29]
Lactobacillus rhamnosus (NBRC 3863)	Lactic acid	[25]
Lactobacillus delbrueckii subsp. *delbrueckii* IFO 3202	D-lactic acid	[30]
Newly isolated LAB	Lactic acid	[31]

2.0 EXPERIMENTAL

2.1 Microorganisms and Growth Condition

Lactobacillus casei ATCC 393 was obtained from the American Type Cell Culture (ATCC, Virginia, USA) and revitalized via spread plate method in De Man, Rogose and Sharpe (MRS) agar. Stock culture was prepared by inoculating a colony in MRS medium with Tween 80 at 37°C for 48 hour. MRS with Tween 80 (peptone, 10 g/L; beef extract, yeast extract, 5 g/L; glucose, 20 g/L; dipotassium hydrogen phosphate (K_2HPO_4) 2 g/L; sodium acetate, 5 g/L; ammonium citrate 2 g/L; magnesium sulphate ($MgSO_4$), 0.2 g/L; manganous sulphate ($MnSO_4$); 0.05 g/L and Tween 80, 1 mL) and MRS agar was obtained from Biolife. Upon reaching stationary phase, glycerol (20%) was added and this stock culture was kept at -20°C until further usage. The inoculum of *L. casei* was prepared by growing the organism without shaking in a 250 mL Erlenmeryer flask with 100 mL working volume of sterile MRS medium with Tween 80 for 22 h at 37°C.

2.2 Preparation of the Rice Bran Medium for Fermentation

The rice bran powder procured from Kilang Beras Bernas, Sungai Besar was sieved at 60-mesh sieve to filter out any foreign matters from the powder. The rice bran powder with specified percentage (w/v) was loaded with distilled water, boiled for 15 minutes, and autoclaved for 15 heat treatment. Then, the solution was cooled down at room temperature, filtered and the extract of the solution was centrifuged at 5000 rpm for 15 minutes to get the particle free medium for fermentation in order to eliminate the interference in bacteria density measurement. Then, the supernatant was autoclaved at 121°C for 15 minutes prior fermentation.

2.3 Fermentation of Rice Bran Medium by *L. casei*

The rice bran medium was inoculated with 2% (v/v) of *Lactobacillus casei.* The range of initial substrate used was from 10% to 20% (w/v). The concentration of sugar in the medium was measured before performing the inoculation. The medium was then, incubated for 22 hours. Three shake flasks with working volume of 100 mL were prepared. At 10 and 22 hours of the incubation periods, samples were taken for viability and growth analysis. For benchmark purpose, similar procedure was conducted using MRS with Tween 80 as the substrate medium.

2.4 Analytical Methods

Samples were taken at 10 and 22 hours of the incubation time. Viable cell counts (CFU/mL) were determined by the standard plate method. The temperature was set at 37°C. Sugar content was analyzed using glucose analyzer. The microbial growth was measured using optical density absorbance at 590 nm and dry weight approach. The pellet obtained after the centrifugation was dried in an oven at 70°C until constant weight was achieved. The medium pH was measured before and after fermentation was conducted.

3.0 RESULTS AND DISCUSSION

3.1 Growth of *Lactobacillus casei* in Rice Bran Medium

Figure 1 presents the growth and glucose concentration profile of *L. casei* in batch fermentation using rice bran extract (10% w/v) as medium fermentation and Figure 2 presents the growth of *L. casei* in different initial rice bran concentration and the comparison with commercial medium, MRS with Tween 80. From Figure 1, it can be observed that *L. casei* is able to grow well in the rice bran medium. Further incubation time may presenting an increase in the growth of *L. casei* in rice bran medium. This is supported by glucose concentration profile, where the glucose inside the medium are not fully consumed by *L.* casei. This shows that, *L. casei* is able to adapt and grow in the rice bran medium, even without additional nutrients. Although adding others nutrient, such as yeast extract [25] has positive effect for *L. casei*, but most of the studies are focusing on the productivity of lactic acid compared to *L. casei* biomass itself. In Figure 2, it can be seen that initial rice bran concentration affects the biomass production of the *L. casei*. The highest biomass produced (0.0076 g/mL) when 20% (w/v) of rice bran was used. The comparison with commercial medium, MRS medium with Tween 80 shows that 20% (w/v) rice bran medium has better performances than commercial medium with 1.5 fold at 22-hour incubations time. This is also in agreement with the findings by Aguirre-Ezkauriatza *et al*., [24]. This finding shows that, rice bran medium can be further optimized as alternatives substrate in the microbial production of *Lactobacillus casei*.

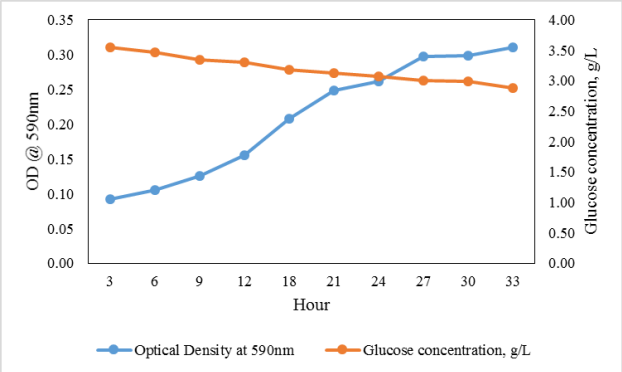


Figure 1 Growth and glucose concentration profile of *Lactobacillus casei* ATCC 393 in rice bran medium (10% w/v), T = 37°C, initial pH = 6.11

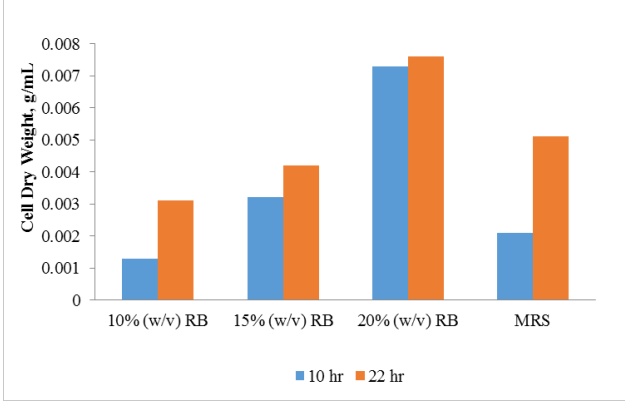


Figure 2 Effect of initial substrate concentration on dry weight of *Lactobacillus casei*, T = 37°C, initial pH = 6.11, (RB = rice bran, MRS = MRS medium with Tween 80)

3.2 Survival of *Lactobacillus casei* in Rice Bran Medium

The survival of *L. casei* in rice bran is shown in Figure 3. The survival or viability of *L. casei* was quantified by measuring the colony form unit in a milliliter. The viability is important in the production of digestive bio-regulator, as consumption of viable microorganisms give more significant health benefits compared to the consumption of dead digestive bio-regulator and for the maximum health benefits, the minimum number of probiotic organisms in a food product should be 10^6 CFU/mL [32].

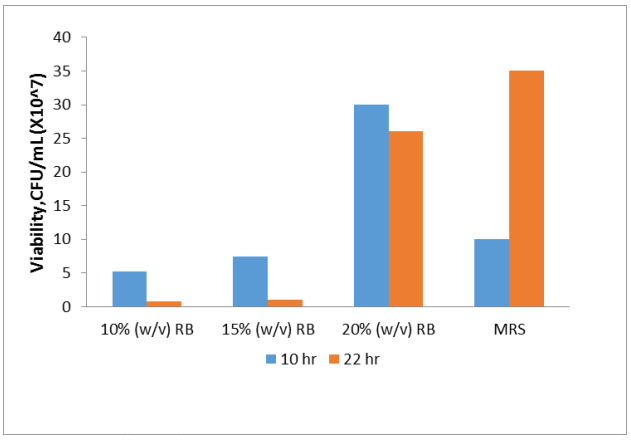


Figure 3 Effect of initial substrate concentration on the viability of *Lactobacillus casei*, T = 37°C, initial pH = 6.11 (RB = rice bran, MRS = MRS medium with Tween 80**)**

From Figure 3, it is observed that the viability of *L. casei* is correlated with the initial rice bran concentrations. It can also be seen that *L. casei* had the highest viability at initial substrate concentration of 20% (w/v) at 10-hour incubation time, which was 3.0 x 10^8 CFU/mL. Moreover, the viability in the rice bran medium of 20% (w/v) is satisfying and can be compared with the viability of *L. casei* by using commercial medium. This is in agreement with the findings by Elok *et al*., [29] that lactic acid bacteria (LAB) is capable in using the nutrient in the rice bran effectively.

4.0 CONCLUSION

The aim of this study is to evaluate the effect of initial rice bran concentrations on the growth and viability of *L. casei* as digestive bio-regulator. From this study, it is concluded that rice bran with no addition nutrients can be used as fermentative medium for the biomass production of *L. casei*. Furthermore, the initial rice bran concentrations had played some roles in the growth and survival ability of *L. casei*. The viability and cell dry weight of *Lactobacillus casei* is increasing when the initial substrate concentration increases. However, others parameters also may play some significant roles in the biomass production of *L. casei*. Hence, further study will be conducted to obtain more viable microbe and optimize the biomass yield of *Lactobacillus casei.*

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