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The Growth of *Lactobacillus fermentum* Isolated from Quail Intestine on Rice Bran Medium

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ABSTRACT

The objective of this study was to investigate the growth of *Lactobacillus fermentum* isolated from the intestine of Japanese quail (*Coturnix japonica*) on medium of rice bran meal. The influence of this medium on the cell growth of *L. fermentum* and its lactic acid product were evaluated every six hours for 36-hours incubation. A randomized block design was used to assign three variation of rice bran medium (6; 9 and 12) %. The result showed that 9% of rice bran medium showed the best medium for *L. fermentum* growth, the cell number reached 5.2 x 1010 CFU/mL and lactic acid concentrations 0.57 % for 12 hour incubation (pH 4.7). We concluded that the optimum growth of *L. fermentum* was in 9 % rice bran medium at 12 hour.

Keywords: *Lactobacillus fermentum, growth, quail, rice bran.*

INTRODUCTION

Product of Japanese quail (*Coturnix japonica*) i.e. egg and meat have a high potential to be developed as a cheaper source of protein, especially in developing countries. In recent years, with growing family incomes, the demand for poultry products has increased in Indonesia. At the same time, consumers are also becoming increasingly concerned about the safety of poultry products. The major concerns are related to the presence of antibiotic residues in poultry products that can cause adverse effects on human health and the possible development of antibiotic resistant bacteria. One key strategy to replace the use of antibiotics in poultry diets is application of probiotic in poultry feed, which are known to exert beneficial effects in the gut, directly to the bird. Probiotics are live microo rganisms which are supplemented to the feed in order to establish as beneficial gut micro flora. Use of these microbes in diets for Japanese quail may enhance the intestinal health by modifying the gut micro flora. Probiotics not only deliver health benefits for the birds that consume them, but also for consumers who purchase the poultry products. In our laboratory, several strains of microbes have been success-fully isolated from the intestinal tract of quails and characterised [1]. The microbial from genus of *Bacillus* was selected for evaluation as a probiotic. The genus is known to produce several enzymes such as protease [2] and amylase [3] and therefore it potentially can be exploited as a pro-biotic. The bacteria was identified as *Lactobacillus fermentum* that promising for probiotic [1]. The spore-forming of the bacteria are stable, resistant to high temperatures and tolerant to bile salts.

Rice bran is animal feed that contain the high concentration of vitamin B, tocopherol, 74% unsaturated fatty and complete amino acid that is the highest compared to other cereals [4]. Soluble fiber content within rice bran is good nutrition for the growth of lactic acid bacteria (LAB) such as *Lactobacillus fermentum*. The character of LAB from the digestive tract of quail can be affected by the condition of the digestive tract and the feed [5]. The quail feed became a source of organic material for the growth and metabolism of LAB in the digestive tract. Nutrients for the growth of *Lactobacillus* can be derived from the host feed and other organic matter in the digestive tract. Therefore we evaluated effect from various concentrations of rice bran mediums on growth of *Lactobacillus fermentum*.

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MATERIALS AND METHODS

The experimental protocol was approved by the Brawijaya University Animal ethics and welfare Committee. *L. fermentum* was isolated from the intestine of Japanese quails and cultured in agar based on the formulations of deMan, Rogosa and Sharpe (MRS) for 48 hours at 37°C. This medium supports growth of lactobacilli from oral, fecal, dairy, and other sources [6]. Then a series dilutions up to 10^{-7} in MRS liquid was performed, followed by spreading on MRS agar that containing 60 mg/kg Bromocresol purple (BCP) and incubated for 24 hours at 37°C. Lactic acid bacteria (LAB) colony was counted based on the change of media to be pale yellow at the location of the growth of bacterial colonies. The characteristic test confirmed that the isolates are LAB, gram positive, catalase negative and negative endospores. Isolation of the species was confirmed with the API 50 CH test Medium Kit [7; 8] and API 50 CHL medium.

To determine it's feasibility as a probiotic, the candidate was then evaluated using several tests, including pH test, pathogen test and bile salt test. Rice bran as medium was weighed (g/v) to accordance three concentration (6%, 9% and 12%), and then dissolved in aquadest and heated at 85°C for 10 minutes. One mL (v/v) isolates of BAL (10^7 cells/mL) was inoculated into 10 mL rice bran medium (6%, 9% and 12%). The speed of growth and the generation of time on the logarithmic phase were calculated with the formula of [9]. A Randomized block design Group was used to assign three variation of rice bran medium (6, 9 and 12) %. Some Variables were observed every six hours for 36 hours of incubation including total lactic acid bacteria, acid levels and the pH of media. Data were statistically analyzed using the analysis of variance. Significant differences between treatment means were separated using Duncan's multiple range test according to [10].

RESULTS AND DISCUSSION

The characteristics of rice bran

The chemical analysis of rice bran shows that the N-amino acids levels of 0.43% and total sugar levels of 0.85%. According to [11] that the rice bran protein is 14% and the carbohydrates is 36 %. The chemical composition of rice bran depends on genetic factors, environmental influences, and the conditions of storage.

The growth of Lactobacillus fermentum

The growth curve of *Lactobacillus fermentum* in media MRS broth (prior to inoculating in the rice bran medium), reaching the higest cell number was at 22 hour. While in 12 % rice bran medium, the maximum cell number was at 18 hour of 10.66 (log10 of $7x10^{10}$ cfu/mL), on 9 % rice bran medium at 12 hours of 10.58 and in 6 % rice bran medium at 18 of 10.3. This shows that the growth of *Lactobacillus fermentum* more optimum on 9% rice bran medium due to more quickly reach the log phase (Figure 1) than in media rice bran 12% or 6%. The addition of substrate will be able to accelerate the speed of reaction until the maximum speed is reached when the enzymes are saturated. After that the addition of substrate will not be able to increase the speed of reaction. According to [12] enzymes will effectively work on conditions determined by the concentration of substrate, pH, temperature and cofactors.

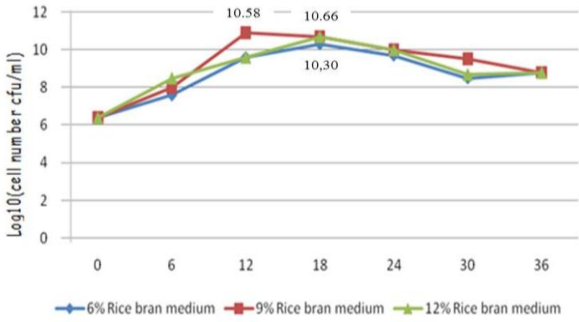

Figure 1. Growth rate of *Lactobacillus fermentum*

Table 1. Generation time of *Lactobacillus fermentum*[1]

Medium	Generation (h)	k(generation/h)
6 % rice bran	1.33 [a]	0.52[a]
9 % rice bran	0.75 [b]	0.92[b]
12% rice bran	1.70 [a]	0.40[a]
Pooled SEM	2.078	1.440

k: ln 2/generation time (constant of growth speed).
[a,b] Different superscripts in a column denote significant difference ($P < 0.05$).
[1] Each value represents the mean of four replicates.

At the 24 total LAB on all media treatment decreased and in 30-36 hours they reach stationary phase. The result is different from research of [13] that the growth of commercial probiotic, the highest cells number at 12 hour in 12 % rice bran medium.

Figure 2 shows that the highest rate of growth occurs in 9 % rice bran medium with constant of growth speed of 0.92 generation/h and generation time 0.75 hour. The cells number of bacteria in the rice bran medium was significantly different at each incubation time.

Nutrients composition of rice bran able to increase the growth activity of *Lactobacillus fermentum* especially soluble fiber content of 12% as well as all the essential amino acids, vitamins B complex, vitamin C, D and minerals such as calcium, phosphate and irons. Vitamin B is the main component of coenzyme that can help to activation the enzymes of microbial cells [14] so that they can accelerate the growth of bacteria.

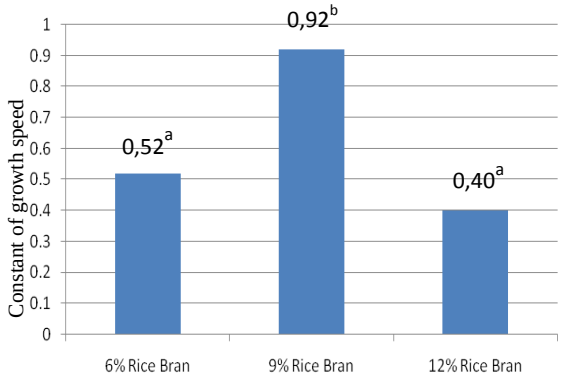

Figure 2. Growth rates of *Lactobacillus fermentum*

The pH of Medium

When bacteria cells are growing, they will produce organic acid due to pH of medium decline. Lactic acid produced by the LAB will be excretion out of cells and will accumulate in the fermentation media (substrate) that would increase the acidity.

The curve shows that at first to18 hour, the pH of treatment medium were significant decline because of *Lactobacillus fermentum* activity high enough. While at the 24 to 36 hour *Lactobacillus fermentum* began decreased activity. This corres ponds to the growth of bacteria cells, at first to18 hour, bacterial cell growth is high and at the 24 to 36 bacteria growth was decreased. According to [14] that incubation temperature optimum leads the process of ionization lactic acid into maximum resulting in increasingly large H^+ ion are exempt, so the pH decreases.

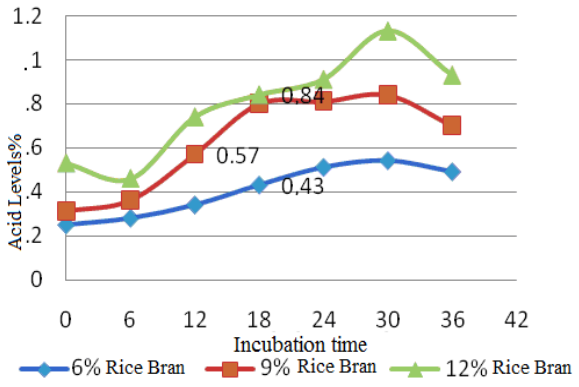

Figure 3. Changes rice bran medium pH

Acid Levels

During the fermentation, LAB breaks down carbohydrates into simple sugar especially glucose and produce lactic acid as energy for cellular activities [15]. Based on the data, lactic acid levels increased from first to 30 hour and then decreased at 36 hour. The highest levels of lactic acid in the treatment is in 12% rice bran medium whereas the lowest levels in 6% rice bran medium. According to [16], the process of cell growth is accompanied by the formation of metabolite in lactic acid resulting from an overhaul of the sugar, proteins and other acids. In addition, the soluble fiber components can be used as a source of rice bran food prebiotic.

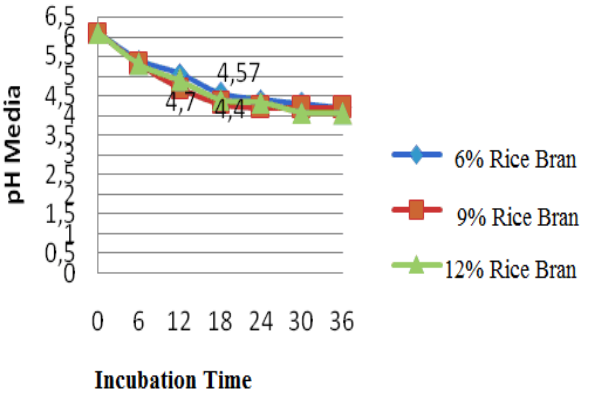

Figure 4. Changes of acid levels in rice bran medium

The acid levels in rice bran medium had significant difference this is in accordance with the number of bacteria cells was significantly different so that affect acid levels. According to [17] that measurement of the pH is a concentration of H^+ ions in form dissociated so that not all components of acid measured. Total measurable titrated acid is categorized into dissociated and associated to overview NAOH binding.

CONCLUSION

The optimum growth of *Lactobacillus fermentum* was in rice bran medium 9 %, where *Lactobacillus fermentum* reached log phase at pH 4.7 at 12 hour was 5.2 x 10^{10} CFU/mL and 0.57% acid levels with constant speed growth of 0.92 generation/hour and time generation 0.75 hours.

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