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Viability And Effectivity Of Encapsulated *Bifidobacterium* sp. With Starch Matrix On Functional Food To White Mice Bulb/C

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ABSTRACT

The development of indigenous species application for enhancing the adding value of local natural resources has been studied by teaching staff and students with intention on isolating, developing, and applying the indigenous species of microorganism. However, the culture collection is still required to be enhanced, especially the potential culture in producing fermentative products, e.g. Bifidobacterium. Fermentation of Bifidobacterium-containing product produces highly-nutrient foods that have a potention as protection againsts gastrointestinal infection, increase the immune response, reduce the cholesterol in blood, and allergies in toddlers. Cell encapsulation should be performed to overcome the problem of cell viability in fermentative product. This study aims to determine the viability and effectivity of Bifidobacterium BBP6 encapsulated by starch matrix in white mice Bulb/C. The result showed that the number of lactic acid bacteria in the intestine of white mice Bulb/C with treatment of *Bifidobacterium* sp. BBP6 by spray and encapsulation for 21 days was not able to increase the number of lactic acid bacteria's cell.

Keywords: Bifidobacterium BBP6, encapsulation, functional food, spray, viability

INTRODUCTION

Important characteristics of Bifidobacterium are Gram-positive, anaerobic obligate (live without O2), non-spore-forming bacteria, 2-8 μm in length, optimum growth temperature of 36-38°C, optimum pH of 6.5, die at 60°C, and catalase negative (Martinez et al., 2013). Bifidobacterium is one of the indigenous bacteria in human and animal gastrointestinal tract (Gagnon et al., 2004). Therefore, thus bacteria are expected to replicate and survive in the digestive tract better than *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Bifidobacterium will perform better to compete with pathogenic bacteria (*Salmonella typhi, Escherichia coli, Staphylococcus aureus* and *Bacillus cereus*) and further inhibit the growth of this bacteria.

Probiotic has been directly applied in food and beverage. It will arise a problem i.e. applied bacterial cells are not entirely alive (viable). Bacterial cells need to be protected from direct effect of environmental factors. The probiotic can be protected by giving a physical barrier using cell encapsulation or cell immobilization on the starch matrix (Agus, 2015).

Encapsulation has the ability to stabilize cells, as well as improve viability during production, storage, and handling. Starch is a material that can be used for encapsulation or capsule-forming material that is cheap and non-toxic. Encapsulation of *Bifidobacterium* sp.

with the starch matrix is expected to increase the viability of probiotic and its effectiveness with different storage temperatue and period.

RESEARCH METHODS

The experiment was conducted experimentally by using Factorial Complete Randomize Design. The treatments were feed type and treatment period as follows:

The first factor, feed type (P)

P1: Feed with probiotics *Bifidobacterium* sp. encapsulated starch matrix

P2: Feed with probiotics *Bifidobacterium* sp. sprayed onto commercial pellets

The second factor, treatment period (L)

L0: 0 days

L1: 7 days

L2: 14 days

L3: 21 days

There are eight treatment combinations with three repetitions. Independent variables are feed type and treatment period. The dependent variable is the viability of the number of probiotic cells in gastrointestinal tract. The main parameter is the number of lactic acid bacteria (LAB) cells in the gastrointestinal tract. The supporting parameter is the total number of bacteria.

DATA COLLECTION

3.1. Probiotic Encapsulation with Extrusion Method (Hsio et al., 2003; Chang & Chiu, 2003)

Cell encapsulation is performed by using single layer coating method. The formula is fish powder, tapioca powder, rice bran, probiotic Bifidobacterium BBP6 (10^8 CFU/mL) and water with a ratio of 4: 2: 1: 2: 3. The ingredients are well mixed. The extrusion method is performed by using a 0.4-0.8 mm syringe on the bottom of container and pressurized to produce droplets of starch suspension. The starch suspension is dried at hot air oven, then sprayed on the white mice Bulb/C.

3.2. Animal acclimation (Smith and Mangkoewidjojo, 1988)

Acclimation of white mice Bulb//C is performed in stainless steel cage (23x13x16 cm) for 1 week. Each cage is contains 5 mice that fed with pellet and *ad libitum feeding* every day. Then, mice are fed with Bifidobacterium BBP6-containing feed as much as 3% of the

average of mice weight. Maintenance of mice is performed for 21 days. Sampling time at the 0, 7, 14, and 21 days.

3.3. Enumeration of Cell Viability

Quantitative enumeration of cell viability is performed by using pour plate method, with dilution to 10^{-8} and incubation for 48 hours at 37^0C. The viability is calculated based on the log ratio of bacterial number per gram sample (after and before storage) and expressed in percent (%) (Lian et al., 2002) as follows:

$$\text{Viability (\%)} = \frac{\text{Log cfu/g dry probiotic base after storage}}{\text{Log cfu/g dry probiotic base before storage}} \times 100\%$$

3.4. Enumeration of bacterial cell of LAB and Total Population of Microbes in Gastrointestinal tract by using Total Plate Count (Lay, 1994)

A gram of intestine sample is meshed and diluted in 9 ml aquadest, then further diluted into 10^{-4}. The population of LAB is cultivated on MRSA medium. The total intestinal microbial population is cultivated in PCA medium with sprea plate method, then incubated for 48 hours at 30^0C. The calculation as follows:

$$\frac{\Sigma \text{ colony 1, 2, 3, etc x 1/dilution factor x sp/pp}}{\text{Petri dish}}$$

Note:

sp = spread plate (0,1 ml)

pp = pour plate (1 ml)

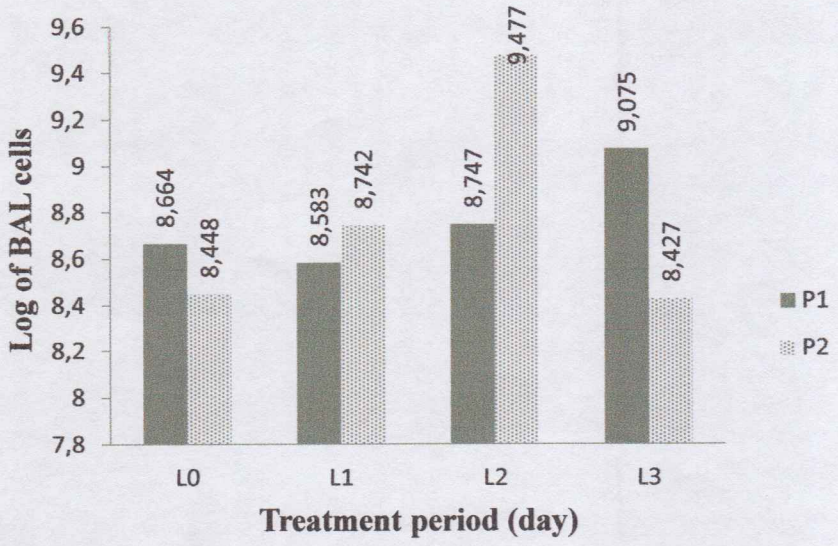
Cell number is expressed in CFU/ml.

ANALYSIS METHOD

The data is analyzed by using Variance Analysis (Anova) at 5% and 1% error level, then continued with the Smallest Real Difference Test (BNT).

RESULTS AND DISCUSSION

The calculation result of the average number of bacterial cell of LAB in gastrointestinal tract of white mice Bulb/C with different treatment and period showed a vary data.



Note:
P1 = encapsulated probiotis
P2 = Probiotic application with spray

L0 = treatment period 0 day (control)
L1 = treatment period 7 day
L2 = treatment period 14 day
L3 = treatment period 21 day

Figure 1. Histogram of LAB cell enumeration in gastrointestinal white mice Bulb/C regarding probiotic uses and different treatment period

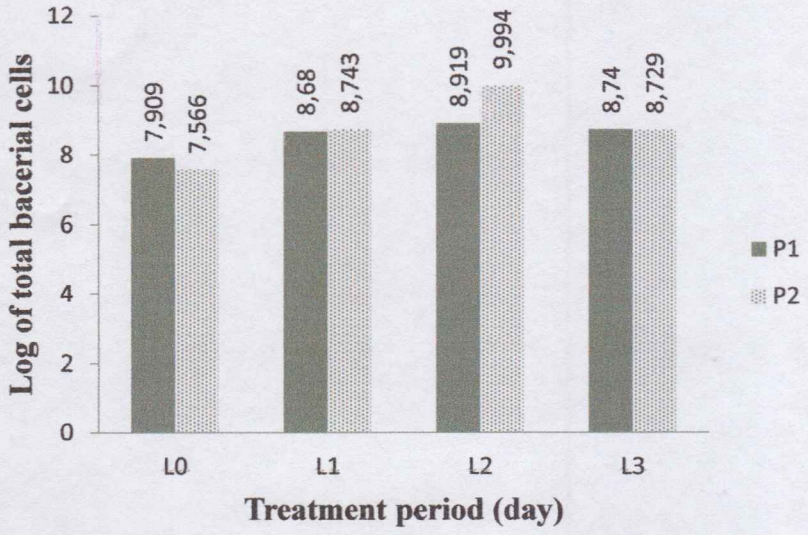
The result of variance analysis showed that the method of applying Bifidobacterium BBP6 and different treatment period had no effect in increasing number of LAB (F arithmetic < F table). Partially, neither the method of applying the probiotic with encapsulation and spray had no effect increasing number of bacterial cells nor treatment period.

The method of applying probiotics i.e. spray or encapsulation has an advantages and disadvantages. The number of probiotic cells present in gastrointestinal tract should work optimally. Meanwhile, direct apply of probiotic causes the cell count to decrease. The direct apply of probiotic by spraying method on the feed is faster and easier, but O2 exposure after spraying probiotics on the feed surface is thought to decrease the number of cells. Oxygen is toxic to LAB, because it can form H_2O_2. Oxygen can fuse through cell membranes and cause oxidative damage in lipids, enzymes, and DNA (Agus, 2015).

Encapsulation technique is able to protect probiotic bacteria from unfavorable environmental conditions, such as heat and chemical substances. Probiotic encapsulation is performed to improve the cell survival and viability during product processing ang stroraging, and also increase the survival in digestive tract. In this study, as statistically, the two techniques of applying probiotics have no effect, although according to Khailasatathy (2002) encapsulation is able to protect the core material from unfavorable factors.

In this study, the encapsulated bacteria is Bifidobacterium BPP6 as core material. According to Silvia (2002), an important characteristic of Bifidobacterium is an anaerobic obligate in primary culture (living without O2, O2 is toxic to this class of bacteria), then become microaerophilic or facultative anaerobes. This might cause the encapsulated LAB to be less viable. It is due to long air expose during product processing that may cause oxidation.

The calculation result of the average number of total bacterial cell in gastrointestinal tract of white mice Bulb/C with different treatment and period showed a vary data. Probiotics application by spray method for 14 days showed the highest bacterial population, but had no significant effect in different number of cells (Figure 2.).



Note:
P1 = encapsulated probiotis
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L0 = treatment period 0 day (control)
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L3 = treatment period 21 day

Figure 2. Histogram of total bacterial cells in gastrointestinal white mice Bulb/C regarding probiotic uses and different treatment period

The group of Gram-negative bacteria from the family Enterobacteriaceae can also live in gastrointestinal tract, beside of LAB. Data showed a balance number between LAB and other bacteria (Figure 2.). Bifidobacterium is one of the original bacterial groups of human and animal gastrointestinal tract (Gagnonet.al., 2004). Therefore, the bacterium is expected to replicate and survive in the digestive tract better than *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Bifidobacterium will be better to compete with pathogenic bacteria (*Salmonella thyphi, Escherichia coli, Staphylococcus aureus* and *Bacillus cereus*) and further inhibit the growth of this bacteria.

Bifidobacteria and Lactobacillus are capable of inhibiting the growth of pathogenic bacteria through their ability to compete on the intestinal surface by producing lactic acid,

acetic acid, and other organic substances, such as H_2O_2 and antimicrobial peptides (Lukiwille et al., 2007). Microorganism composition in caecum described the microoranism composition in consumpted food. Caecum in mice is the place for fermentation of food substances by intestinal microflora as well as colon in human intestine (Liong and Shah, 2006).

According to Suskovic et al. (2001), the benefits of LAB and *Bifidobacterium* spp. can be described by three main mechanisms. First, suppress pathogenic microorganisms in the intestinal tract by producing antibacterial substances including primary metabolites, such as lactic acid, acetic acid, carbon dioxide, diacetyl, acetaldehyde, hydrogen peroxide and bacteriocin which are protein compounds that have an influence on antibacterial activity. Second, it alters the metabolism of microbes in the intestinal tract by increasing useful enzyme activity and decreasing the activity of carcinogenic enzymes. Third, it stimulates the immune system due to the introduction of LAB as an important step for the development of the normal mucosa and immune system.

CONCLUSION

The application of probiotics by encapsulation and spray on feed can not directly use, it need further formula to fit the animal. Encapsulation with starch matrix is more effective than fish powder. The encapsulation of Bifidobacterium requires a fast product proseccing to prevent oxidation.

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