The effect of encapsulated *Pediococcus lolii* L2 on its cell viability and α-glucosidase inhibition activity

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Abstract. Alpha-glucosidase is an enzyme that catalyzes the breakdown of α-glycosidic bound in carbohydrates, cause increasing of blood sugar level. Hyperglycemia conditions can bring an adverse impact on people with diabetes mellitus. One therapy to control blood sugar levels is to suppress the activity of α-glucosidase activity. *Pediococcus lolii* L2 is a potential probiotic that produce an α-glucosidase inhibitor. We need to maintain the viability and functionality of probiotics when applying to food industries. One of the methods for protecting the cell viability of bacteria and the inhibition activity is microencapsulation using spray dry. This research aimed to know the viability and α-glucosidase inhibition activity of *Pediococcus lolii* L2 after spray dry. We used maltodextrin, arabic gum, sodium alginates (SA), SA + maltodextrin, and SA + arabic gum as a carrier for probiotics for our research. The result showed that microencapsulated bacteria have higher viability with >85% of encapsulation efficiency. Likewise the ability of α-glucosidase inhibition. There is no significant difference of five materials used to encapsulate the cell with the inhibition activity.

1. Introduction

The α-glucosidase is one of the enzymes that have a role in the process of digestion of carbohydrates in the small intestine. The enzymes will break down the bonds of α-glycosidic on carbohydrates so it will increase the level of sugar in the blood. In patients with type 2 diabetes (T2DM), the activity of α-glucosidase can trigger hyperglycemic conditions. Patients with T2DM, insulin failed to positively respond by the body, resulting in insulin resistance or decreased insulin secretion by pancreatic beta cells [1]. Various strategies are carried out to treat T2DM such as increasing insulin ability in target tissues, stimulating insulin secretion, using oral hypoglycemic agents, and inhibiting carbohydrate degradation by the α-glucosidase enzyme [2].

Alpha glucosidase inhibitor compounds have been produced to overcome the condition of hyperglycemia in patients with diabetes mellitus, including acarbose, voglibose, nojirimycin, and 1-deoxyxojirimycin. However, its use currently causes side effects on the body as insomnia, nausea & vomiting, headache, flatulence, and diarrhea [3]. Therefore research on the development of this inhibitor continuously done by exploration of microbes such as *Streptomyces* sp., *Lactobacillus* sp. and *Bacillus* sp.; from plants such as *Nelumbo nucifera* and from fungi such as *Ganoderma lucidum* [4-8].
Lactic acid bacteria is one of the microbial groups that have a wide range of benefits for health and food sectors such as bio-preservative, enhance immunity and maintain the natural microflora in the digestive system [9]. Some types of strain L. casei 2607, L. casei 15286, L. acidophilus 4461, B. longum 5022, Pedicoccus lolii L2 isolated from Ganoderma lucidum, Lactobacillus pentosus isolated from Muntingia calabura, lactic acid bacteria isolated from Ganyong (Canna edulis) and Kimpul (Xanthosoma sagittifolium) have an inhibitory activity of alpha glucosidase in-vitro [10-14]. In the process of food, the application allows the lactic acid bacteria exposure experienced to unfavorable condition, so we need a protective one by encapsulation with a spray drying method.

Encapsulation is a process of wrapping a substance to other substances, or the process to produce a particle size in nanometer (nano-encapsulation), micrometers (microencapsulation), or millimeter (milliencapsulation)[14,15]. The sample which encapsulated can be better protected. Some food industry using spray drying encapsulation techniques for easy and inexpensive production costs[16]. Spray drying process of α-amylase encapsulation can maintain their activity[17]. The use of a spray drying method against lactic acid bacteria has been widely carried out, to maintaining viability and the physiological characteristics[18].

The presence of lactic acid bacteria which has the potential as an α-glucosidase inhibitor allows the application of these bacteria in the food sector, especially for diabetes management. In the application process, changes in unfavorable environmental conditions can cause changes in the cell viability and the activity of the enzyme. Therefore, the encapsulation process needs to be carried out to determine its effect on the ability of and cell viability after spray drying. This research aims to assess the effect of the spray drying process on the cell viability and the α-glucosidase inhibition activity.

2. Material and methods

2.1. Preparation of Bacterial Culture
The bacterial culture used in this research was Pedicoccus lolii L2 that isolated from Ganoderma lucidum or lingzhi and has alpha-glucosidase inhibitor activity [12]. The isolate was grown in MRS broth medium at 37ºC for 24 hours. The volume of inoculum was 10% of the total volume of culture. The pour plate method was carried to find out the number of bacterial cells. The three highest level dilutions were inoculated in MRSA medium, then incubated at 37ºC for 48 hours.

2.2. Encapsulation creates of Lactic acid Bacteria
Two percent sodium alginate [16], arabic gum and maltodextrin was dissolved in distilled water (20% w/v). Encapsulant was sterilized at 121 ºC for 15 minutes. There were five variants of encapsulant including sodium alginate, arabic gum, maltodextrin, gum arabic+sodium alginate, and maltodekstrin+sodium alginate. The sterilized encapsulant material were mixed with bacterial biomass. We used 1:1 for mixed encapsulation (sodium alginate+arabic gum, sodium alginate+maltodextrin). The spray dryer was set at an inlet temperature of 120 ºC, an aspirator rate of 90%, and a pump rate of 22%. The spray-dried powder is stored in ziplock plastic and stored at 4 ºC until it is used for analysis. The method used in the efficiency of encapsulation is carried out according to Rajam et al.[20], by using comparison of the number of bacterial cells before (No) and after (N) encapsulation. The formula for encapsulation efficiency (EE) is:

\[
EE = \frac{N}{No} \times 100
\]

2.3. Alpha glucosidase inhibition activity (AGI)
Alpha-glucosidase inhibition activity by lactic acid bacteria was done based on the method [21]. The sample used was a 24-hour age Pedicoccus lolii L2 growth supernatant separated by 5000 rpm centrifugation for 15 minutes, at 4ºC. The reaction was carried out on a 96-well microplate by mixing 25 µL 0.1M PBS pH 6.8, 25 µL 10 mM p-Nitrophenyl-alpha-D-gluco pyranoside, and 50 µL samples
in the form of bacterial culture supernatants or 1.5% acarbose. Incubation was carried out for 10 minutes at 37°C, then 50 μL of α-glucosidase 1 U / mL was added. Incubation was done at 37°C for 30 minutes. To stop the reaction, 100 μL sodium carbonate was added. Absorbance was measured using a spectrophotometer with a wavelength of 405 nm. As a corrector, the blank solution was made by replacing the α-glucosidase enzyme to 0.01 M PBS pH 6.8. Positive controls were made using PBS and the α-glucosidase enzyme, while negative controls only used PBS. The following formula calculated the percentage of inhibition activity:

\[
% \text{ inhibition} = \left(1 - \frac{\text{abs sample} - \text{abs blank}}{\text{positive control} - \text{negative control}}\right) \times 100\%
\]

2.4. Morphological Characterization using Scanning Electron Microscope (SEM)
The spray dried encapsulant was placed on the carbon tip that has been pasted above the stub. Sample was then coated with gold using the appliance ion sputter for 10 minutes, 10 mA and vacuum pressure of 60 Pa. Morphological observation using SEM used walking distance 6.7 mm.

3. Results and discussions

3.1. Cells viability after encapsulation
The total number of Pediococcus lolii L2 grown on MRS broth medium after an optimum incubation period of 16 hours was \(1.44 \pm 0.74 \times 10^9\) CFU/mL. Based on table 1, the cell viability before encapsulation varies between the use of encapsulant materials. Because of a decrease in the number of bacterial cells during the process of transferring culture biomass into a mixture of encapsulant material. According to Huang et al. (2017), other factors that can play a role in cell viability before spray drying are a bacterial strain, medium, temperature, pH, growth phase and the initial number of bacteria.

| Encapsulant           | The number of lactic acid bacteria (log CFU/mL) | Encapsulation Efficiency (%) |
|-----------------------|-----------------------------------------------|------------------------------|
|                       | Before Encapsulation | After Encapsulation        |                              |
| Maltodextrin          | 8.69 ± 0.3          | 8.12± 0.0                   | 93.49 ± 0.27 c               |
| Gum Arabic            | 9.14 ± 0.1          | 8.21 ± 0.6                  | 87.25 ± 0.73 a               |
| Sodium Alginates      | 8.89 ± 0.1          | 8.02 ± 0.0                  | 90.22 ± 0.11 b               |
| Sodium Alginates +    | 8.91 ± 0.2          | 8.51 ± 0.8                  | 95.46 ± 0.85 d               |
| Maltodextrin          | 9.13 ± 0.3          | 8.04 ± 0.0                  | 86.40 ± 0.24 a               |

Cell viability after encapsulation in table 1 has decreased. Decreasing bacterial number after spray drying is common because cells experience stress when exposed to high temperatures which cause cells to become dehydrated and to change cell osmosing. Other stress conditions that can be experienced by bacterial cells during spray drying are stress due to oxidative stress and mechanical stress [22]. The percentage of encapsulation efficiency in this study ranged from 86.40% - 95.46%, and the highest value was found in the use of encapsulant material in the form of sodium alginate+maltodextrin. Sodium alginate is the most polysaccharide used as encapsulating material of lactic acid bacteria, due to ease of handling, non-toxic nature, and low cost, besides increasing the
viability of these bacteria when exposed to different conditions [15]. Yonekura et al. [23] got encapsulation efficiency of 89% for Lactobacillus acidophilus NCIMB 701748 encapsulation with sodium alginate.

3.2. Alpha glucosidase inhibition activity after encapsulation

Although the use of encapsulant material affects the cell viability in culture, the activity of α-glucosidase inhibition was not significantly different from each encapsulant material. In figure 2, the inhibition activity of maltodextrin, arabic gum, sodium alginate, and SA + maltodextrin, SA + arabic gum showed results ranging from 75.57% - 95.52%. It means the use of maltodextrin, arabic gum, sodium alginate, SA+maltodextrin, SA+gum arabic was still able to maintain the alpha-glucosidase inhibition activity, despite a decrease in cell number. The highest activity founded in SA+maltodextrin, while the lowest activity founded in arabic gum.

Several studies related to the metabolic ability of lactic acid bacterial cells after encapsulation show mixed results. One of them is in research conducted by Pradipta et al. [24], poultry indigenous lactic acid bacteria encapsulated by spray dry with encapsulant materials such as maltodextrin and skim (20% v/v) showed a decrease in the ability to fight pathogenic bacteria Salmonella enteritidis and E. coli. The use of lactic acid bacteria that can grow and inhibit α-glucosidase after encapsulation has excellent potential as a functional food product along with increasing public awareness of health.

![Figure 1](image-url) Alpha-glucosidase inhibition activity encapsulated probiotic
Description: In the trunk of the histogram bar is a standard mean error; AGI (%) between samples is not different between the real test on the DMRT column with the significance of 5 percent

3.3. Morphological characterization using SEM

Observation of the sample’s morphology was carried out using SEM. The samples used were encapsulant powders which were not yet dissolved and spray drying powder. The microstructure between the encapsulated material and spray drying powder showed differences (figure 2). The encapsulated powder of sodium alginate does not show holes or pores but is round with a rough surface. Differences in morphological appearance and particle size are strongly influenced by parameters when spray dry such as atomizing pressure, spray nozzle type, and feeding rate [25]. Whereas in other materials the form of powder tends to be curved, with the addition of a small round structure attached to the surface of the powder. Curves that appear can occur due to dehydration process when heating takes place, water molecules that are bound to the solution of the encapsulant material with cell biomass evaporate as they pass through the drying chamber. In SA+gum arabic
curvature powder is more visible and deep, this allows a low percentage of the efficiency of the encapsulation because the cell is dehydrated.

![Figure 2](image_url)

**Figure 2.** Microstructure of (1) maltodextrin powder (2) spray drying powder with maltodextrin (3) spray drying powder with SA+maltodextrin (4) arabic gum powder (5) spray drying powder with arabic gum (6) spray drying powder with SA+arabic gum (7) sodium alginate powder (8) spray drying powder with sodium alginate.

4. Conclusion
Probiotic encapsulation using spray drying method with arabic gum, maltodextrin, sodium alginate and a mixture of SA+gum arabic, SA+maltodextrin, can maintain the cell viability of 86.40% - 95.46%. Besides, the spray drying process is also able to sustain alpha-glucosidase inhibitor activity of 75.57% - 95.52%. The use of sodium alginate+maltodextrin as an encapsulant can provide the highest and best encapsulation efficiency and maintaining enzyme activity.
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