

Prebiotic ability of *Musa balbisiana* colla stems on the growth of *Pediococcus acidilactici* in vitro and probiotic encapsulation

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Abstract. *Musa balbisiana* Colla banana is a plant that has many benefits, one of which is useful as a traditional medicine using its stems. Banana stems have potential as a source of prebiotics because they contain carbohydrates and oligosaccharides which can support the growth of good bacteria in the digestive tract. Probiotic encapsulation was carried out to determine the role of *Musa paradisiaca* stems as a prebiotic in digestion. The encapsulation process is carried out using chitosan, carrageenan, and maltodextrin. The results showed that the prebiotic banana stem *Musa paradisiaca* could increase the growth of *Lactobacillus* sp. significantly at certain concentrations, with the most optimal results at a concentration of 6.89% compared to the control. Based on test results, *Musa paradisiaca* stem flour contains a fructan percentage of $0.14 \pm 0.001\%$. The presence of fructan compounds makes *Musa paradisiaca* stem flour function as an effective source of natural prebiotics. The test results showed that the biocapsulation contained 2.7×10^3 cfu/gram of lactic acid bacteria. This shows that the bioencapsulation process of lactic acid bacteria was successful. Encapsulation can protect probiotics from extreme conditions during processing and storage, as well as from acidic pH in the digestive tract.

1 Introduction

Balinese people have various ways of processing vegetables to meet their needs, which are grouped as vegetables without soup, vegetables with little soup, and vegetables with a lot of soup. Banana stem vegetable and *roroban* vegetable are two types of Balinese cuisine that have a lot of soup made from young banana tree trunks. Banana trunk is another name for banana tree saplings or banana trees with young trunks in Bali.

For people outside Bali, they may not know that banana stems or young banana trees can be processed as side dishes. Banana stems are a favorite food of the Balinese people which are cooked when there is a big celebration or ceremony involving many members of the

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community. So, it is cooked in large portions, with complete spices (*basa genep*) and lots of gravy, so that it can meet the needs of many people [1]

Banana is a plant that has many benefits, one of which is useful as a traditional medicine by using its stem. Ambon Banana Stem is used as a natural ingredient to reduce the use of synthetic drugs. The stem has active compounds, namely flavonoids and tannins, which play a role in preventing the side effects of indomethacin. Banana stem flavonoids can function as antioxidants and anti-inflammatories. Flavonoids work by slowing down the inflammation process through an inhibitory effect on the arachidonic acid metabolism pathway and the formation of prostaglandins. While tannins play a role in stopping bleeding [2].

Prebiotics are food ingredients that cannot be digested in the small intestine and when they reach the large intestine will be fermented by colon bacteria into short chain fatty acids (SCFA) such as acetate, lactate, butyrate, propionate, and also gases (such as CO₂, methane, and hydrogen). These short chain fatty acids will be absorbed and metabolized by the body [3]. Prebiotics are generally in the form of dietary fiber [4]. In addition, the most potential prebiotics also consist of carbohydrates. Food ingredients that are prebiotics can be vegetables, tubers, and fruits [5]. Several previous studies on prebiotic sources or products have used many high dietary fiber and carbohydrate ingredients. One of them is from fruit, namely banana flour, which in previous studies has been shown to have benefits for the growth of the probiotic *Lactobacillus casei*. There is also other research on the use of prebiotic extracts from Rumbia fruit (*Metroxylon sago* Rottb), the results of which show the influence of prebiotic sources on ration consumption, final weight gain, and other positive effects on broiler chickens [6]

So far there has been no in-depth information or research on the potential of banana stems as a source of prebiotics. Prebiotic tests can use LAB (Lactic Acid Bacteria), especially from the genus *Pediococcus* which is a normal flora. One of them used in this study is *Pediococcus acidilactici* which is a bacteria with the ability to inhibit contamination from pathogenic microorganisms and toxin producers because it can produce lactic acid and lower pH. And has the ability to produce bacteriocins that function as antibiotics [7]. Based on the explanation above, this study was conducted with the aim of determining the ability of banana stem prebiotic sources based on their prebiotic tests on the growth of *Pediococcus acidilactici* in vitro. The results of this study are expected to provide additional information on the benefits of banana stems and increase the use of natural ingredients, one of which is banana stems as a source of prebiotics.

2. Research methods

2.1 Research Location

This research was conducted at the Laboratory of the Faculty of Agriculture, Warmadewa University, the Laboratory of Dhyana Pura University and the Analytical Laboratory of Udayana University. Banana stems were obtained from Antap Village, Selemadeg District, Tabanan.

2.2 Tools and materials

The materials used in this study include: banana stem fruit, Inulin Powder, cold water (0°C), MRS agar media (de Man, Rogosa, Sharpe), MRS broth media, distilled water, 0.85% NaCl, and *Pediococcus acidilactici* bacterial isolates. The tools used in this study include: petri dishes, test tube racks, test tubes, LAF (Laminar Air Flow), tubes, spectrophotometers,

micropipettes, beakers, blenders, knives, 80 mesh sieves, Erlenmeyer flasks, ovens, autoclaves, vortexes, bunsen, ose needles, analytical scales, tips, stirrers, hotplates, colony counters, measuring cups, spatulas, aluminum foil, rubber, paper, plastic, cotton, and trays.

2.3 Research Procedures

Research Stages of Making Banana Stem Flour are prepared, namely selected from the unripe *Musa balbisiana* Colla banana variety. Furthermore, washing, peeling, slicing approximately 22 mm, and weighing as much as 500 grams. Then given a soaking treatment in cold water 0 °C for 10 minutes, then drying in an oven for approximately 3 days at a temperature of 45 °C-50 °C. After drying, flour is made using a blender and sieved using an 80 mesh sieve. Culture Rejuvenation Bacterial culture rejuvenation begins with the preparation of a growth medium, namely MRS broth media by weighing 2.6 grams of media powder and dissolving it in 50 ml of distilled water. Furthermore, the media is homogenized with a magnetic stirrer, then 5 mL of media is poured into a test tube and sterilized with an autoclave at a temperature of 121 ° C for 15 minutes. Furthermore, the media is removed and cooled. The frozen bacterial culture stock was taken as much as 1 loop and inoculated into 5 mL of MRS broth media then incubated for 24-48 hours at 37°C. Growth was indicated by turbidity in the media [8].

Carrageenan 1%, maltodextrin 1.5% and flour 1.5% were dissolved in 30 mL of distilled water (Solution A), then heated at 96°C for 5-6 minutes. The solution was cooled to 45°C while stirring. Making a 2% chitosan solution is by weighing 2 grams of chitosan and dissolving it with 1% acetic acid (Solution B). Furthermore, the addition of *Pediococcus acidilactici* bacterial cell suspension (1:6) to the mixture of solution A and solution B. The mixture of ingredients was stirred with a magnetic stirrer for 2 hours and then ovened for 24 hours at 40°C until the banana stem flour bioencapsulation powder was produced.

2.3 Prebiotic Characteristics Test

The prebiotic characteristic test in this study was carried out by observing the amount of *Pediococcus acidilactici* bacterial growth in banana stem flour bioencapsulation powder. The test began with the preparation of MRS broth media, weighing 15.6 grams of media and dissolving it in 300 mL of distilled water and homogenizing it. The banana stem flour bioencapsulation powder was inoculated into 10 mL of MRS broth media that had been added with banana stem flour bioencapsulation powder. Furthermore, it was incubated at a temperature of 37°C for 24-48 hours. The population of *Pediococcus acidilactici* in banana stem flour was calculated using the spread method [8]. The bacteria that grew were then gram stained to ensure that the growth of the bacteria was lactic acid bacteria.

3. Results and Discussion

Musa paradisiaca stem flour has the ability as a prebiotic because of its carbohydrate and oligosaccharide content that can support the growth of good bacteria in the digestive tract. The yield of banana flour bioencapsulation was $0.5 \pm 0.02\%$. The low yields produced are caused by several factors. The inoculum concentration used in the bioencapsulation process has a significant effect on the results. Using optimal concentrations can increase the number of microorganisms involved thereby increasing the production of the desired metabolite. Factors such as pH, temperature and fermentation time also greatly determine the results. Lactic acid bacteria grow well at pH 5-6, where they can produce effective lactic acid and bacteriocins. A decrease in pH below 4.5 can kill pathogenic bacteria, while a pH above 6.5

can inhibit the growth of Lactic acid bacteria itself [9]. At a temperature of 37°C, Lactic acid bacteria shows optimal growth. On the other hand, at temperatures below 10°C or above 45°C, the growth of these bacteria is inhibited or does not occur at all. Probiotic encapsulation aims to protect Lactic acid bacteria from unfavorable environmental conditions, including temperatures [10]. Encapsulation aims to protect bacteria from high temperatures that can kill bacterial cells or damage their cellular structure. The substrate used for bioencapsulation must be rich in nutrients and suitable for microbial growth. Substrate quality can influence the interaction between microorganisms and their binding materials, as well as the availability of nutrients necessary for optimal growth [11]. In this stem research, the substrate used was banana flour.

Based on the test results, *Musa paradisiaca* stem flour contains a percentage of fructan of $0.14 \pm 0.001\%$. Fructans can be found in various food sources, including bananas, which contain inulin and FOS [12]. The presence of fructan compounds makes *Musa paradisiaca* stem flour function as an effective source of natural prebiotics [13]. The requirements for good prebiotics are that they cannot be hydrolyzed in the upper gastrointestinal tract, can be digested by good bacteria in the colon, so that they can suppress the growth of pathogenic bacteria. Lactic acid bacteria have the enzyme alpha-galactosidase which can break down alpha-galactosidase bonds which can break down polysaccharides into galactose, glucose, and fructose [12].

Probiotic encapsulation was conducted to determine the role of *Musa paradisiaca* stems as prebiotics in digestion. The encapsulation process was carried out using chitosan, carrageenan, and maltodextrin. The encapsulation results were tested for the presence of lactic acid bacteria using the spread method. The test results showed that the bioencapsulation contained 2.7×10^3 cfu/gram of lactic acid bacteria. This indicates that the bioencapsulation process of lactic acid bacteria was successful. The higher the total growth of lactic acid bacteria microbes, the better the bioencapsulation process was. Encapsulation can protect probiotics from extreme conditions during processing and storage, as well as from acidic pH in the digestive tract. Encapsulation can increase the stability of probiotics during storage and distribution of products. The encapsulating material used is chitosan which can form a matrix that protects probiotic cells from damage [14], [15]. By using encapsulation techniques, probiotics can be added to various food products without changing the taste or texture [16], [17]. This will make it easier to consume probiotics in more diverse forms, thus increasing consumer compliance with probiotic consumption [18]. Encapsulation protects probiotic cells from detrimental factors such as stomach acid, emping salts, and high temperatures that can reduce their viability. Research shows that encapsulated *Lactobacillus plantarum* and *Streptococcus thermophilus* bacteria have better resistance to stomach acid conditions compared to non-encapsulated ones, with a lower decrease in viability [19]. The encapsulation process creates a protective layer around the probiotic cells, thereby helping to maintain their integrity and stability during storage and processing [7]. This is especially important in food products that undergo heating or storage for long periods. Encapsulation using materials such as chitosan and alginate has been proven to be able to maintain the number of bacterial colonies in simulated gastric acid fluid [20]. Encapsulation allows control of the release of probiotics in the digestive tract. By using appropriate encapsulant materials, probiotics can be released gradually according to pH conditions in the gastrointestinal tract, ensuring that more probiotic cells reach the large intestine, where they can provide health benefits [21]. The results of probiotic encapsulation were continued with gram staining to ensure that lactic acid bacteria were well encapsulated. Lactic acid bacteria are gram-positive bacteria in the form of bacilli (rods) which can be seen in Figure 4.



Fig 1. Probiotic Encapsulation

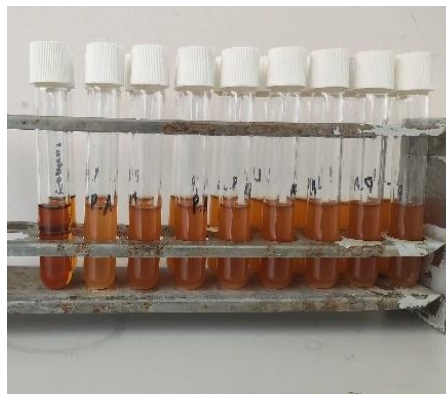


Fig 2. Growth of lactic acid bacteria from probiotic encapsulation

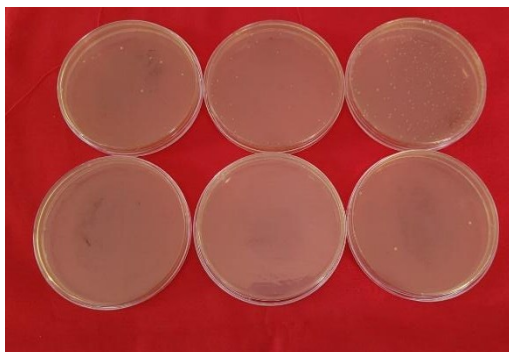


Fig 3. Total Lactic Acid Bacteria Testing using the TPC method

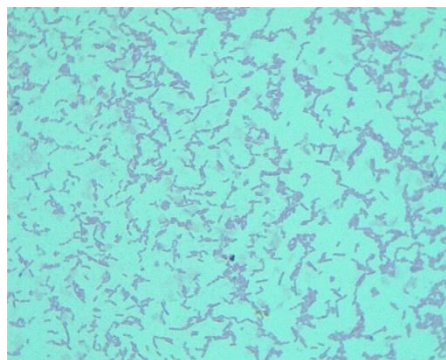


Fig 4. Results of gram stain test of lactic acid bacteria in probiotic encapsulation.

4. Conclusion

The results showed that the probiotic banana stem *Musa paradisiaca* could increase the growth of *Lactobacillus sp.* significantly at certain concentrations, with the most optimal results at a concentration of 6.89% compared to the control. Based on test results, *Musa paradisiaca* stem flour contains a fructan percentage of $0.14 \pm 0.001\%$. The presence of fructan compounds makes *Musa paradisiaca* stem flour function as an effective source of natural prebiotics. The test results showed that the biocapsulation contained 2.7×10^3 cfu/gram of lactic acid bacteria. This shows that the bioencapsulation process of lactic acid bacteria was successful.

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