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Full Length Article

The Potency of Inulin Extract from Mangrove Apple as a Prebiotic for *Lactobacillus bulgaricus* Growth *In Vitro*

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Abstract

Prebiotic sources can be developed from natural materials such as the inulin of mangrove apples (IMA). The objective of this research was to evaluate the efficacy of inulin from mangrove apples on the growth of *Lactobacillus bulgaricus* at an incubation time of 24 h. The experiment was conducted in the completely randomized design with 4 replicates using five different concentrations of IMA. The IMA concentrations applied in this study were 0, 2.5, 5.0, 7.5 and 10% w/v. The concentration of IMA of 10% had significantly ($P<0.05$) revealed to have higher viability of *L. bulgaricus* bacteria, presenting the growth value of 9.63±0.61 log CFU/mL, producing 1.55±0.02% of total lactic acid and resulting the low pH value at 3.46±0.01. *L. bulgaricus* could produce different amounts of short-chain fatty acid content *In vitro*. IMA could be a candidate as prebiotics to support the survival of *L. bulgaricus.* © 2024 Friends Science Publishers

Keywords: Inulin; *Lactobacillus bulgaricus*; Mangrove apple; Prebiotic

Introduction

Prebiotic is non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth of bacteria in the colon and stimulating native microflora in the gut (Ashaolu *et al*. 2021; El-Sayed *et al*. 2021; Tawfick *et al*. 2022). The prebiotic also could confer a health benefit (Slizewska and Chlebicz-Wójcik 2020; Zabihollahi *et al*. 2020; El-Sayed *et al.* 2021). Prebiotics not only have protective effects on the gastrointestinal system but also on other parts of the body, such as the central nervous system, immune system, and cardiovascular system (Davani-Davari *et al*. 2019). Prebiotic-based substances are derived from the sources natural such as inulin, oligofructose, fructo-oligosaccharide, galacto-oligosaccharide, mannooligosaccharide, etc. (Zhang *et al*. 2022; Parsana *et al*. 2023). Inulin is a natural and polydisperse β-(2-1) fructans (Zhang *et al.* 2015). Inulin is type of prebiotics which can be added food to stimulate the proliferation of lactic acid bacteria (Valero-Cases and Frutos 2017; Davani-Davari *et al*. 2019). Inulins are carbohydrates and act like dietary fiber (Zhang *et al*. 2015). Inulin-type fructans are dietary fiber that cannot be digested by human digestive enzymes (Sun *et al*. 2020). According to a recent study in 2021, mangrove apples were revealed to be soluble dietary fibers that significantly contain inulin, which can be a source to natural prebiotics (Wibawanti *et al*. 2021).

Fermentation of prebiotics by gut microbiota produces short-chain fatty acids (SCFAs), such as propionic acid, butyric acid, and acetic acid. They add to SCFAs role in human body, and maintaining intestinal health (Boger *et al.* 2018; Davani-Davari *et al*. 2019; Sun *et al*. 2020). There are abundant studies to determine the prebiotics activity by employing probiotics bacteria *Lactobacillus* is a strain of lactic acid bacteria that can utilize prebiotics of inulin (Paula *et al*. 2020). Prebiotics from inulin and fructo-oligosaccharide support the growth of *L. acidophilus* bacteria (Masroor *et al*. 2019). Prebiotics have been investigated in several studies to support the growth of lactic acid bacteria. Lesser yam inulin was stimulated on the growth of *Lactobacillus* and *Bifidobacterium* (Winarti *et al.* 2013). Carrot and orange juice fortified with inulin was supports the survival of *L. plantarum* (Valero-Cases and Frutos 2017). Various concentrations of prebiotic inulin was stimulated on the growth of *L. bulgaricus*, *L. acidophilus*, and *Streptococcus thermophilus* (Setiarto *et al*. 2017). *L. bulgaricus* as gram positive are used for dairy products in yogurt (Wibawanti *et al*. 2022; Wibawanti *et al*. 2023).

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To date, the information about the use of inulin from mangrove apples as a source of prebiotics is still limited. The aim of this study was to investigate the efficacy of inulin extracts from mangrove apples as type a prebiotic source for *L. bulgaricus* growth using *In vitro* fermentation experiments.

Materials and Methods

Bacteria treatment

The probiotic bacteria used in this research was *L. bulgaricus* (FNCC 0041) containing 10^8 CFU/mL. The bacteria were obtained from Food and Nutrition Culture Collection, Center for the Study of Food and Nutrition, Universitas Gadjah Mada, Yogyakarta, Indonesia. The purified colonies were introduced to de Man Rogosa and Sharpe (MRS) broth and incubated at 37°C for 24 h.

Inulin of mangrove apple treatment

The mangrove apple inulin with the extraction method was carried out according to the method used by Wibawanti *et al*. (2022) method. The mangrove apple was sliced thin and heated in hot water 90°C for 60 mins with 1: 4 ratios. The filtrate was precipitated with 40% ethanol and kept at-18°C for 6 h. The filtrate was defrosted at room temperature. The filtrate was centrifuge for 5 min at 5000 rpm and supernatant was collected.

The experiment was principally set up based on a completely randomized design. The research was randomly divided into five treatment groups with four replicates. The different concentrations of inulin of mangrove apples (IMA) were 0, 2.5, 5, 7.5 and 10% (w/v) in the Man Rogosa Sharpe (MRS) Broth of media culture.

Assessment of prebiotic activity

The prebiotic activity of inulin from mangrove apple was determined *In vitro* by counting the bacterial population of *Lactobacillus bulgaricus* (Shubha *et al*. 2022). MRS agar was used to count lactic acid bacteria (LAB) and the plates were anaerobically incubated at 37°C for 24 h. Total number of colonies was recorded as CFU/mL. It was calculated in plates containing 25–250 colonies.

Determination of pH values

The pH values of the inulin from mangrove apple were determined using a pH meter that had previously been calibrated with pH 7.0 and 4.0 standard buffers (Rezende *et al*. 2022). All analyses were performed in duplicate at 20°C.

Determination of titratable acidity (TA)

The TA value of inulin of mangrove apple was determined using the method described by Zhao *et al*. (2022). A few drops of phenolphthalein were added as the colored indicator solution and titrated with 0.1 M NaOH solution.

SCFAs analysis

The SCFAs were measured following the method of Ashwar *et al*. (2021). The High-Performance Liquid Chromatography (HPLC; Shimadzu GC-2010, Japan) system was modified to examine the short chain fatty acids (SCFA). Together with vigorous shaking, 20 mL of 0.4%. HCl and about 1 mL of the fermentation broth were combined. Then it underwent the centrifugation process (3000 rpm, 4°C) for 10 mins. Syringe filters with a 0.2 m pore size were used to filter the supernatant. Acetonitrile and 0.1% H_3PO_3 were used as the mobile phase and at a flow rate of 1 mL/min during in chromatographic separation on an Agilent Zoffax Eclipse Plus C18 column (4.6 100 mmol/L, 3.5 m).

Statistical analysis

All experimental data were presented in mean values with ± standard deviation (SD). Statistical differences among the results were analyzed using one way analysis of variance (ANOVA) using SPSS software. Differences were considered statistically significant at $P < 0.05$.

Results

The effect of IMA on the growth of *L. bulgaricus* is presented in Fig. 1. Based on the results, total lactic acid with different concentration of IMA had significant (P<0.05) effect on the viability of *L. bulgaricus.* The amount of probiotic *L. bulgaricus* varied following the incubation for 24 h. The highest growth rate of *L. bulgaricus* the MRS Broth media added with 10% IMA (P<0.05) with value 9.63±0.61 log CFU/mL. However, concentrations of 5 and 7% of IMA did not show significant (P>0.05) difference in the viability of *L. bulgaricus* with a value of 8.96±0.67 and 9.09±0.61 log CFU/mL, respectively. Total population of *L. bulgaricus* with 0 and 2.5% was 8.28±0.5 and 8.77±0.47 log CFU/mL, respectively (P>0.05).

The pH values of IMA are represented in Fig. 2. Based on the results, *L. bulgaricus* exhibited trends of pH reduction at higher concentrations of IMA. The pH value of IMA with a concentration of 10% was the lowest compared with another concentration 3.46±0.01 (P<0.05). The pH value of 7.5% IMA was also lower (P<0.05) compared to other treatments. However, concentrations 0, 2.5 and 5.0% of IMA did not show distinct differences (P>0.05) in the pH value, where 3.6±0.01; 3.58±0.01, and 3.55±0.03 were marked, respectively.

There was an increase in lactic acid levels during the inulin fermentation by *L. bulgaricus* for 24 h in the control and concentration IMA of 2.5, 5.0, 7.5, and 10%. Fig. 3 shows the titratable acidity of inulin from mangrove apples. The highest concentrations of lactic acid were observed when the *L. bulgaricus* was grown in the presence of 10% of IMA with an average amount of 1.55±0.02% ($P<0.05$). However, the titratable acidity value of the IMA concentration of 7.5% (1.50±0.01%) was not significantly different ($P>0.05$) with the value obtained by the IMA concentration of 10%. The IMA concentrations 0, 2.5 and 5% of IMA were not different ($P>0.05$) in the titratable acidity value, 1.48±0.01, 1.47±0.07 and 1.48±0.03%, respectively.

The content of acetic acid during fermentation of IMA for 24 h incubation is shown in Fig. 4. The result of acetic acid fermentation from *L. bulgaricus* with the different IMA concentrations of 0, 2.5, 5.0 7.5 and 10% as much as 30.79, 30.22, 29.47, 27.53 and 29.58 mM, respectively. The content of propionic acid during fermentation at incubation time 24 h of IMA are shown in Fig. 5. The result of propionic acid fermentation from *L. bulgaricus* with the diverse IMA proportions of 0, 2.5, 5.0, 7.5 and 10% as much as 0.07, 0.58, 0.39, 0.21 and 0.24 mM, respectively. The content of butyric acid during fermentation at incubation time 24 h of IMA are shown in Fig. 6. The result of acetic acid fermentation from *L. bulgaricus* with the addition of IMA with the various IMA amounts of 0, 2.5, 5, 7.5 and 10% as much as 0.34, 0.73, 0.85, 0.18 and 0.38 mM, respectively.

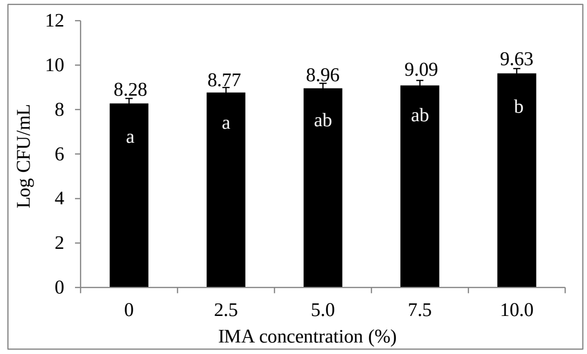

Fig. 1: The addition of IMA on the growth of *L. bulgaricus*

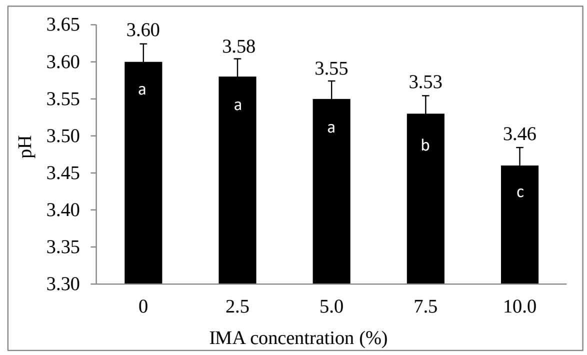

Fig. 2: The addition of IMA on the pH value

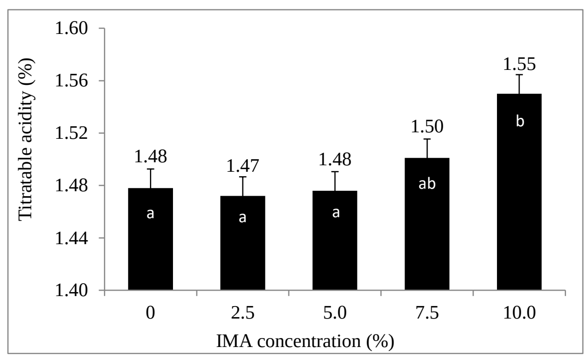

Fig. 3: The addition of IMA on the titratable acidity

Discussion

In this study, increasing total viability of *L. bulgaricus* was associated with the concentration of the IMA as a prebiotic (Fig. 1). This means that *L. bulgaricus* growth could be stimulated by IMA, which could thus be used as an energy source in correlation with decrease of pH and the increase of lactic acid content. Kanjan and Hongpattarakere (2017) reported that inulin could support the growth of *L. paracasei*. Further, Setiarto *et al*. (2017) claimed that the addition of inulin could increase the growth of *L. bulgaricus* bacteria. Valero-Cases and Frutos (2017) also claimed that the addition of inulin could support on the viability of *L. plantarum*. Palacio *et al*. (2014) reported that the activity of prebiotics which could act as a carbon source so as to stimulate the growth of probiotic. In the literature Kanjan and Hongpattarakere (2017) observed a significant grow of *Lactobacilli* and *Bifidobacteria* along with an increase in the amount of lactic acid the decrease of pH, and SCFAs. The improving of probiotic stability by addition of inulin can be explained the formation of interactions (hydrogen bonds) between inulin and polar head groups of membranes phospho-lipids of probiotic bacteria (Zabihollahi *et al*. 2020).

The presence of *L. bulgaricus* resulted in a decrease in the pH values of the culture media. In this research, a decrease in pH affected the lactic acid produced by *L. bulgaricus* (Fig. 2). Reduction in pH, which results in a higher level of organic acid produced, may indicate greater fermentability. Oluwatosin *et al*. (2022) reported that the pH reduction of *L. plantarum* cultivated in 2% (m/v) glucose-free MRS media of inulin with value of 4.78. Rezende *et al*. (2022) reported that the decrease in pH values indicates metabolic activity of probiotic microorganisms and production of organic acid. Napisah *et al*. (2022) reported that *L. brevis* was also found to reduce pH in media containing inulin and fibersol-2.

An increase in the titratable acidity value may be due to the decrease in pH value (Fig. 3). Lactic acid is also used as a carbon source by *L. bulgaricus* in improving growth by increasing metabolism. Napisah *et al*. (2022) reported that

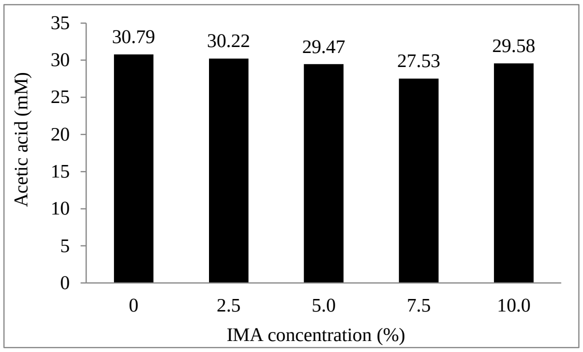

Fig. 4: The addition of IMA on the acetic acid

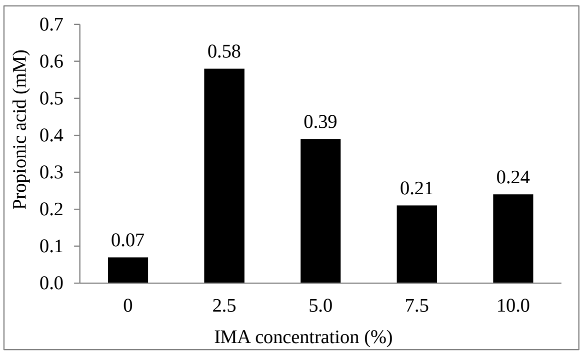

Fig. 5: The addition of IMA on the propionic acid

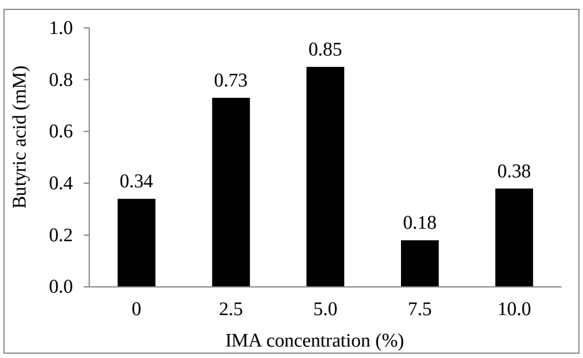

Fig. 6: The addition of IMA on the butyric acid

lactic acid is the main end product of LAB's use of carbohydrates during fermentation. Winarti *et al*. (2013) reported that lesser yam inulin was used *L. acidophilus* for produces lactic acid production during fermentation.

Different amounts of acetic acid, propionic acid and butyric acid were produced by the fermentation of *L. bulgaricus* with the addition of IMA (Figs. 4, 5 and 6). IMA is one of the substrates, which is fermented in the intestine and is known to stimulate the production of SCFA. Johnson *et al*. (2015) reported that inulin is one of the substrates that is fermented in the intestine and is known to stimulate the production of SCFAs. Ashwar *et al*. (2021) reported that varying propionic acid, acetic acid and butyric acid were produced from the fermentation of resistant starch by *Lactobacilli*. Buruiana *et al*. (2017) investigated the prebiotic of xylo-oligosaccharides from corn stover giving effect to the amount of SCFA produced by *Lactobacillus*, *Enterococcus* and *Bacteroides* genera.

Conclusion

The IMA at 10% concentration stimulated the growth of *L. bulgaricus* with a value of 9.63±0.61 log CFU/mL, pH value 3.46±0.01 and total lactic acid with a value of 1.55±0.02%. Fermentation by *L. bulgaricus* with IMA produced different amounts of acetic acid, propionic acid and butyric acid. The IMA may serve as a potential source of prebiotics that may help *L. bulgaricus* survive.

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Author Contributions

JMWW planned the experiments, conducted lab works, statistically analyzed the data, and interpreted the results, Z interpreted the results, LMS and HK reviewed and revised the manuscript and SP conducted field work.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper

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