Lactic Acid Bacteria Producing Inhibitor of Alpha Glucosidase Isolated from Ganyong (Canna Edulis) and Kimpul (Xanthosoma sagittifolium)

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Abstract. Type 2 diabetes is a disease caused by the failure of insulin secretion by the beta cells of the pancreas and insulin resistance in peripheral levels. One therapy for diabetics is by inhibiting the activity of α-glucosidase. Lactic acid bacteria have the ability to inhibit of α-glucosidase activity. The aims of this research was to isolation and screening of lactic acid bacteria from ganyong tuber (Canna Edulis) and kimpul tuber (Xanthosoma sagittifolium), which has the ability to inhibit the activity of α-glucosidase. Eighteen isolates were identified as lactic acid bacteria and all of them could inhibit the activity of α-glucosidase. The GN 8 isolate was perform the highest inhibition acivity.

Keywords. Lactic acid bacteria, inhibitor α-glucosidase, Canna Edulis, Xanthosoma sagittifolium

1. Introduction
Diabetes mellitus (DM) is a disease characterized by the occurrence of hyperglycemia and disfunction metabolism of carbohydrates, fats, and proteins. DM is associated with an absolute deficiency or relative deficiency of insulin or insulin secretion [1,2]. Indonesia is the 4th country with the highest prevalence of diabetes in the world after India, China and the United States. Even the number of people with diabetes continues to increase from year to year, especially for type 2 diabetes is caused by the failure of insulin secretion by the beta cells of the pancreas and insulin resistance in peripheral levels [3]. Based on the latest estimate of IDF (International Diabetes Federation) [4], in 2013 diabetics in the world reached 382 million people. By 2035, diabetics are expected to increase to 592 million people. In Indonesia, diabetes mellitus reaches 12 million people counted in 2013 [5].

Various therapeutic strategies used to treat type 2 diabetes, including the stimulation of insulin secretion, increase insulin action in target tissues, the use of oral hypoglycemic agents such as sulfonylureas and biguanids and inhibition of starch degradation by the enzyme α-glucosidase. Alpha glucosidase is an enzyme that plays a role in the hydrolysis of food carbohydrates into glucose and other monosaccharides [6,7]. The excess production of monosaccharides and the lack of sugar removal by the
body can cause diabetes [8]. Therefore, one way to inhibit postprandial hyperglycemia is to inhibit the activity of the α-glucosidase enzyme [9]. Some synthetic drugs have been used to inhibit the activity of α-glucosidase, for example acarbose as a treatment for type 2 diabetes mellitus. These synthetic compounds have limited, non-specific and cause side effects such as indigestion, bloating, nausea, abdominal discomfort and diarrhea [10,11,12,13].

Some recent researches say that lactic acid bacteria has the ability to inhibit the action of the enzyme α-glucosidase [14,15,16,17,18]. Therefore, this study aims to isolation and screening of lactic acid bacteria from ganyong tuber and kimpul tuber, which has the ability to inhibit the activity of α-glucosidase enzyme. Such lactic acid bacteria can be developed as probiotics with antidiabet ability.

2. Material and Methods

2.1. Materials

The samples used in this study were Ganyong (Canna Edulis) and kimpul (Xanthosomasagittifolium). The sample was obtained from farmers in Merelu village, Gedangsari district Gunungkidul Regency.

2.2. Isolation of Acid Producing Bacteria

The tuber sample was peeled to remove the skin, then sterilized the surface with 70% alcohol soaked for 1 minute. The sample was smoothed, then taken as much as 20 grams and fermented with MRS Broth medium 80 ml at 37°C for 48 hours. Isolation was done by plating using Pour Plate method on MRSA + CaCO₃ medium. Colonies that form clear zones are thought to be acid producing bacteria. Clear zones are formed because the acid produced by the bacteria will react with CaCO₃, so that initially cloudy will be clear. Several colonies of different shapes and sizes were selected and purified on the MRSA medium by streak plate method. Incubation at 37°C for 48 hours. The experiment is done 2-3 times until the pure or single isolate was obtained.

2.3. Screening of Lactic Acid Bacteria

The screening of lactic acid bacteria refers to Bergey”s Manual Determination of Bacteriology by biochemical test. There are 4 key characteristics of lactic acid bacteria: gram positive, non-motile, negative catalase, and do not form spores.

2.4. Preparation of Cell Free Supernatant

The isolates of Lactic acid bacteria were inoculated in 5 ml MRSB, then incubated for 24 h at 37°C. Supernatant was obtained by centrifugation at 4°C, 5000 rpm for 15 min. Then separated between supernatant and cell biomass. The supernatant was used for measurement of α-glucosidase inhibitor.

2.5. Measurement of Alpha Glucosidase Inhibitor

This method refers to Zheng et.al. [19]. The test was performed by adding 25 μl 10 mM p-nitrophenyl-α-D-glucopyranoside (PNPG), 25 μl PBS (pH 6.8) and 50 μl sample or 50 μl acarbose 1.5% (15mg/ml), then incubated for 10 min at 37°C. Thereafter, 50 μl α-glucosidase from Saccharomyces cerevisiae 1 U/ml was added and incubated for 30 min at 37°C. The reaction was terminated by adding 100 μl 0.1M Na2CO3, and the absorbance of sample was measured at 405 nm with microplate Reader. Each test sample was analyzed in duplo, and the absorbance value was corrected against the blank sample where α-glucosidase was replaced with PBS (0.1 M, pH 6.8). Positive control (α-glucosidase activity without inhibitor) and negative control (no α-glucosidase activity) was performed using PBS (0.1 M, pH 6.8), instead of samples and α-glucosidase solution. The results of α-glucosidase inhibition were calculated by :

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\% \text{ inhibition} = \left( 1 - \frac{\text{sample} - \text{blank}}{\text{positive control} - \text{negative control}} \right) \times 100\%
\]
3. Result and Discussion

3.1. Isolation and Screening of Lactic Acid Bacteria

There were 9 isolates of acid producing bacteria from ganyong (Canna edulis) and 10 isolates of acid producing bacteria from kimpul (Xanthosoma sagittifolium). These acid producing bacteria were obtained by formation of clear zone in MRS agar + CaCO3 medium. However, one isolate (GN 6) from ganyong was known not include to lactic acid bacteria because its positive result on catalase test and gram negative. Screening for lactic acid bacteria based on Bergey’s Manual Determination of Bacteriology: gram staining, catalase test, endospore staining and motility.

Among 19 isolate, there were 18 isolates identified which perform gram positive, catalase negative, non motile and negative endospore. The screening result are presented on Table 1.

| Isolate | Gram staining | Catalase test | Motility | Endospore staining | identity |
|---------|---------------|---------------|----------|-------------------|----------|
| GN 1    | +             | -             | -        | -                 | LAB      |
| GN 2    | +             | -             | -        | -                 | LAB      |
| GN 3    | +             | -             | -        | -                 | LAB      |
| GN 4    | +             | -             | -        | -                 | LAB      |
| GN 5    | +             | -             | -        | -                 | LAB      |
| GN 6    | -             | +             | -        | -                 | Not LAB  |
| GN 7    | +             | -             | -        | -                 | LAB      |
| GN 8    | +             | -             | -        | -                 | LAB      |
| GN 9    | +             | -             | -        | -                 | LAB      |
| KM 3.7  | +             | -             | -        | -                 | LAB      |
| KM 6.7  | +             | -             | -        | -                 | LAB      |
| KM 9.7  | +             | -             | -        | -                 | LAB      |
| KM 4.7  | +             | -             | -        | -                 | LAB      |
| KM 1.7  | +             | -             | -        | -                 | LAB      |
| KM 5.7  | +             | -             | -        | -                 | LAB      |
| KM 10.7 | +             | -             | -        | -                 | LAB      |
| KM 2.7  | +             | -             | -        | -                 | LAB      |
| KM 7.7  | +             | -             | -        | -                 | LAB      |
| KM 8.7  | +             | -             | -        | -                 | LAB      |

The characteristics of lactic acid bacteria are the cells reacting positively to gram staining, reacting negatively to catalase, not forming spore and non-motile [20]. Positive catalase is characterized by forming air bubbles when dropping hydrogen peroxide to lactic acid bacteria. This indicates that lactic acid bacteria have catalase enzymes. The catalase enzyme serves to neutralize the bactercidal effect of hydrogen peroxide. Catalase accelerates the breakdown of hydrogen peroxide ($\text{H}_2\text{O}_2$) into water and oxygen ($2\text{H}_2\text{O}_2 + \text{catalase} \rightarrow 2\text{H}_2\text{O} + \text{O}_2$). This reaction is evidenced by the rapid formation of bubbles. In gram staining, lactic acid bacteria include gram-positive bacteria. Gram positive bacteria will retain the violet crystalline dye and will therefore look purple under the microscope. In the motility test, the lactic acid bacteria only growing around the puncture. This was showed negative test results [21]. For endospore staining, all isolates are red. Endospores will look green due to malachite green coloration. Lactic acid bacteria do not form endospores.
3.2. Measurement of alpha glucosidase inhibitor

Cell free supernatant of each lactic acid bacteria isolates was used for measurement of α-glukosidase inhibitor. The result showed that 8 isolates from ganyong and 10 isolates from kimpul were able to inhibit the activity of α-glukosidase at varying degrees (Table 2).

Table 2. Inhibition of Alpha glucosidase by LAB from Ganyong (Canna edulis) and Kimpul (Xanthosoma sagittifolium)

| Isolate | % Inhibition  | Isolate | % Inhibition |
|---------|--------------|---------|--------------|
| GN 2    | 80.97 ± 0.95<sup>def</sup> | KM 9.7 | 56.26± 4.18<sup>ef</sup> |
| GN 3    | 78.60 ± 0.06<sup>de</sup> | KM 4.7 | 7.48 ± 1.87<sup>i</sup> |
| GN 9    | 81.48 ± 4.45<sup>def</sup> | KM 1.7 | 84.88± 0.23<sup>cde</sup> |
| GN 5    | 34.95 ± 0.21<sup>b</sup> | KM 5.7 | 96.19± 0.13<sup>ab</sup> |
| GN 7    | 32.61 ± 1.50<sup>b</sup> | KM 10.7 | 90.22± 6.34<sup>bc</sup> |
| GN 1    | 63.10 ± 0.10<sup>f</sup> | KM 2.7 | 80.60± 5.05<sup>def</sup> |
| GN 4    | 93.82± 2.04<sup>b</sup> | KM 7.7 | 90.68± 8.32<sup>bc</sup> |
| GN 8    | 103.18± 3.96<sup>a</sup> | KM 8.7 | 93.75± 2.70<sup>b</sup> |
| KM 3.7  | 75.62± 5.99<sup>ef</sup> | Acarboza | 87.60±0.01<sup>cde</sup> |
| KM 6.7  | 84.15± 0.16<sup>cde</sup> | 15mg/ml |

Each value represent mean ± SD from 2 independent treatments.

The α-glukosidase inhibitor of each isolates is different. The highest inhibition activity is found in GN 8 isolate, while the lowest inhibition activity is KM 4.7. Even the inhibition activity of GN 8 is higher than inhibition activity of acarboza 15 mg/ml. This may be due to the difference in concentration between acarboza and the cell free supernatant of each lactic acid bacteria. The cell free supernatant content extracellular metabolite which associated with α-glukosidase inhibitor.

Based on the research of Bajpai et.al [8], that lactic acid bacteria capable of inhibiting alpha glucosidase. Inhibition of α-glucosidase is derived from exopolisakarida and inulin produced by lactic acid bacteria [15]. The ability of lactic acid bacteria isolated from ganyong (Canna edulis) in inhibiting α-glucosidase may be caused by ganyong including essential tuber crops in herbal medicines as anti-diabetic [22,23]. Ganyong is a plant that has a high fiber content, high fiber serves to prevent diabetes. While the ability of lactic acid bacteria isolated from kimpul in inhibiting α-glucosidase can be caused by bioactive compounds from kimpul that positively affect the absorption of blood glucose such as water-soluble polysaccharide (PLA), dietary fiber and diosgenin [24]. Kimpul has low-carbohydrate, low fat, and low glucose content so it can be used for diet of diabetic people [25]. Endophytic microorganisms from a plant have the same metabolites as their host which potentially for exploitation in medicine, agriculture and industry [26].

The percentage of inhibitor activity in this study showed higher results when compared with studies conducted by Zeng et al. [19]. The study reported that the percentage of activity of α-glucosidase inhibitors from lactic acid bacteria, especially the Lactobacillus strains, ranged from 25%-34%. Other studies have also reported that lactic acis bacteria strains have a 9% -26% of α-glucosidase inhibitor activity [17].

4. Conclusion

The current study showed that there were 8 isolates that isolated from Ganyong and 10 isolates that isolated from kimpul. Eighteen isolates were identified as lactic acid bacteria and all of them could inhibit the activity of α-glukosidase. The GN 8 isolate was perform the highest inhibition activity (103,18 %). These isolates were potentially to be developed as antidiabet probiotic.
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