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The Potential of Yacon Root (Smalanthus sonchifolius (Poepp.et Endl.) H.Robinson) as Prebiotics to Stimulate Growth of Lactobacillus plantarum B1765

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***Abstract*— Yacon *(Smalanthus sonchifolius Poep p. Endl.)* root is a source of inulin and fructooligosacharide (FOS) which potent as a prebiotic. Inulin and FOS hydrolyzed to glucose and fructans by inulinase which is secreted by probiotics bacteria and further metabolized to short chain fatty acids (SCFA) that were known as bioactive compound to prevent some degenerative diseases. This research observed the potential of yacon roots as prebiotics to stimulate the growth of *L.plantarum B1765*, a probiotic bacteria candidate, for some aspect including the number of lactic acids bacteria (LAB), pH, acidity and the profile of SCFA producing. Enumeration of LAB was done by total plate count using MRS medium + CaCO3, acidity by titration, and the profile of SCFA were analyzed by HPLC. The growth of *L.plantarum B1765* in yacon medium for 48 hours increased from 1,12x106 to 3,25x108 CFU/g, pH decreased to 3.28, and total acids increased from 0.04% to 0.45%. The yields of SCFA for 7 days of fermentation are propionic acid 1182.30 ppm, acetic acid 803.28 ppm and butyric acid 375.41 ppm. Yacon roots are potential as prebiotics especially for the growth of *L.plantarum B1765* as the probiotic candidate.**

Keywords*— *Yacon roots, L.plantarum B1765, prebiotic, probiotic, SCFA

I. INTRODUCTION

Prebiotics are defined as non-digestible food ingredients that selectively stimulate the growth and/or the metabolism of health-promoting bacteria in the intestinal tract, thus improving an organism's intestinal balance [1], [2]). Yacon root has potency as prebiotic because of their fructooligosacharides (FOS) and inulin containing substances. More than 90% of carbohydrates containing substances in yacon are FOS and inulin [3], [4]. Some lactic acid bacteria (LAB) strains could use FOS and inulin as growth medium [5], [6], [7] within the group of LAB, some of *Lactobacillus plantarum* strains had been studied had an ability to ferment inulin and FOS [5]. Inulin and FOS were hydrolyzed to glucose by inulinase which secreted by probiotic bacteria and further metabolized to SCFA, a bioactive compound which very useful to prevent some degenerative disease [8]. SCFAs are saturated aliphatic organic acids that consist of one to six carbons of which acetate (C2), propionate (C3), and butyrate (C4) are the most abundant ($\geqslant$95%) [9]. *L. plantarum B1765* is an isolate of LAB of bekasam (traditional Indonesian fermented fish) isolate. Its potency as a probiotic candidate had been evaluated (data not published). In this study the potency of yacon as a prebiotics for some aspects including the growth of *L.plantarum B1765*, pH, acidity, and SCFA production were studied.

II. METHODS

A. Culturing of L.plantarum B1765

L.plantarum B1765 were obtaining from personal collection. It is the LAB isolate from *bekasam* (traditional fermented fish from Indonesia). The isolate bacteria from frozen glycerol and Man Rogosa Sharpe (Oxoid) (1:1) stock at temperature 2°C was subculture twice. About 1% of isolate was inoculated to MRS broth (Oxoid) and incubated (Memmert) at 37^0C for 20 h at anaerobic state. The suspension then was centrifuged (Eppendorf) for 15 minutes at 3500 rpm, supernatant were discarded and the pellet were suspended in 50 mL of NaCl 0.85% sterile used as a culturing starter of LAB bacteria for *yacon* pickle.

B. Pickle yacon and samples preparation

Yacon were harvested from Senduro, East Java, Indonesia. The roots with no physical damaged then was selected, peeled, washed and processed as described by [10] with some modification. *Yacon* roots were cut ± 1 cm x 1 cm x 1 cm in size, and then approximately 200 g were soaked in a glass jar that contained about 500 mL of 3% brine (NaCl). 10 % (V/W) of starter culture then was inoculated after brine fermentation for 2 hours, and fermented for 7 days at 37^0C. For sample preparation, about 10 g approximately of bacon pickles was homogenate aseptically in stomacher by add of 90 mL of 0.86% NaCl. Sample withdrawn at 0 h, 3 h, 24 h and 48 h for determination of number of LAB, acidity and pH, and only at 48 h for SCFA assay.

C. Enumeration of total LAB

Enumeration of LAB was done by modification method of [11] using enumerate the viable cells in the homogenate samples by serially diluted and plated aseptically using poured method on solid MRS agar (Oxoid) that contains 1% CaCO3, and incubated anaerobically for 48 h at 37 °C.

LAB growth was expressed in colony forming units per milliliter (cfu/mL).

D. SCFA analysis

About 5 mL of homogenate sample was centrifuged at 8500 rpm for 10 minutes. Supernatant was filtered using millex, then 20 µL of filtrate injected into HPLC. The SCFA components of *yacon* pickle were detected by MethaCarb H Plus coulomb, flow rate of 0.6 mL/minute, equipped with photodiode array detector, coupled to spectrophotometer at 215 nm wave length. The mobile phase was 0.005M H2SO4. SCFA profiles and lactic acids were determined qualitatively and quantitatively using standards of acetic acid, propionic acid, butyric acids and lactic acids from Sigma.

E. Acidity and pH determination

Acid total test was done by acid base titration using NaOH 0.1 N as a standart solution. 10 mL sample was dilution to 100 mL in a volumetric flask, then 20 mL sample solution was taken and put in an erlenmeyer flask, 2-3 drops of phenolphthalein were dropt as an indicator. The mixture then was titrated with 0.1 N NaOH until the change of pink colors was happened. The volume of NaOH needed then was noted, and the acid total was represented as a lactic acid percentage. pH of samples was measured using Eutech pH meter.

III. Results and Discussions

A. Results

1) Total LAB, acids production and pH of pickle yacon : The total LAB, acid production and pH of yacon pikle at fermentation time is showed at Table 1.

TABLE I. TOTAL LAB, ACIDITY (%) AND PH OF PICKLE YACON DURING THE FERMENTATION PROCESS

No	Fermentation time (h)	Total LAB (CFU/mL)	Acidity (%)	pH
1	0	1.12 x 10^6 a	0,044a	6.15 a
2	3	4.20 x 10^6 a	0.110 b	5.14 b
3	24	6.90 x 10^7 b	0.296 c	3.43 c
4	48	3.25 x 10^8 c	0.452d	3.28 c

During the fermentation process of pickle *yacon* using a culture starter of *L.plantarum* B1765, the number of LAB showed no significant changes until 3 hours fermentation, but increased sharply for 2 log cycle from 1.12 x 10^6 CFU/mL to 3.25 x 10^8 CFU/mL after 48 hours of fermentation. The acidity increased significantly, started from 0.044% at 0 h fermentation to 0.452 % at the end of fermentation for 48 h. Increasing of acidity was allowed by reducing of pH. The pH reduced significantly from 6.15 to 3.43 during 24 h fermentation, but didn't show a significant reducing until 48 h fermentation.

2) Profil of SCFA : Analysis of SCFA profile was conducted using High Performance Liquid Chromatography (HPLC). SCFA containing in homogenate of extract yacon pikle for 7 days fermentation was evaluated. Figure 1 show the retention time of SCFA standard analysis by HPLC at 14.590, 16.648, 19.253 and 22.930 minutes for lactic acid, acetic acid, propionic acid and butyric acid standard respectively.

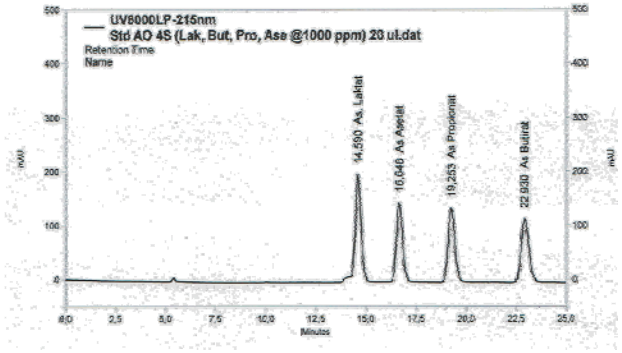

Fig. 1. Retention time of some SCFA standart at chormatogram of HPLC

The SCFA profiles of yacon pickle extract are showed in Fig.2. It could be seen from this figure that yacon pickle extract showed some retention times as the same as SCFA standard. It means that yacon pickle produced lactic acid, acetic acid, propionic acid and butyric acid.

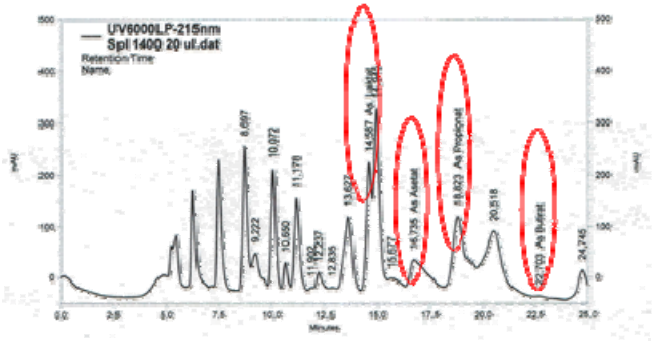

Fig. 2. The SCFA profile of yacon pickle fermented for 7 days.

The yield of SCFA containing in yakon pickled were analyzed quantitatively. The result showed that among the SCFA's containing, propionic acid produced in the highest level 1182.3 mg/L, allowed by acetic acid 803.8 mg/L, butyric acid 375,1 mg/L and lactic acid 371.01mg/L as shown at Figure 3.

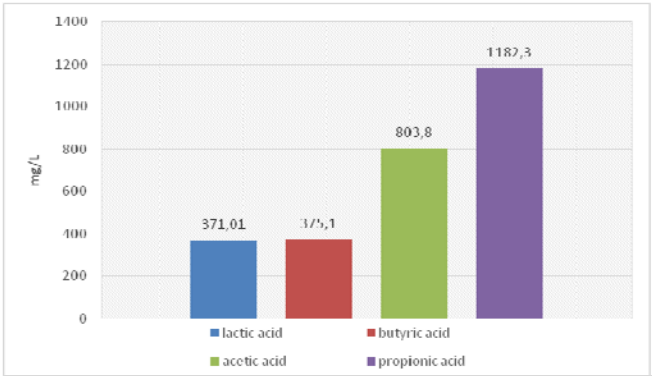

Fig. 3. The yield of SCFA containing in yakon pickled

B. Discussion

The number of LAB was increased to 3.25 x 10^8 CFU/mL during the fermentation process for 48 h of pickle yacon using *L.plantarum B1765* as a culture starter. It attributed that yacon pickle as a good source of prebiotics especially for the growth of *L.plantarum* B1765, a prebiotic candidate that was isolated from *bekasam*. The growth of LAB followed by increasing acidity and reducing pH to 3.43. This is suported with the study of characterization microbial diversity in yacon fermentation with 2% NaCl spontaneously, that the number of LAB increasing to 10^8CFU/g for 3 days of fermentation and reduced gradually after 7 days of fermentation [12].LAB could metabolized yacon which was showed by drops the pH to 3.7 [13] Yacon roots is rich with inulin and FOS, both of these compounds couldn't be hydrolyzed by digested enzyme, but they fermented by the microbial at intestinal tract. *Lactobacillus plantarum* is a Gram positive lactic acid bacterium commonly found in fermented food and in the gastro intestinal tract [14]. It is an evident that FOS and inulin in yacon roots were metabolized by *L.plantarum* B1765 yielding some SCFA that increasing the acidity and reducing pH in yacon pickle. This agreed with some study that *L.plantarum* could metabolize saccharide including FOS and inulin [5],[6],[7]Fermentation of pickle yacon by starter culture of *L.plantarum B1765* produced lactic acids and some SCFA including propionic acids, acetic acids and butytic acid. Profile of SCFA showed that propionic acids were the highest level of SCFA that were found in pickle yacon. In fermentation process, fructans will be degraded into glucose and fructose polimer by β-fruktofuranosidase at α (1-2) glycoside bond [15]. Fructose polymers then were hydrolyze by inulinaseat β(2-1) bond, glycolized to phosphoenolpyruvate (PEP) and metabolized to SCFA furthermore [5],[8].This studied was supported by some research. *L. plantarum* WCFS1 from human saliva has β-Fructofuranosidase that attacked linkage of β-2, 1 [6]. *L. plantarum* ST-III Kimchihas β-Fructofuranosidase which attacked linkage of β-2, 1; β-2, 6 [16]. From this research we inform that pickle yacon is a good source of prebiotics, especially for the growth of *L.plantarum* B1765, a probiotic candidate that was isolated from *bekasam*. In future days this pickled has a big chance to develop as an symbiotic agent and health food because some benefits i.e. promoting health bacteria in intestinal track, and SCFA producing is useful to prevent some degenerative diseases.

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