Hydrolysis of Local Genotype Taro (Colocasia esculenta) Starch by Crude Amylase from Brevibacterium Sp. for Maltooligosaccharides Production

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Abstract. Maltooligosaccharides are starch-based carbohydrate oligomers linked by α-1.4 glycosidic bonds with a degree of polymerization between three and ten. Maltooligosaccharides can be produced by hydrolysis of starch by α-amylase. The enzymatic hydrolysis can be affected by several factors such as substrate and enzyme concentration, pH, temperature and incubation time. In this present study, the hydrolysis of starch from local genotype of Taro (Var. Bentul) was conducted by optimization of 3 parameters: substrate concentration, amount of enzymes, and hydrolysis time. Taro starch concentrations were 10%, 15%, and 20%. Total Enzymes used for hydrolysis were 17.5, 14, and 7 U, respectively. The results showed that an amylase produced by Brevibacterium sp could hydrolyze taro starch into maltooligosaccharides with the most optimum hydrolysis conditions using 20% substrate (w/v) and total enzyme 14 U. Based on HPLC and TLC analysis, Maltotriose revealed as predominant oligosaccharides found in the hydrolysis product with concentration of 8621.7 µg/ml.

Keyword: maltooligosaccharides, local genotype taro, hydrolysis enzymatic, α-amylase

1. Introduction
Indonesia has been well-known as one of countries that has high diversity on tubers as potential crop products such as porang, dahlia tubers, local cassava, local taro, etc [1-4]. Among them, taro is one of the tubers which have many varieties of local cultivar. In Indonesia, Maxiselly dan Karuniawan (2011) has been reported that more than 31 accessions of taro were found in west java, Indonesia [5]. High diversity of local taro is potential to be developed in order to support national food security in Indonesia. One the potential cultivar to be developed is Taro Bentul. This cultivar has good taste, good texture, and round shape. Recently, this cultivar has been developed by many local farmers in Indonesia due to the ability to adapt in various environmental conditions [6].
In order to increase added value of local taro, product diversification is necessary to be conducted. The processing of taro to be food products is in line with the Indonesian government program in food diversification using local food commodity. Several research have been conducted to convert local taro into several high economical products, both intermediate products such as flour, paste, etc and also final ones such as snack, noodle, cakes, etc [7]. In the present study, we converted taro bentul into maltooligosaccharides (MOS) via enzymatic hydrolysis using amylase complex produced by *Brevibacterium* sp. MOS are composed of glucose monomers, with an overall average degree of polymerization (DP) from 3-7. MOS can be used use as replacements for sugar, fat, artificial preservatives, antistaling agents, and as encapsulating food ingredients [8]. Ketabi and Dieleman (2011) reported that MOS has prebiotic characteristics due to their stimulation on intestinal microflora of rats and the growth of *Lactobacilli* [9].

In the present study, we used amylase produced by marine bacteria, *Brevibacterium* sp. Based on previous study, this species has been reported as α-amylase producer [10]. The enzyme could hydrolysis starch into smaller molecule of sugar such as MOS and glucose as final products. Optimization of substrate concentration and the enzyme added into solution were conducted to investigate the best condition for MOS production from taro bentul based starch.

2. Method

2.1. Materials and Chemicals

Dinitrosalycylic acid (DNS) for α-amylase assay was purchased from Sigma Aldrich Co., Ltd. Artificial sea water (ASW), agar, starch, yeast extract, and peptone purchased from Himedia. Taro Bentul (*Colocassia esculenta*) was obtained from Laboratory of Plant Cell and Tissue Culture, Research Centre for Biotechnology LIPI.

2.2. Microorganisms

The bacterial strain used in this study was *Brevibacterium* sp. The strain was obtained from collection of Laboratorium Biocatalyst and Fermentation (LBF)-RC Biotechnology LIPI. The strain was cultivated at 37°C on ASW-starch agar medium for 24 hours in a disposable plastic Petri dish and maintained at 4°C prior to use.

2.3. Starch Extraction of Taro Bentul

The extraction of starch from Taro Bentul was conducted using method developed by Suhery et al. (2015) [11]. Taro Bentul was grinded and added with water (1/3 of taro weight) to form suspension. Suspended material (starch) was filtered and decanted for 24 hours until 2 layers were formed. Starch layer was centrifuged (10,000 rpm for 20 minutes). The starch pellet was dried using oven (T=40°C) for 12 hours (over night). The starch pellet was grinded and filtered to obtain starch powder 100 mesh.

2.4. Production of amylase from *Brevibacterium* sp

Protocol for amylase production was modified from method by Rahmani et al. (2011) [10]. The medium for production consist of ASW, agar, starch, yeast extract, and peptone. The sterile medium was inoculated with *Brevibacterium* sp and incubated for 96 hours (agitation 150 rpm and T=30°C). The culture was harvested and separated from bacterial cell by centrifugation (10,000 rpm) for 20 minutes at T= 4°C.
2.5. **Enzyme assays**

Amylase activity was assayed described by Rahmani et al. (2011) [10] using fraction mixture comprising of 1 mL of crude enzyme, 1 mL of soluble starch solution (0.5% w/v). The mixture was incubated at 30°C for 15 minutes and terminated by addition DNS solution and incubation in boiling water for 15 minute. Reducing sugars liberated were estimated by the DNS methods. 1 unit enzyme was defined as 1 µmol of substrate which was converted by enzyme per minute.

2.6. **Hydrolysis of taro starch by α-amylase derived Brevibacterium sp for MOS production**

The experiment was conducted in a 100-mL Erlenmeyer flask containing starch solution (10, 15, and 20% w/v) in 20-mL phosphate buffer (pH 6.6). Prior to hydrolysis, the solution was gelatinized in boiling water for 5 minutes. Amylase with various activities was added into starch solution (17.5, 14, and Amylase Unit (AU). The solution was incubated in bioshaker at temperature 30°C and agitation 150 rpm. Sampling was conducted at 0, 3, 6, 9, and 24 h. The hydrolysis product was treated for further quantitative and qualitative analysis.

2.7. **Qualytative and quantitative analysis of MOS**

Qualitative analysis was conducted using TLC analysis method [12]. Silica gel plate was used as static phase and mix solution of n-butanol, acetic acid, and aquadest as mobile phase (24:12:12). Four microlitre of sample and standards were injected into the TLC plate. After elution, TLC plate was added using DAP (diphenylamine phosphate) solution. After drying process, TLC plate was put in oven (T=120°C for 15 minutes) until dark spot detected. For quantitative analysis, reducing sugar [13], total sugar [14], and HPLC analysis [15] were conducted.

3. **Result and Discussion**

As the increasing starch concentration, total sugar tends to increase in assayed solution (10-20%). Total sugar describes all sugar content in the solution such as monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Total enzyme added into reaction also contributes to the total sugar detected in the reaction products (Figure 1). Budiarti et al. (2016) [16] stated that substrate concentration affected the sugar yield produced. For the key parameter to detect the successful of polysaccharides hydrolysis into lower molecular sugar such as MOS is reducing sugar analysis. Reducing sugars define as sugars that can have the carbonyl group “exposed” such as glucose, fructose, galactose, lactose, maltose, and also MOS [17]. Based on analysis, reducing sugars tends to increase as the increasing incubation time and enzyme amount added into reaction. Susmiati et al. (2011) and Zhao et al. (2009) also have reported similar result [17-18]. The maximum reducing sugar was detected at 24th hour using concentration of taro starch and total enzyme are 15% (w/v) and 17.5 U, respectively (Figure 2). The total reducing sugar was more than 15,000 µg/ml (15 mg/ml). It means that approximately 10% of taro starch was converted into lower molecular sugar. The result shows that the yield conversion was still low. Use of unpurified amylase might cause uncomplete hydrolysis of taro starch in this study. Moon and Choo (1997) have reported that by using commercial purified α-amylase could enhance MOS yield of meltapentaose up to 70% [19].
Figure 1. Total sugar in various starch concentration (a) 10%, (b) 15%, and (c) 20% and total enzyme: 17.5 Unit, 14 Unit, dan 7 Unit.

Figure 2. Reducing sugar in various starch concentration (a) 10%, (b) 15%, dan (c) 20% and total enzyme: 17.5 Unit, 14 Unit, dan 7 Unit.

Figure 3. Chromatogram for thin layer chromatography analysis of starch hydrolysis into lower molecular sugar (a) conc. 10%, (b) conc. 15%, and (c) conc. 20%, with sugar standards: glucose (G), maltose (M2), maltotriose (M3), and maltoheptaose (M7).

For qualitative data, TLC analysis was conducted using silica gel 60 (F254-Merck) as static phase and mix solvent (butanol:acetic acid:mili-Q=2:1:1) as mobile phase. Based on the result, 3 clear spots were detected in the sample solution: glucose, maltose, and maltotriose. Maltose and maltotriose have revealed as the two predominant spots detected on TLC plate (Figure 3). It could
indicate that α-amylase might be as predominant enzyme in the crude enzyme used for reaction since major products resulted. α-amylase catalyze the hydrolysis of the internal α-1,4-glycosidic linkages in starch, converting starch into low-molecular-weight products such as glucose, maltose, and maltotriose units [20]. We also found that the thickness of spots increase as the increasing incubation time indicating enzymatic hydrolysis was still undergoing until 24 hour.

![Figure 4](image1.png)

**Figure 4.** Chromatogram for HPLC analysis of starch hydrolysis into lower molecular and maltoheptaose (M7)

Figure 4 shows chromatogram of HPLC analysis of hydrolysis product. Five peaks were found in HPLC chromatogram in all samples. Three of them were identified as glucose, maltose, and maltotriose. These results strengthen the TLC analysis data. Maltotriose was found as the only MOS produced from enzymatic hydrolysis with the optimum concentration was 8621 µg/ml at substrate concentration and enzyme added were 20% (w/v) and 14 U, respectively.

![Figure 5](image2.png)

**Figure 5.** HPLC analysis of starch maltotriose as major MOS produced by the enzyme in various starch concentration (a) 10%, (b) 15%, dan (c) 20% and total enzyme: 17.5 Unit, 14 Unit, and 7 Unit

Several reports have been reported regarding to MOS production method. Wu and Chen (2014) investigated that maltotriose could be produced in high yield (93%) by hydrolysis of pullulan by pullulanase with optimum condition: 9.40 h, 47.88 °C, pH 4.92, 10 U/g pullulanase [21]. In the present study, the yield was still low since only 2 parameters were investigated (concentration of taro starch and amount of enzyme added). In the next study, effect of other parameters such as pH and temperature are necessary to be conducted.

Gaston et al (2012) also reported MOS production using different approach [22]. They stated that MOS production was optimized in a batch process reaching a yield of 60% by cyclodextrin glycosyltransferase and cyclodextrin as substrate. Rahmani et al (2015) reported that the optimum hydrolysis of cassava starch (cultivar kuning) was obtained substrate concentration 4.5% (b/v),
comparison of substrate: enzyme 1:2, temperature reaction 30°C with reducing sugars concentration of 13.359 ppm (13.4 g/L). The hydrolysis products of cassava starch cultivated variety Kuning were maltoligosaccharides mixture, yielding maltose, maltotriose, maltotetraose, maltopentaose [23]. Based on the study, the key parameter of MOS production are temperature, pH, substrate concentration, enzyme (amount and enzyme type).

This result showed that taro bentul starch potential for MOS production. However, for increasing the yield of MOS from this taro starch, optimization of other parameters (such as pH and temperature of reaction) and enzyme used for the reaction (purified type) are necessary to investigate for next comprehensive study.

4. Conclusion
Starch origin from Bentul taro and crude amylase derived *Brevibacterium* sp could be used as material for MOS production with the highest concentration was 8621 µg/ml (maltotriose). The optimum condition for MOS production were starch concentration 15% (w/v), enzyme amount 17.5 U, and incubation time 24 hour.

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