ISSN 0126-0472 EISSN 2087-4634
Accredited by DGHE No: 66b/DIKTI/Kep/2011

**Media Peternakan, December 2014, 37(3):190-197**
DOI: 10.5398/medpet.2014.37.3.190
Available online at http://medpet.journal.ipb.ac.id/

# Enzymatic Hydrolysis of Porang by S*treptomyces violascens* BF 3.10 Mannanase for the Production of Mannooligosaccharides

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*(Received 13-06-2014; Reviewed 07-07-2014; Accepted 12-09-2014)*

## ABSTRACT

**Porang (*Amorphophallus muelleri* Blume) is an indigenous Indonesian plant containing high hemicellulose as a source of glucomannan. An alternative way to produce a good quality of mannooligosaccharides was through hydrolysis of glucomannan by endo-β mannnase from actynomicetes. Based on 16S rRNA analysis, BF 3.10 isolate, isolated from Bukit Duabelas National Park soil, Jambi was identified as *Streptomyces violascens* BF 3.10. Reducing sugar was analyzed by dinitrosalicylic acid methods. The highest reducing sugar was achieved at the 72 hours of incubation. Mannanase of isolate BF 3.10 had the highest activity at pH 6 and temperature of 70 °C with enzyme activity of 16.38 U/mL and was stable at 4 °C for 48 h. During 5-hour of hydrolysis with substrate concentration of 0.25%, 0.5%, and 1% porang glucomannan dissolved in 10 mL enzyme, mannooligosaccharides were produced with the degree of polymerization of 2-3. Visualization of the products by using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) methods showed that mannooligosaccharides produced comprised of glucose, mannobiose, mannotriose, and mannotetraose. The degree of polymerization and the simple sugars produced indicated that mannanase produced by *S. violascens* actively catalyzed the hydrolysis of 1.4-β-D-mannoside linkage from β-1.4-mannan backbone, that eventually produced simple sugars of mannooligosaccharides.**

*Key words: glucomannan, mannanase, mannooligosaccharides, porang, Streptomyces violascens*

## ABSTRAK

**Porang (*Amorphophallus muelleri* Blume) merupakan tanaman asli Indonesia yang mengandung kadar hemiselulosa dalam bentuk glukomanan yang tinggi. Salah satu alternatif untuk memperoleh manooligosakarida dengan kualitas baik adalah melalui hidrolisis glukomanan oleh enzim endo β-mananase dari aktinomisetes. Berdasarkan analisis 16S rRNA isolat BF 3.10 yang diisolasi dari tanah Taman Nasional Bukit Duabelas, Jambi teridentifikasi sebagai *Streptomyces violascens* BF 3.10. Kadar gula pereduksi ditentukan menggunakan metode asam dinitrosalisilat (DNS). Kadar gula pereduksi tertinggi dihasilkan pada jam ke-72 inkubasi. Mananase *S. violascens* mempunyai aktivitas tertinggi pada pH 6 dan suhu 70 °C dengan aktivitas enzim sebesar 16.38 U/mL dan stabil pada suhu 4 °C selama 48 jam. Selama 5 jam waktu hidrolisis, reaksi dengan konsentrasi substrat 0,25%, 0,5%, dan 1% glukomanan porang yang dilarutkan dalam 10 mL enzim menghasilkan manooligosakarida dengan derajat polimerisasi 2-3. Visualisasi produk hidrolisis mengggunakan metode *thin layer chromatography* (TLC) dan *high performance liquid chromatography* (HPLC) menunjukkan bahwa jenis manooligosakarida yang dihasilkan adalah glukosa, manobiosa, manotriosa, dan manotetrosa. Derajat polimerisasi dan gula-gula sederhana yang terbentuk menunjukkan bahwa mananase *S. violascens* bekerja secara aktif mengatalisis reaksi hidrolisis ikatan 1,4-β-D-manosida dari rantai utama β-1,4-mannan sehingga menghasilkan gula-gula sederhana manooligosakarida.**

*Kata kunci: glukomannan, mananase, manooligosakarida, umbi Porang, Streptomyces violascens*

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## INTRODUCTION

Porang (*Amorphophallus muelleri* Blume) is one of 27 *Amorphophallus spp* in Indonesia out of a total of 170 types known in the world (Poerba *et al*., 2008). This corm is considered wild-type plant and has not been widely used despite its abundant availability in Indonesia (Sumarwoto, 2004). Currently, porang is commonly exported in the form of porang flour with a low quality and a relatively low price. However, the difficulty in processing due to its high oxalic acid content caused porang to be considered as a material with limited function as food and feed.

Porang is found to be the source of alternative carbohydrate with the highest amount of glucomannan out of all *Amorphophallus* in Indonesia (Sumarwoto, 2004). High amount of glucomannan is found in the corm part of porang plant. The structure of glucomannan composed of β-(1.4) linked D-mannose and D-glucose residues in certain molar ratio (Albrecht *et al*., 2009). The ratio of mannose to glucose in glucomannans depends on the origin of glucomannan (Al-ghazzewi *et al*., 2007; Chua *et al*., 2010) and ranges from around 1.5:1 to 4.2:1 (Tester & Al-ghazzewi, 2013). Glucomannan content of porang ranges from 35%-55% (Sumarwoto, 2004). High mannan content in porang becomes economically potential to be explored further.

Glucomannan with its main component as D-mannose, is important for several industries, including food, feed, and feed stocks (Dhawan & Kaur, 2007). Glucomannan can be broken down by enzymes mannanase into simple sugars. Mannooligosaccharides can be produced by hydrolysis of mannan by a synergistic action of endo-mannanases and exo-acting β-mannosidase. Mannooligosaccharides are non-digestible oligosaccharides and could be potentially applied as dietary fiber and prebiotics (Yopi *et al*., 2006).

According to Roberfroid (2007), prebiotics are defined as selectively fermented ingredients allowing specific changes, both in the composition and/or activity in the gastrointestinal microflora, that give benefits upon the well-being and health of the host. Feed supplemented with mannooligosaccharides (MOS) will significantly affect the intestinal microflora of chicken by increasing the *Bifidobacterium spp.* and *Lactobacillus spp.*, and decreasing the *Enterobactericeae* groups (Fernandez *et al*., 2002). MOS also positively affected on the fecal microbial population by reduction of *E. coli* and *Salmonella* number (Khanongnuch *et al*., 2006). The effect of MOS supplementation in the diet of broilers shows a significantly higher body weight gain and improvement in feed efficiency as compared to the control diet (Shendare *et al*., 2008). Moreover, mannooligosaccharides are potential to be used as non-nutritional and functional food additives for selective growth of intestinal beneficial microflora, reduction of dietary fat absorption, and inhibition of the increased blood pressure (Jian *et al*., 2013).

The aim of this study was to produce mannooligosaccharides through enzymatic hydrolysis of porang glucomannan and to analyze mannooligosaccharides product by thin layer chromatography and high performance liquid chromatography. This study also determined the optimum production time and characterized the optimum conditions of mannanase that can be used to produce prebiotic mannooligosaccharides from porang.

## MATERIALS AND METHODS

### Physico-chemical Analysis of Porang Flour

Porang flour was purchased from PT Ambico Surabaya, East Java, Indonesia. The particle size of porang flour obtained was 80 meshs. Porang flour was weighed and subsequently processed for physico-chemical properties analysis i.e., moisture, protein, lipid, crude fiber, and ash contents of the isolated samples (AOAC, 1995).

### Rejuvenation of Isolate and Preparation of Inoculum

Actinomycetes BF 3.10 isolate from Bukit Duabelas National Park soil, Jambi has been known to possess mannanolitic activity. The isolate was rejuvenated in solid media containing of 0.5% glucomannan and incubated at 30 °C for 4 d.

### Mannanase Production and Mannanase Assay

The medium for mannanase production consisted of 0.5% porang glucomannan, 0.05% yeast extract, 0.075% peptone, 0.14% $(NH4)_2SO_4$, 0.2% $KH_2PO_4$, 0.03% $MgSO_47H_2O$, 0.03% CO $(NH_4)_2$, 0.03% $CaCl_2$, 0.0005% $FeSO_47H_2O$, 0.00016% $MnCl_27H_2O$, 0.00014% $ZnSO_47H_2O$, and 0.0002% $CoCl_2$ (Mendels & Sternberg, 1976). The media were sterilized at 121°C for 15 min. Mannanase production by isolate BF 3.10 was induced by cultivating 1 corckborer microorganism (diameter ±1 cm, 4 days old) in 100 mL of production liquid media in a 500 mL Erlenmeyer. The culture was incubated in a shaking incubator (Taitec) with 200 rpm agitation at 30 °C for 5 d. The crude mannanase enzyme preparation was obtained in the form of culture supernatant by centrifugation at 12000 rpm for 10 min at 4 °C temperature.

Mannanase activity was evaluated by measuring the amount of reducing sugars produced by the 3.5-dinitrosalicylic acid (DNS) method (Miller, 1959) by using mannose as a standard. The mannanase activity was assayed by incubating the enzyme solution into substrate of 0.5% locust bean gum (Merck, Darmstadt, Germany) prepared in 50 mM phosphate buffer with pH 6 for 30 min. The reaction was stopped by immersing the test tube in boiling water for 20 min. The yielded reducing-sugar substance was assessed by spectrophotometer with a wavelength of 540 nm (Hitachi, U-3900H, Tokyo, Japan). One unit of mannanase activity was defined as the amount of enzyme which produces 1 μmol mannose per minute under the assay condition.

The curve of bacterial growth was drawn based on cell's biomass dry weight. The culture of bacteria was inoculated into 30 mL mineral media of 0.5% porang glucomannan, shaken at 200 rpm and 30 °C for 5 d. Sample

was harvested every day, and then centrifuged at 12000 rpm with 4 °C temperature for 20 min. The pellet was sieved and dried in an oven at 50 °C. The biomass was weighed and plotted to incubation time to obtain bacterial growth curve.

### Mannanase Characterization

Enzyme characterizations conducted in this experiment were determination of optimum pH and temperature as well as the stability of crude extract of mannanase enzyme. Optimum pH was determined by analyzing the activity of crude extract enzyme at range of pH 3-10 with 1 interval by using the following buffer systems (50 mM): sodium citrate (pH 3-5), sodium phosphate (pH 5.5-7.5), Tris HCl (pH 7.5-9), and glycine-NaOH (pH 9-10). Optimum temperature was determined by reacting the enzyme and substrate at various temperatures, ranging from 30-100 °C with a 10 °C interval, at the optimum pH obtained before. The stability of crude extract of mannanase enzyme was analyzed by incubating the enzyme (without substrate) at three different temperatures (30 °C, 4 °C, and optimum temperature). Crude enzyme extract was analyzed every hour (from 1$^{st}$ to 5$^{th}$ hour) and every 24 h at its optimum pH and temperature with 0.5% mannan substrate.

### Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out at various substrate concentrations (w/v) i.e., 0.25%, 0.50%, 1% in enzyme solution and various reaction times i.e., 3, 5, and 24 h. The reactions were carried out in 50 mL Erlenmeyer flasks containing 10 mL of reaction mixtures in rotary shaker (Stuart orbital incubator S1500, Staffordshire, United Kingdom) at 30 °C. The samples were taken at regular intervals (after 3, 5, and 24 h) and the reactions were stopped in boiling water for 15 min. The samples were then centrifuged at 12000 rpm for 15 min and the supernatant, as the product of hydrolysis, was analyzed both quantitatively and qualitatively.

### Analysis of Mannooligosaccharides

The product of hydrolysis was analyzed by calculating the reducing sugar, total sugar content, and the degree of polymerization. Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) were used for analysis of mannooligosaccharides. Analysis of total sugar was performed by applying phenol-sulphuric acid method with modifications described by Dubois *et al.* (1956). Reducing sugar was analyzed by DNS method (Miller, 1959). The degree of polymerization was calculated from the ratio of total sugars and reducing sugars yielded.

The types of simple sugars were analyzed by thin layer chromatography (TLC) method on silica gel $60F_{254}$ plates (Merck Art20-20 cm, Darmstadt, Germany) by using a solvent of n-butanol:acetic acid:aquades (12:6:6, by volume). The product of hydrolysis was applied on silica plate comprising of 4 µL sample and 2 µL standard solution. Acting as standards were glucose and mannooligosaccharides (mannose, mannobiose, mannotriose, mannotetraose, mannopentose, and mannohexose). The spots formed were visualized by heating at 121 °C for ±15 min after spraying the plate with staining solution consisted of $\alpha$-diphenylamine 0.2 g, 10 mL of acetone, 1.5 mL of phosphate acid, and 0.2 mL of aniline. Mannooligosaccharides products were analyzed by HPLC under the following conditions: column 250 x 4.6 mm i.d. Zorbax SIL coated with 3-amino propyl silane, mobile phase was acetonitrile and distillated water in ratio of 75:25 (v/v), column temperature (30°C), flow rate (1.4 mL/min) and detector Agilent Technologies 1200 series refractive index monitor (RID).

## RESULTS AND DISCUSSION

### Chemical Composition of Porang Flour

Chemical composition obtained from proximate and Van Soest analysis of porang flour is shown on Table 1. Proximate and Van Soest analysis showed that porang contained high hemicellulose (75.72% polysaccharides), presumably most of them was glucomannan. This composition resembled the glucomannan content in konjac flour, ranged from 70%-90% (Thomas, 1997). On the other hand, cellulose was found in very little amount in porang.

### Determination of Optimum Production Time and Activity of Mannanase

Porang flour contained approximately 75% mannan polysaccharides that could be used as the carbon source for bacterial growth. The highest activity of mannanase was achieved by *S. violascens* BF 3.10 on its optimum growth i.e., at the 72 h incubation reached 0.76 U/mL at substrate concentration of 0.5% glucomannan porang, 200 rpm, and 30 °C (Figure 1). After 72 h incubation, the cell number decreased, followed by an insignificant decrease in enzyme activity.

Table 1. Chemical composition of porang flour

| Analysis | Constituent | % |
|---|---|---|
| Proximate[a] | Moisture | 12.28 |
| | Ash | 3.61 |
| | Lipid | 0.36 |
| | Crude protein | 1.61 |
| | Crude fiber | 0.34 |
| | Nitrogen free extract | 81.80 |
| Van Soest[b] | Dry weight | 86.47 |
| | NDF | 78.72 |
| | ADF | 3.00 |
| | Hemicellulose | 75.72 |
| | Cellulose | 2.90 |
| | Lignin | 0.08 |

Note: [a]Laboratory of Feed Science and Technology, Department of Nutrition and Technology, Faculty of Animal Science, Bogor Agricultural University; [b]Research Centre for Bioresources and Biotechnology, Institute of Research and Community Empowerment, Bogor Agricultural University.

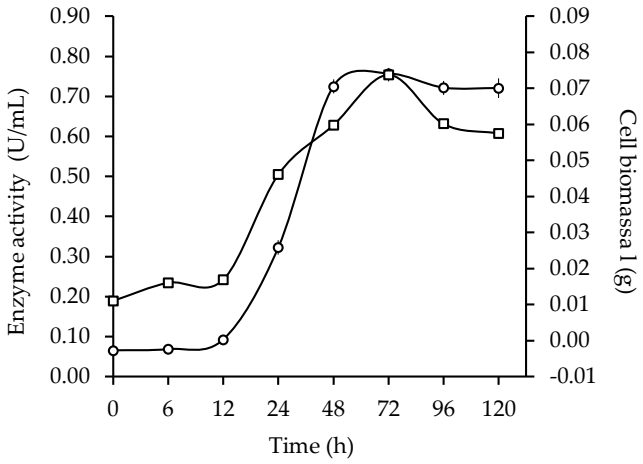


Figure 1. Time course of mannanase production by *Streptomyces violascens* BF 3.10 in 0.5% porang glucomannan at 30 °C, pH 6, 200 rpm: mannanase activity; (-o-), cell biomassa ; (-□-).

Bacterial growth was affected by aeration and the amount of nutrition. Low oxygen solubility and slow oxygen replacement through diffusion process were two inhibiting factors for aerobic bacteria to live (Madigan *et al*., 2006). Porang is highly viscous substrate in water. During the first 24 h, the bacterial growth and activity were low because of the low oxygen availability. But, they started to increase significantly after 24 h when the viscosity of 0.5% of porang substrate got lower than that of the previous hour. Agitation could accelerate oxygen replacement in the medium (Meryandini *et al*., 2008). An agitation speed of 200 rpm in 0.5% porang substrate in this current study may be able to provide a good condition for bacterial growth to produce enzyme with a high activity. Bhoria *et al.* (2009) reported that *Streptomyces* sp. PG-08-03 produced mannanase with activity of 15 U/mL in 0.5% guar gum at 72 h, 37 °C, and 200 rpm agitation. The optimum time in producing mannanase was then used as a standard harvesting time for the next mannanase production.

**Mannanase Characterization**

Changes in pH gave various effects on mannanase activity produced by *S. violascens* BF 3.10. At the range of pH 3-10, the activity of mannanase produced by *S. violascens* BF 3.10 showed the highest activity at pH 6 (Figure 2). Low pH or more alkaline condition led to a significant decrease in enzyme activity. In acidic condition (pH<5), the activity of enzyme decreased to less than 80% of its maximum one. Enzyme is a polyionic polymer and thus is highly affected by the changes in pH. Changes in pH can alter the spread of charges in the active site of all parts of protein molecule's surface (Illanes, 2008). At optimum pH, the structure and active site of an enzyme are in the most suitable condition to bind substrate, resulting in a maximum activity. Microorganism has high diversity in the sensitivity to pH changes, i.e., *Streptomyces galbus* NR has an optimum pH of 6.5 (Kansoh & Nagieb, 2004), whereas for bacterias, *Bacillussubtilis* WY34 the optimum pH was 6 (Jiang, 2006). For fungal, *Aspergillus niger* and *Trichoderma spp*,the highest activity was found at pH of 5 (Adesina *et al*., 2013).

Enzyme is highly sensitive to temperature changes. This study showed that mannanase *S. violascens* BF 3.10 as a thermostable enzyme has an optimum temperature at 70 °C at pH 6 with a maximum activity up to 16.38 U/mL (Figure 3). Similar to β-mannanase thermophilic actinomycetes *A. aculeatus* have optimum temperature at 70 °C (Pham *et al.,* 2010), whereas for bacteria, mannanase of *Bacillus agaradhaerens* strain HMTS15 had an optimum temperature at 75 °C pH of 10 (Liao *et al.,* 2011). The enzyme activity decreases slowly as the temperature increases. Enzyme inactivation by temperature occurs because the intermolecular strength weaken, affecting the enzyme's three dimensional stability and reducing its catalytic ability (Bommarius & Broering, 2005).

Different temperatures affected differently on mannanase stability as shown when it was incubated at 4, 30, and 70 °C (Figure 4). At its optimum temperature, mannanase activity decreased significantly at the first hour of incubation and at the 5 h incubation, the activity was lost by 98%. On the other hand, it appeared that the en-

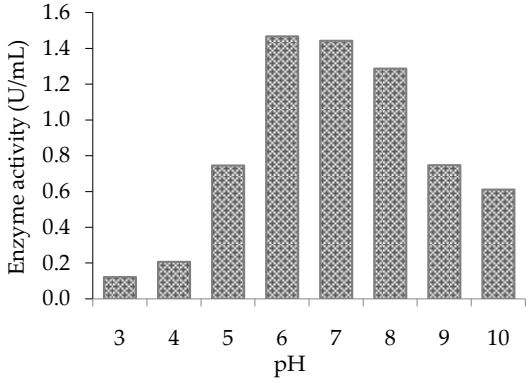


Figure 2. The effect of pH on mannanase *Streptomyces violascens* BF 3.10 activity measured at 30 °C using 50 mM of different buffers

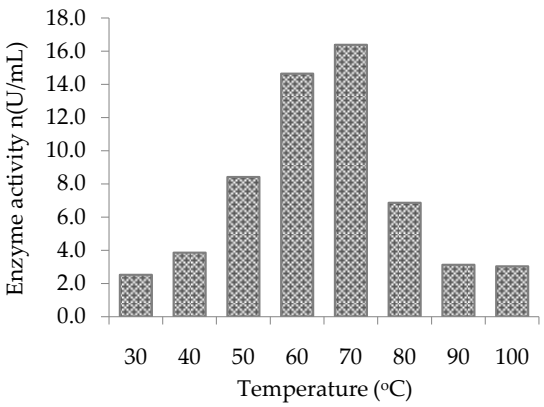


Figure 3. The effect of temperature on the activity of mannanase *Streptomyces violascens* BF 3.10 measured at pH 6

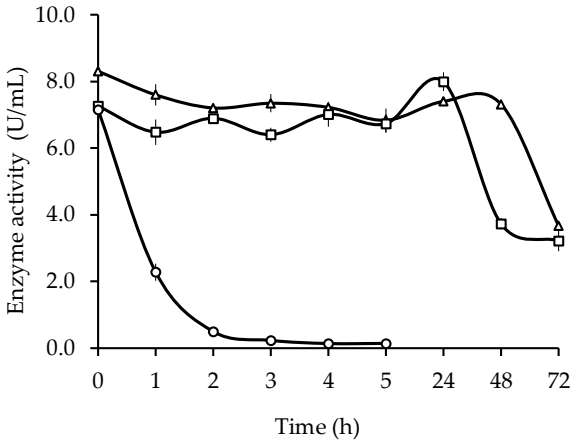


Figure 4. Mannanase stability of *Streptomyces violascens* BF 3.10 at different storage temperatures measured at pH 6. Enzyme stability was measured at three storage temperatures : 4 °C (-Δ-), 30 °C (-□-), and 70 °C (-o-).

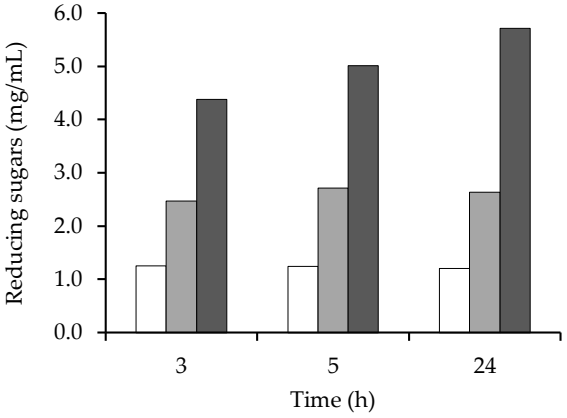


Figure 5. Analysis of reducing sugars as product of hydrolysis, hydrolyzed by mannanase enzyme of *S. violascens* BF 3. 10 with porang glucomannan concentration of 0.25%, 0.50%, and 1% in 10 mL enzyme solution, 30 °C. Concentration of porang substrate of 0.25% (□), 0.5% (▤), 1% (■).

zyme had longer half-life time at incubation temperature of 4 and 30°C. Incubation at 4 and 30 °C could retain the enzyme activity for 48 and 24 hour of incubation, respectively, then the activity significantly decreased by 50%. Temperature has a high impact on enzyme activity and its stability. As the temperature increases, not only chemical reaction rate will increase but also the inactivation rate (Illanes, 2008). This explains why mannanase has a relatively shorter half-life at 70 °C. Mannanase of *Penicillium occitanis* was reported to lose 50% of its activity after 4-hour incubation at 60 °C. The immobilized β-mannanase of *Penicillium occitanis* retained 40% of its activity after 30 min of incubation at 70 °C (Blibech *et al.*, 2011).

### Hydrolysis of Porang Flour by Mannanase *S. violascensi* BF 3.10

Porang flour is an economic raw material for MOS production. Enzyme ability and substrate concentration are important factors for polymer hydrolysis process (Jian *et al.*, 2013). In this study, the maximum concentration of porang was set at 1% due to its high viscosity. High viscosity will reduce enzyme ability to hydrolyze component linkage within substrate (Jian *et al.*, 2013). Hydrolysis time was determined based on mannanase stability, for 3, 5, and 24 h at 30 °C. For ≤ 24 h, mannanase could retain up to ≥ 90% of its activity. The amount of reducing sugar produced was monitored to determine the ability of mannanase in hydrolyzing porang. At the same reaction time, the higher substrate concentration the more reducing sugar was produced (Figure 5). The highest reducing sugar produced was observed at substrate concentration of 1%. They were 4.375, 5.008, and 5.708 mg/mL at the incubation for 3, 5, and 24 h, respectively.

Hydrolysis of glucomannan at various substrate concentrations and reaction times resulted in nearly the same degree of polymerization (DP) i.e., 2-3 (Table 2). The same DP value from the three different substrate concentrations indicated that the ability of mannanase

Table 2. Degree of polymerization (DP) analysis with variations of porang glucomannan concentration (0.25%, 0.5%, and 1% in 10 mL enzyme solution, at 30 °C and 150 rpm)

| Substrate concentration | Time (h) | Total sugar (mg/ML) | Reducing sugar (mg/ML) | DP |
|---|---|---|---|---|
| 0.25% | 3 | 2.513 | 1.253 | 2.00 |
| | 5 | 2.595 | 1.247 | 2.08 |
| | 24 | 2.504 | 1.207 | 2.07 |
| 0.50% | 3 | 5.774 | 2.470 | 2.34 |
| | 5 | 6.319 | 2.710 | 2.33 |
| | 24 | 4.874 | 2.640 | 1.85 |
| 1.00% | 3 | 13.077 | 4.375 | 2.99 |
| | 5 | 11.668 | 5.008 | 2.33 |
| | 24 | 11.714 | 5.708 | 2.05 |

enzyme of *Streptomyces violascens* BF 3.10 might be restricted to only degrade substrate into mannobiose and might not be able to degrade further into their monomer forms (mannose). The same degree of polymerization was observed at hydrolysis of konjac's glucomannan with DP ranging from 1-4 and the main product spreaded at DP of 2 (Kurakake *et al.*, 2006). Konjac flour hydrolyzed by mannanase of *Bacillus* sp. MSJ-5 produced mannooligosaccharides with DP 2-6 (Zhang *et al.*, 2009).

### Analyzing Mannooligosaccharides by Using TLC and HPLC

Based on thin layer cromatography (TLC) analysis, mannooligosaccharides produced by mannanase *S. violascens* BF 3.10 consisted of glucose, mannobiose, mannotriose, and mannotetraose with mannotriose as the main product (Figure 6). It indicates that mannanase belongs to endo-mannanase type. Endo-mananase can degrade mannan polysaccharide to mannotetraose, mannotriose, and mannobiose (Jiang *et al.*, 2006). The

results of the analysis of hydrolysis of 0.5% porang glucomannan with incubation time for 5 hours using HPLC showed five peaks (Figure 7). Mannose monomers were detected in the first peak with a retention time of 4.176 min. Mannooligosaccharides products detected were mannobiose (5.179 min), mannotriose (5.991 min), mannotetraose (6.738 min), and mannohexose (10.648 min).

Endo β-mannanase is an important enzyme in degrading polysaccharide due to its ability to catalyze random hydrolysis of β-D-1,4-mannopyranosyl linkage (Alonso-Sande *et al.*, 2009). Hydrolysis on this linkage from a glucomannan backbone will produce oligosaccharides in the form of mannobiose and mannotriose (Addemark *et al.*, 1998). The ability of β-mananase to degrade glucomannan backbone depends on several factors, including number and distribution of the substituents on the backbone and the ratio of glucose and mannose (Mc Cleary *et al.*, 1986). Recent studies reported that mannanase from certain bacteria can hydrolyze mannan into certain oligosaccharides, such as mannooligosaccharide production from palm kernel by mannanase of *Brevibacillus borstelensis* (Utami *et al.*, 2013), also from copra pulp by recombinant of *A. niger* (Cuong *et al.*, 2013), and from *locust bean gum* by using immobilized *Penicillium occitanis* mannanase (Blibech *et al.*, 2011). Hydrolysis of porang glucomannan by mannanase of *S. violascens* BF 3.10 in this study produced the same products compared to hydrolysis of konjac flour by endo-β-mannanase MAN5 (Zhang *et al.*, 2009). More than 90% polysaccharides were hydrolyzed by endo-β-mannanase MAN5 into oligosaccharides with DP 2-6 (Zhang *et al.*, 2009). Some fungal mannanase hydrolyze mannotetraose to mannotriose and mannobiose through transglycosylation reaction (Puchart *et al.*, 2004). The presence of mannose (monosaccharide) in the hydrolyzed mixture is the product of β-Mannosidase. β-Mannosidase, an exo-type enzyme, cleaves β-1,4-linked mannosides, releasing mannose from the non-reducing end of mannans and mannooligosaccharides (Dhawan & Kaur, 2007). The presence of glucose product on the re-

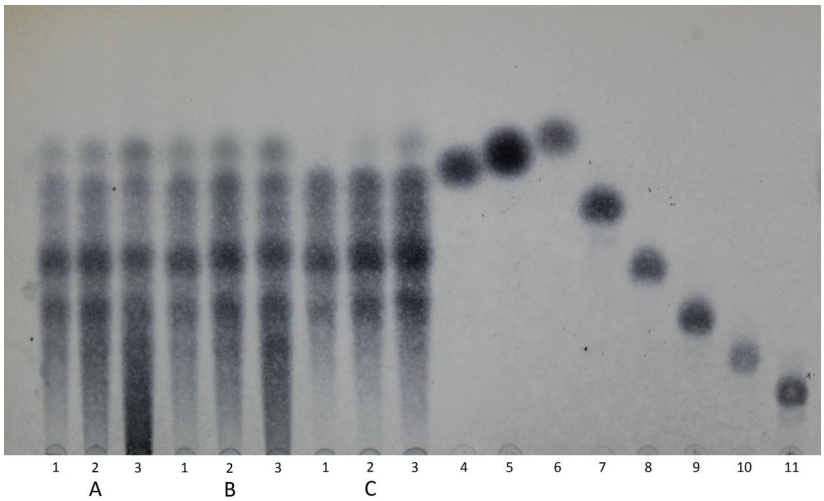


Figure 6. Thin layer chromatography analysis of the porang flour hydrolazed by *Streptomyces violascens* mannanase at the reaction time of 3 h (A), 5 h (B), and 24 h (C). 1= 0.25% substrate concentration; 2= 0.50% substrate concentration; 3= 1.0% substrate concentration; 4= standard galactose; 5= standard glucose; 6= standard mannose;7= standard mannobiose; 8= standard mannotriose; 9= standard mannotetraose; 10= standard mannopentose; 11= standard mannohexose.

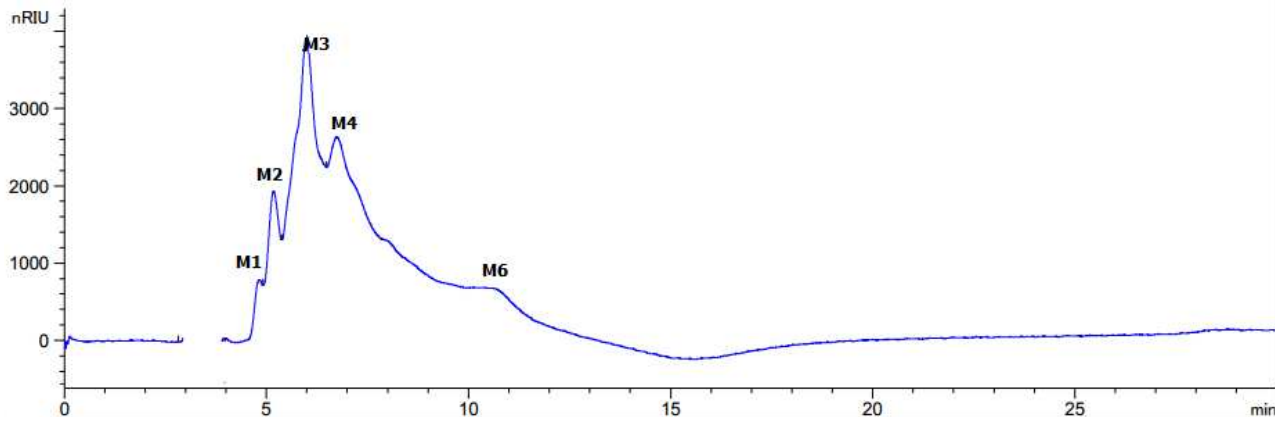


Figure 7. High performance liquid chromatography analysis of 0.5% porang glucomannan with incubation time for 5 hours hydrolyzed by mannanase *Streptomyces violascens* BF 3.10. M1= mannose; M2= mannobiose; M3= mannotriose; M4= mannotetraose; M6= mannohexose.

sult of TLC analysis indicates that enzyme β-glucosidase cuts glucose terminal resulting in glucose side chain (Alonso-Sande *et al*., 2009).

Based on this study, porang could be an efficient substrate for mannanase production by *S. violascens* BF 31.0 to produce mannooligosaccharides. Manooligosaccharides are often used as ingredients in the formulation of functional feed, for example in cattle. Mannooligosaccharide produced by mannanase of *S. violascens* BF 3.10 is potentially developed as functional food or feed, acting as a prebiotic. Mannooligosaccharide should comply with many requirements to be classified as a prebiotic : resist gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; fermented by the intestinal microflora; stimulates selectively the growth and/or activity of intestinal bacteria associated with health and wellbeing (Gibson & Roberfroid, 2004). Endo-β-mannanase allows a large molecular weight glucomannan to be depolymerized into low molar weight fractions with lower DP (prebiotics belong to this group). Based on the chemical structure, the compounds of mannooligosaccharide are not absorbed in the upper part of the gastrointestinal tract or hydrolyzed by digestive enzymes, so it could potentially developed as prebiotic. Broiler chicks fed with prebiotics were increased in weight gain, feed conversation efficiency, nutrient digestibility, while the jejunal content viscosity was decreased (Sundu *et al*., 2006). On the other hand, dietary supplementation with prebiotics reduced some negative effects of heat stress in broilers (Sohail *et al*., 2010).

## CONCLUSION

The corms of Porang (*Amorphophallus muelleri* Blume) was subjected to enzymatic hydrolysis by local isolate BF 3.10 (*S. violascens* BF 3.10) for the production of high quality mannooligosaccharides to develop its functional application as prebiotics. Characterization of the hydrolysis product afforded many kinds of mannooligosaccharides with DP 2-3. The result of this study suggested the potential of porang to be applied as prebiotics in the feed industry.

## ACKNOWLEDGEMENT

Part of this research was funded by Start-up Program from CRC 990 for BO2 for Anja Meryandini.

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