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Prebiotics Activity of Laminaran Derived from *Sargassum Crassifolium*

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ABSTRACT

The objectives of this study were to evaluate the prebiotic activity based on the change in cell biomass of the probiotic strain after 24 hours growth in the presence of Laminaran from *Sargassum crassifolium* with the methods of Laminaran Acid Extract (LAE) and Laminaran Modified Extract (LME), inulin, or glucose-relative against the change in cell biomass of *Escherichia coli* FNCC 0091 grown under the same condition. Prebiotic activity was calculated for *L. plantarum* FNCC 0051 and *Bifidobacterium longum* FNCC 1081. The results showed that the increasing cell number of *L. plantarum* was higher in both substrates LAE and LME (0.58 and 2.03 log cycle), whereas that of B. longum was lower. The higher prebiotic activity score obtained for L. plantarum and B. longum grown on LME were positive (0.26 and 0.96 log cycle), whereas the lowest score was for *L. plantarum* and *B. longum* grown on LAE, which were negative (-0.35 and -0.31 log cycle), but the higher prebiotic activity score was obtained for inulin (4.08 and 4.78 log cycle). It could be concluded that Laminaran Modified Extract (LME) had potential as prebiotic source, but its potential was lower than inulin.

Keywords: Laminaran Acid Extract, Laminaran Modified Extract, prebiotic activity score.

INTRODUCTION

Laminaran, one of short-chain polysaccharides of brown algae, could be classified as Non-digestible Oligisaccharides (NDOs), the non-digested oligosaccharides which may function as a prebiotic (Lambertus et al., 2008). Horse et al. (1998) stated that laminaran could be fermented by some strains of bacteroides and clostridium. It was supported by Salyers et al. (1977) stating that laminaran, β-(1.3)–glucan similar to those found in the cell walls of higher plants, is fermented by several species of anaerobic bacteria from the human colon and is an important source of carbohydrates for the bacteria of the colon. Thus, it could be classified as a prebiotic, which is defined as food ingredient that is not digested giving health benefit to a host by selectively stimulating growth and or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Currently prebiotic carbohydrates are often added to many processed foods to influence both and composition or activity of digestive tract microbial. Composition of digestive tract microbia affects the digestive tract health and host's nutrition by supplying nutrients, convert metabolites, and interact with host cells (Flint et al., 2007). In addition, the existence of a micro floral colonies as associated with the presence of certain diseases such a is inflammatory bowel disease (IBS), gastroenteritis, and colon cancer (Steer et al., 2000; Venter, 2007). Prebiotic development becomes more rapid along with the growing consumer awareness that there is a link between health and diet. One of possibe ways to evaluated candidate prebiotic is by observingits prebiotic activity. Prebiotic activity indicates the ability of substrates in supporting growth relative to that of other

organisms and the relative growth of non-prebiotic substrates such as glucose.

The aim of this research was to evaluate the prebiotic activity of Laminaran Acid Extract (LAE) and Laminaran Modified Extract (LME), which was produced from brown algae *Sargassum crassifolium* originated from Talango Island, Sumenep Regency, Madura, Indonesia.

MATERIALS AND METHODS

Raw materials and supporting materials

Raw materials used in this study were brown algae *Sargassum crassifolium* obtained from Talango Island, Sumenep Regency, Madura, Indonesia, which was harvested from nature with unknown agein natural habitats at depths of 50-250 cm of tidal area in February 2009. Raw materials used were clumps that had *talli* between 40 and 100 cm.

Reagents for prebiotic activity test were M-9 minimal media (Na2HPO4-7H2O, KH2PO4, NaCl, NH4Cl, MgSO4, CaCl2), glucose or other sugar source (LAE, LME and inulin). In addition, peptone, powder lab. lemco, yeast extract, MRS broth, gas generating kit (Oxoid), glucose, NaCl, Tween 80, MgSO4.7H2O, MnSO4.7H20, triamonium citrate, CH3CH2Na.3H2O (Sigma-Aldrich), skimmed milk brand Lactona and agar were also used.

Microbial culture used in this study was obtained from the Center for Food and Nutrition Studies Gadjah Mada University namely *Escherichia coli* 0091 FNCC, *Lactobacillus plantarum* 0051 FNCC and *Bifidobacterium longum* FNCC 1081, while *Bacteroides fragilis* ATCC 23745 was obtained from Health Laboratory Special Territory of Yogyakarta Province, Indonesia. The bacteria were stored at -20^{o}C in 10% skim milk (w/v) and 10% glycerol (w/v). For prebiotic activity assay, frozen culture sof *Lactobacillus plantarum* and *Bifidobacterium longum* were grown with streaking method in MRS agar Merck), and using similar method that of *E. coli* was grown in Nutrient Broth (Merck) followed by solid incubation for 24-48 hours at 37^{o}C. A single colony from each plate was transferred into 10 mL MRS broth (Merck) or Nutrient Broth and was incubated overnight.

Preparation of prebiotics laminaran

Brown algae *S. crassifolium* was extracted by modifying the previously developed method by Yvin (1999) and Mohsen et al. (2007). Laminaran produced in the first extraction (extraction using sulfuric acid solution) was called LAE, whereas the second extraction (extract from the residue reextraction with aquadest) was referred as LME, as described in Figure 1.

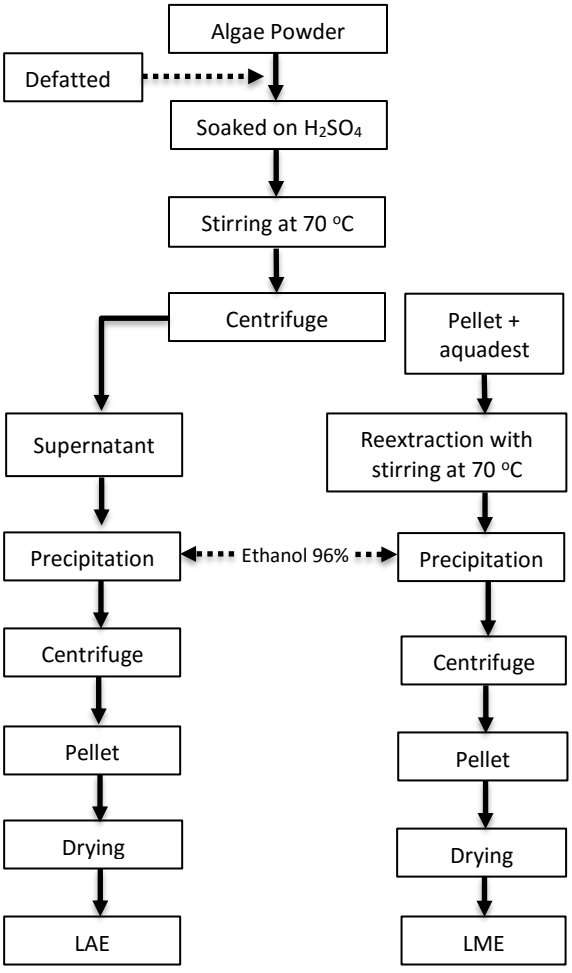

Figure 1. Schematic Process of the Preparation of Prebiotic LAE and LME.

Prebiotic Activity Test

Laminaran prebiotic activity assay was performed using a method developed by Huebner et al. (2007). Test was done by adding 1% (w/v) laminaran (LAE and LME) and inulin [inulin-S Glu-α-(1.2), (β-Fruc-1.2)n] in MRS Broth media (media made without sugar) or 1% (w/v) glucose. Strains of *B. longum* and *L. plantarum* were incubated at 37^{o}C in anaerobic conditions. After incubated for 0 and 24 hours, samples were enumerated on MRS agar media and then their numbers of colonies were counted.

E. coli strain was inoculated in M9 broth media (artificial media), which contained 1% (w/v) glucose or 1% (w/v) LAE and LME and inulin. Cultures were incubated at 37^{o}C in anaerobic conditions for 0 and 24 hours, and then were enumerated on Tryptic Soy Agar (TSA) media. Upon 48 hours, the total number of growing colonies were counted. Carbohydrate will have a positive score if (i) it is well metabolized by probiotics, (2) selectively is metabolized by probiotics but not metabolized by another microbial colon. Prebiotic activity was calculated using the equation below:

$$Prebiotic\ activity = \frac{[(A\ -\ B)\ /\ (C\ -\ D)]}{[(E\ -\ F)\ /\ (G\ -\ H)]}$$

While prebiotic activity score was calculated using the equation below:

$$Prebiotic\ activity\ score\ = \left[\frac{A-B}{C-D}\right] - \left[\frac{E-F}{G-H}\right]$$

Where:

A = Colonies of probiotics with prebiotics carbon source (LAE, LME or inulin), 24 hours incubation.

B = Colonies of probiotics with prebiotics carbon source (LAE, LME or inulin), 0 hour incubation.

C = Colonies of probiotics with carbon source glucose, 24 hours incubation.

D = Colonies of probiotics with carbon source glucose, 0 hour incubation.

E = Total colony of enterobacteria with prebiotic carbon source (LAE, LME or inulin), 24 hours incubation.

F = Total colony of enterobacteria with prebiotic carbon source (LAE, LME or inulin), 0 hour incubation.

G = Total colony of enterobacteria with carbon source glucose, 24 hours incubation.

H = Total colony of enterobacteria with carbon source glucose, 0 hour incubation.

Growth of Lactobacilli, Bifidobacteria and Enteric Bacteria on Prebiotic Laminaran.

Increase of cell density of strain *L. plantarum* and *B. longum* growing (24 hours) in media with carbon sources 1% (w/v) laminaran (LAE and LME) or inulin [inulin-S Glu-α-(1.2), (β-Fruc-1.2)n] was compared with that growing in media with carbon sources 1% (w/v) glucose as seen in Table 1.

RESULTS AND DISCUSSION

Table 1. Increase of Cell Density (Log Cycle) Between 0 and 24 Hours for Strains of Bacteria Growing on Various Substrates.

Bacterial culture	Glucose	LAE	LME	Inulin
B. longum	0.29	0.12	0.35	0.30
L. plantarum	0.26	0.11	0.49	3.35
E. coli	0.28	0.21	0.26	0.10

Based on Table 1, it was shown that cell density of *B. longum* and *L. plantarum* growing on medium LME had a quite high value (0.35 and 0.49 log cycle), which means the used substrate was able to support the growth of these probiotics. However, the ability of LME to support growth was better than that of LAE (0.12 and 0.11 log cycle). In general, *L. plantarum* strains showed higher growth ability compared to *B. longum* did in algae. It was possibly because the polysaccharide laminaran was difficult to directly fermented by Bifidobacteria. Horse et al. (1998) stated that the *Bifidobacterium* is unable to benefit from laminaran, but degraded products from laminaran by *C. ramosum* could be utilized by the Bifidobacteria *in vivo* and *in vitro*.

Number of colonies on LME carbon source was higher when compared to that on glucose media, which means that the LME and glucose could be used by *B. longum* or *L. plantarum* to induce their growth. However, LME was able to support growth better, so that growth *of B. longum* and *L. plantarum*s strains was higher.

Number of colonies on LAE and LME was far less than that on inulin. This is due to inulin is a prebiotic that have been tested. According to O'Sullivan et al. (2010) prebiotics are selectively fermented components, which support growth and activity of digestive macrobiotic that are beneficial. When compared, *B. longum* and *L. plantarum* induced by inulin showed a comparable growth level (3.30 and 3.35 log cycle) showing that inulin is able to support the growth of beneficial probiotic.

E. coli which was grown on media LME and LAE showed quite high growth (0.26 and 0.21 log cycle) showing that the LAE and LME are less selective. This was likely because *E.coli* has the high ability to grow even on a minimal substrate, or with the presence of enzymes capable of degrading hardly degraded components by other bacteria. Hartemink et al. (1997) showed that *E. coli* has the ability to use prebiotics as a carbon source. Some strains of microbes could produce extra cellular enzymes capable of hydrolysis of oligo or polysaccharides, but their effectiveness also depended on the length of the chains.

When *E. coli* was substituted with glucose, the colony increase was higher meaning that the ability of both to support growth of *E. coli* is still lower than glucose. Inulin inoculated with probiotic *B. longum* or *L. plantarum* produced very high growth compared to the LAE and LME, and even glucose. In the beginning, growth of these beneficial microbes was slow enough on 0 hour compared to the media, but after 24 hours, inoculation was able to achieve the highest growth. The low growth in the beginning was probably due to an adaptation phase of microbial towards the carbon source inulin used, because the inulin used was a natural chikori inulin from fresh roots (DP 2-60, the average DP of 12) which might take more time for the microbes to degrade than when using inulin type Fructan or orafti 1 (Roberfroid, 2007).

After 24 hours, the inulin showed its existence as an already tested prebiotic, which could grow very quickly over other substrates. On the other hand, it was able to suppress the growth of enteric microbial such as *E. coli* with growth hour difference from 0 to 24 hours sat the lowest number. This shows that inulin is a selective prebiotic. This is because *E. coli* could not break inulin, which has β-(2.1) bonds and could not produce inulinase (β-fruktosidase) (Damian et al., 1999 in Morrison et al., 2006).

Prebiotic Activity Score

Prebiotic activity score is the difference between number of colonies of probiotics

and prebiotic medium after 24 hours per difference in the number of probiotic colonies with glucose medium after 24 hours minus the difference between the number of enteric colonies in prebiotic medium after 24 hours per difference in the number of enteric colonies with glucose medium after 24 hours. The values of prebiotic activity score could be seen in Figure 2.

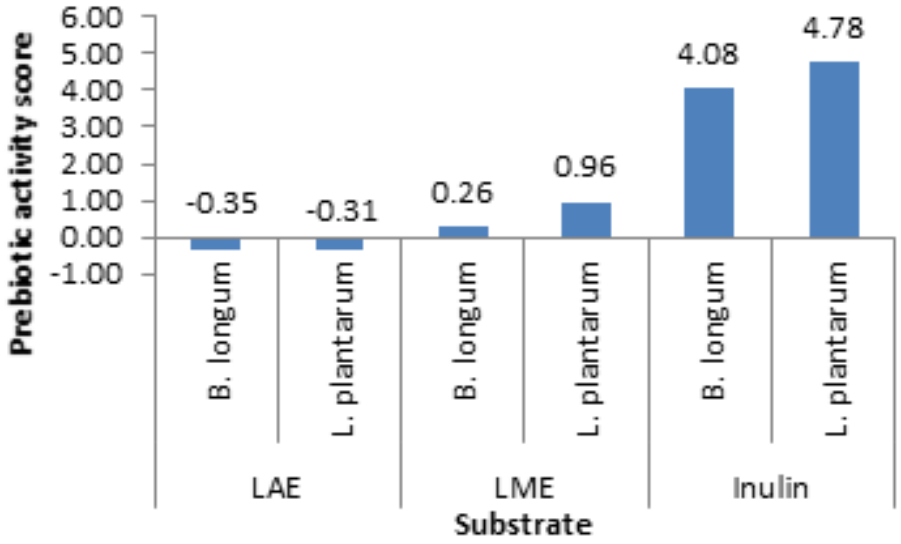

Figure 2. Prebiotic Activity Score of *B. Longum* and *L. Plantarum i*n Various Carbon Sources

As seen in Figure 2 prebiotic activity scores for *B. longum* or *L. plantarum* with carbon source LAE showed negative values (-0.35 and -0.31). This means that the substrate LAE was less able to support the growth of probiotic bacteria. The negative prebiotic activity value was obtained when the bacteria grow more poorly on probiotic than or their growth is lower than that of enteric on prebiotic substrates (Huebner et al., 2007).

Meanwhile, LME carbon source produced positive AP scores (0.26 and 0.96), which means that the LME could support the growth of probiotic bacteria. Compared to results of Moore et al. (2011), which scores for inulin, FOS, GOS and RS substrates were 0.49: 0.37: 0.21and 0.14, respectively, then the prebiotic activity score of LME was higher, particularly for probiotic *L. plantarum*. Such high AP score indicates that the LME fit for use as a candidate prebiotic.

Carbon source inulin has positive probiotic activity score for *B. longum* and *L. plantarum* (10.95 and 12.42), which means inulin very well encouraged the growth of probiotic bacteria. Using inulin, Huebner et al. (2007) reported AP score for B. longum and *L. plantarum* were 0.41 and 0.30, respectively. It means scores obtained in this study are quite high. This is likely because inulin and probiotic strains used are different.

CONCLUSION

Based on the results of this study, it was obvious that both laminaran LAE and LME has the ability to promote the growth of bacteria. Hence, they were able as a prebiotic. Unfortunately, they were less selective because *E. coli* could use laminaran as carbon source.

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