The Potency of Corn (Zea Mays) Cob Waste as a Prebiotic Candidate to Support the Growth of Bifidobacterium longum: a Preliminary Study

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Abstract

Corn cob is one of the most common agricultural wastes in Indonesia with high content of xylan. Xylan can be used to produce xylitol which cannot be digested by human digestive enzymes thus, make it a potential candidate of prebiotic. This preliminary research aimed to study the potency of corn cob in supporting the growth of *Bifidobacterium longum*. Corn cob was produced into powder and used as the carbon source in the growth medium of *B. longum*. Total plate count method was done to count the growth of *B. longum* in various concentrations of bacteria solution. The result showed that crude corn cob powder can support the growth of *B. longum* from bacterial enumeration with the value of 2.01 x 10^6 CFU/mL. The study indicated that corn cob has a potential to be used as prebiotic.

Keywords: Corn cob, prebiotic, *Bifidobacterium longum*, xylan

Introduction

Corn (*Zea mays*) cob is one of the agricultural wastes whose number continues to increase in Indonesia. Currently, corn cob is being used for bio-ethanol, animal feeding, fertilizer, feedstock for cellulosic ethanol, and as base product for various industries (Pennington, 2020). Corn cob is a lignocellulose material with 12.4% of xylan, the highest among agriculture wastes (Richana *et al*., 2007). Xylan can be hydrolysed to produce xylose and further fermentation will produce xylitol (Mardawati *et al*., 2018; Fairus *et al*., 2016). Xylitol can be used to substitute sucrose and one of its characteristics is it cannot be digested by digestive enzyme so, it can be used by *Lactobacillus* and *Bifidobacterium* in human gut to support their growth and act as prebiotic (Lugani & Sooch, 2017).

Prebiotic is an ingredient that resistance to digestive enzymes in the upper part of gastrointestinal track (GIT), can be selectively fermented by intestinal microbiota, and stimulate the growth of health-promoting bacteria (Gibson *et al*., 2004) such as *Bifidobacterium longum, Lactobacillus casei*, and *Bacillus lactis*. *Bifidobacterium longum* is a probiotic and non-pathogenic bacterium that in adequate amount can give health benefits to the host. They can be found naturally in human GIT and vagina. The growth of *B. longum* can be stimulated by sufficient amount of prebiotic intake as their carbon source (Wong *et al*., 2019; Vliagoftis *et al*., 2008; Palframan *et al*., 2003).

Those, corn cob has a potency to act as prebiotic to support the growth of probiotic because it contains xylan which cannot be degraded by human digestive enzymes in upper GIT (Despres *et al*., 2016). The aim of this research was to study the potency of corn cob waste as prebiotic candidate to support the growth of *Bifidobacterium longum*.

Material and methods

Corn cobs were obtained from the waste of street roasted corn seller in Yogyakarta City, Indonesia. The isolate of *Bifidobacterium longum* FNCC 0210 was purchased from Pusat Studi Pangan dan Gizi Universitas Gadjah Mada Yogyakarta. All chemicals and reagents used were analytical grade.

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Corn cob preparation

The sample was prepared according to the method from (Fairus *et al.*, 2016) with modification. Corn cob waste was brushed, cleaned using flowing water, cut using knife (5-10 mm in thickness) and then dried inside the oven (60 °C) to reach its constant weight. Dried corn cob then grind, sieved (40 mesh) and put inside the closed place for further step (Mardawati *et al.*, 2018). Water content of the sample was calculated according to the following equation as described by AOAC (Anonim, 1984) with the following equation.

$$\text{Water content (\%)} = \frac{initial\ weight - final\ weight}{sample\ weight} x100\%$$

Re-culture of Bifidobacterium longum

Stock of *Bifidobacterium longum* FNCC 0210 was inoculated into *de Man Rogossa Sharpe* (MRS) broth aseptically and incubated for 24-48 h at 37 °C until it shows white sediment of the bacteria colony in the bottom of the tube. Then homogenized by vortexing and inoculated using ose loop into MRS agar. The medium was then incubated for 24 h in 37 o^C under anaerobic condition (Amer *et al.*, 2014).

Bacterial isolate confirmation

Confirmation of *Bifidobacterium longum* isolate was done using Gram staining method by (Schlegel & Jannasch, 2006). Cleaned glass object added with sterile aquadest and 1 ose of the isolate that were collected from the MRS agar from the previous step. Fixation process was done using bunsen to evaporate the remaining aquadest and glued the bacteria to the slide. Primary stain (crystal violet) was added using drop pipette and left for 1 min. Washed the slide and let it dry. Iodine was added into the slide and left for 1 min then washed and dried. Combination of acetone and ethanol used for decolorized and removed the primary stain, left for 45 s then washed and dried. A counter stain (safranin) is applied for 1 min, washed and dried. Fixation of the slide was done by passing it through the flame, washed using aquadest and dried. Slide was observed under the microscope to see the cell morphology and uniformity.

Serial dilution of Bifidobacterium longum

Bacterial dilution was done using test tubes containing 9 mL of 0.1% peptone. Serial dilutions were of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}. About 1000 µL of bacteria from re-culture step was added into the first test tube (10^{-1}), homogenized, then taken (1000 µL) and put into the second test tube (10^{-2}). The step was repeated up to the seventh test tube (10^{-7}). The method was performed to know the optimum dilution for the bacterial to grow in the range of 30-300 CFU/mL (Widodo *et al.*, 2015; Sutton, 2012).

Bacterial enumeration by Total Plate Count method

Total plate count (TPC) method was performed by replacing the carbon source of the medium (MRS agar) with the sample. Medium contained per 250 mL were 2.5 g peptone, 2.5 g triptone, 1.25 g yeast extract, 0.5 g ammonium citrate, 1.25 g sodium acetate, 25 mg magnesium sulphate, 12.5 mg manganese sulphate, 0.5 g dipotassium phosphate. All the composition of were put into Erlenmeyer. Tween 80 solution (250 µL), L-cysteine HCl (125 µL), 5 g sample and 3 g agar were added. Aquadest (10 mL) was added, and the solution were mixed using hotplate and magnetic stirrer, then sterilized by autoclave. Total plate count method was done by adding 100 µL of bacterial solution from the serial dilution of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} into empty-sterile petri dish, then poured the MRS agar medium (15 mL), homogenized and incubated for 24 h (37 °C). The number of colonies growing in the medium was counted by observing the colony shape which were seen as clear and round in the media. Bacterial are enumerated by conventional CFU counting in the range of 30 to 300 colonies per Petri dish for its optimal counting (Sieuwerts *et al.*, 2008) because more than 300 colonies would be called as too numerous to count (TNTC) and less than 30 colonies is too small to represent the original sample. The value of colony forming unit per millilitre was calculated according to the following equation (Soesetyaningsih & Azizah, 2020).

$$CFU/mL = \Sigma colony\ per\ petri\ x\ \frac{1}{dilution\ factor}$$

Result

Corn cob preparation

In this study, the powder of corn cob was produced by drying the cleaned corn cob chunks using oven under the temperature of 60 °C for 44 h to reach its constant weight (Figure 1). Corn cob chunks were weighed and calculated for its water content before being processed into powder.

Figure 1. Corn cob chunks during the drying process

Re-culture and confirmation of Bifidobacterium longum isolate

Bifidobacterium longum isolate was re-cultured to obtain pure isolate using MRS broth and incubated for 48 h. The growth of the colony can be seen as the white sediment in the bottom of the tube is shown in Figure 2. There was no contamination during the incubation process. The bacterial culture was inoculated into MRS agar without corn cob as its carbon source using streak plate method and showed the uniform colony with the characteristics of white round colonies without unpleasant odour or mucus surround it (Figure 3).

The single colony formed in MRS agar was taken and confirmed using Gram stain method. The result showed that the colonies observed under microscope were in basil shape and in violet colour and indicated that the colonies of this bacteria

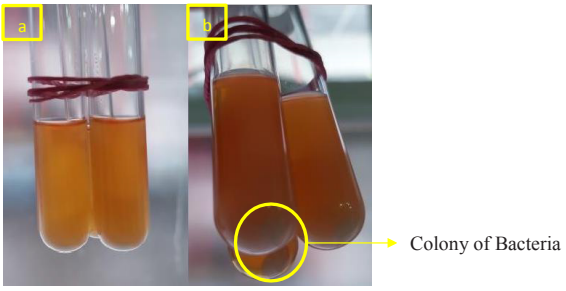

Figure 2. The colony of *Bifidobacterium longum* in MRS broth during 24 h (a) and 48 h (b) incubation

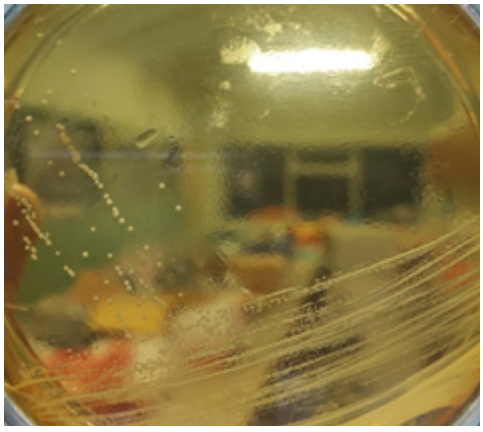

Figure 3. The colony of *Bifidobacterium longum* in MRS agar

are in the group of Gram-positive bacteria (Figure 4).

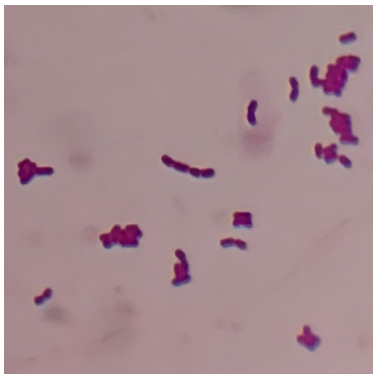

Figure 4. Gram stain of *Bifidobacterium longum* under the microscope (zoomed in at 400x magnification)

The growth of Bifidobacterium longum

Corn cob powder was used to know its potency in supporting the growth of *Bifidobacterium longum* using total plate count method in MRS agar by replacing its carbon source using corn cob powder. Bacterial solution in various concentrations that have

been made from serial dilution (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}) were tested and showed in Figure 5.

The crude powder of the sample was in yellow color, almost similar with MRS agar as the medium and made it cloudy. In this study, this caused a bit problem in the colony counting of the bacteria. The higher dilution level of bacteria solution, the lower number of the bacteria will grow. The number of bacteria counted using TPC method after calculated was 2.01 x 10^6 CFU/mL

Discussion

Corn cob chunks were calculated the water content for each replicates. The average water content value of dried corn cob chunks obtained in this research was 39.3%. This corn cob chunks processed into powder then used as the carbon source in MRS agar to see its potency in supporting the growth of *Bifidobacterium longum*.

The bacteria isolate that will be used in this research need to confirm for its characteristic and morphology. Kongo (2013) stated that *Bifidobacterium longum* were in basil shape and in violet colour. Gram-positive bacteria maintained the crystal violet colour while Gram-negative bacteria coloured in red or pink. Their result is comparable with our result.

The effect of corn cob as carbon source to support the growth of bacteria isolate were calculated using total plate count method. Total bacteria counted using TPC method was calculated using the equation from Soesetyaningsih & Azizah (2020) by only putting the colony in the range of 30 to 300 colonies, and the value is 2.01 x 10^6 CFU/mL. Prebiotics can alter the gut microbiota thus affected the host health (Sarbini & Rastall, 2011) and contribute to promote the growth of Bifidobacteria and Lactobacilli (Connolly *et al*., 2010). Thus, based on our result, corn cob can support the growth of *Bifidobacterium longum* and can be used as an alternative prebiotic. According to (Davani-Davari *et al*., 2019), carbohydrate especially oligosaccharide groups are including in prebiotic which can increase the growth of lactic acid bacteria. As a bio-product of agriculture, corn cob is high in fiber, carbohydrate, and xylan (12.4 – 31.94 %) and thus make it potential as a prebiotic (Eylen *et al.*, 2011). Bifidobacteria is a lactic acid bacteria which can utilize various carbohydrate by heterofermentative metabolism to produce

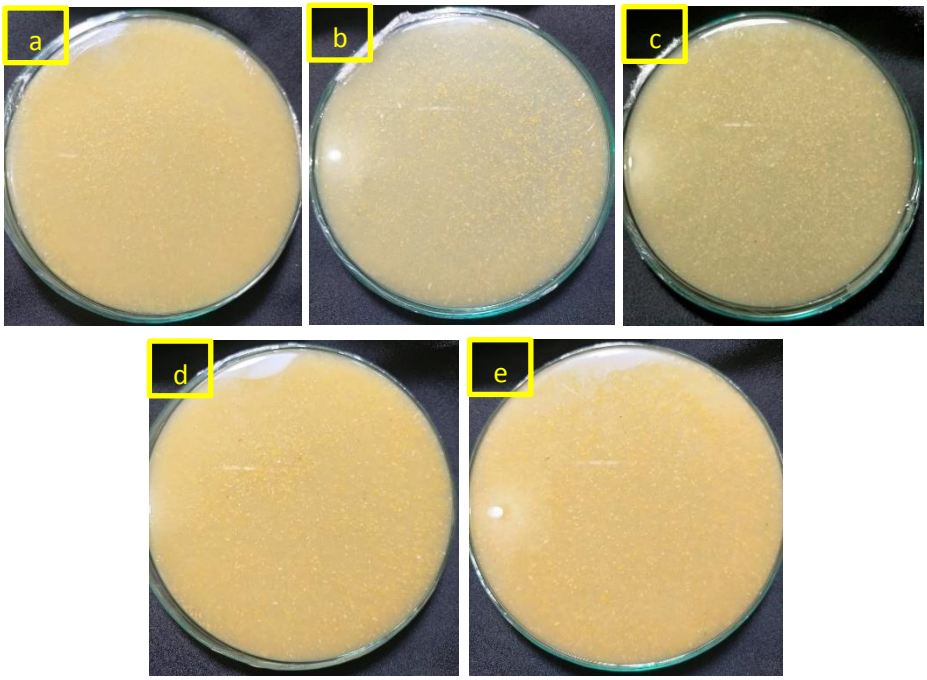

Figure 5. The growth of *Bifidobacterium longum* in various concentrations of 10^{-3} (a), 10^{-4} (b), 10^{-5}, (c) 10^{-6} (d), 10^{-7} (e) in MRS agar using corn cob powder as its carbon source

lactic acid and it can beneficially affected the host health (Pokusaeva *et al.*, 2011).

According to Sutton (2012), the number of colonies counted in one petri dish range from 30-300 CFU/mL. In the present study, our result was comparable with them. The result from total plate count method in our study showed that the crude powder of corn cob can be used by *B. longum* to support their growth. Prebiotic properties from some ingredients can be determined by seeing its characteristic to increase the number of beneficial bacteria (Ariestanti *et al.*, 2019)

Conclusion

Our result from this preliminary study indicated that corn cob has a potential to be used as prebiotic because it can support the growth of *B. longum*. Purification and extraction process to obtain the pure extract and identify the type of carbohydrate in corn cob should be performed.

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