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Viability of Lactic Acid Bacteria Isolated from Kombucha Tea Against Low pH and Bile Salt

Ketahanan Bakteri Asam Laktat yang Diisolasi dari Teh *Kombucha* terhadap pH Rendah dan Garam Empedu

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ABSTRACT

Kombucha tea is a functional drink fermented by various types of microbes. *Kombucha* tea is also a source of lactic acid bacteria that can maintain the balance of the microflora of the digestive tract which can improve the health of the human body. Lactic acid bacteria can act as a probiotic if it is able to survive to the human gastrointestinal tract, where in order to reach the digestive tract, lactic acid bacteria has to be resistant to the low pH in the stomach and bile salts. The purpose of this study was to determine the level of resistance of lactic acid bacteria in *kombucha* tea against low pH and bile salts. This study uses 20 isolates, each of these isolates were tested to the resistance of low pH 2.0 and 0.5 % bile salts with incubation time of 4 hours. The results indicated that from 20 isolates of lactic acid bacteria that were obtained from kombucha tea, 15 isolates were resistant to low pH and 13 isolates were resistant to bile salts. The isolates have a huge potential to be developed as a probiotic candidate that can contribute greatly to the health of the digestive tract.

Keywords: *kombucha tea, lactic acid bacteria, viability, low pH , bile salts*

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INDTRODUCTION

Lactic acid bacteria has been widely used as a starter for the fermentation of meat, vegetables, and beverages. In fermentation products, lactic acid bacteria has a role in the formation of aroma, color, texture, and nutritional quality of fermented products. According to Hayakawa (1992), lactic acid bacteria are a group of beneficial bacteria that can ferment sugar as an energy source to produce lactic acid in large quantities and does not form putrefactive compounds (an odor compound) when separated by protein. In food products, generally lactic acid bacteria are harmless and meet the GRAS status (Generally Recognized As Safe). These bacteria can also provide beneficial effects for humans because of the metabolites components it produces can inhibit bacterial enteric pathogens, improve digestion of lactose, reduce cholesterol, antimutagenic and anticarcinogenic levels and improve the immune system (Surono, 1998).

Since 3000 years ago, *kombucha* tea is well known as a health drink and is thought to have come from China through trading which eventually spread all over the world (Naland, 2004). Basically, *kombucha* tea is a symbiotic culture between bacteria and yeasts grown in tea. The combination between bacteria and yeast is then called SCOBY (*Symbiotic Culture of Bactery and Yeast)* that consists of: *Bacterium xylinum, Bacterium xylinoides, Bacterium gluconicum, Sacharomyces ludwigii*, varieties of *Sacharomyces apiculatus, Schizosaccharomyces pombe, Acetobacter ketogenum,* varieties of *Torula,* and *Pichia fermantans.*

Kombucha tea has a wide opportunity as a natural resource for the growth of beneficial bacteria and opens great opportunities to explore the microbes contained in the tea namely lactic acid bacteria. So far there has been no research that indicates the presence of lactic acid bacteria in *kombucha* tea, but from the results of previous studies 84 isolates of lactic acid bacteria were obtained (Arihantana dan Puspawati, 2015 *unpublish*). Health benefit/clains of lactic acid bacteria as probiotics causes the quest in seeking LAB strains from various natural sources such as the gastrointestinal tract of humans and animals as well as traditional fermented foods. According to Shortt (1999), the conditions required for lactic acid bacteria as a probiotic isolate is survival to the stressful environment of the stomach, acid and bile resistance and survival during gastrointestinal transit. Therefore, it is necessary to conduct a study of the viability of lactic acid bacteria isolated from *kombucha* tea against low pH and bile salts that could be developed as a probiotic candidate.

MATERIALS AND METHODS

The materials used in this study are: 19 isolates of LAB isolated from *kombucha* tea, MRS Broth (Oxoid), MRS Agar (Oxoid), alcohol 96%, NaCl, oxgall, HCl (Merck), cotton, aluminum foil, resistant plastic heat, lugol, safranin, crystal violet, oil immersion.

The equipments used are: autoclave, incubator, vortex, analytical balance, test

tubes, petri dishes, measuring pipettes, test tube racks, measuring cup, bunsen, ose needles, glass objects, cover glass, microscope, rod bent, gloves, water laminar flow, hot plate, magnetic stirrer, colony counter, eppendorf, and hoky stick.

Experiment Procedure

Twenty LAB isolates obtained from *kombucha* tea were tested for resistance to low pH and bile salts. Tests were conducted by growing the LAB isolates on MRSB media with the pH set to 2 and bile salts with the concentration of 0.5%, then incubated for 0 and 4 hours. Resistance to low pH and bile salts was conducted by calculating the difference between the number of colonies of log units that grow on the incubation time of 0 hours to 4 hours.

Analysis Method

1. Resistance to Low pH (Modified by Chou dan Weimer, 1999; Zavaglia *et al*., 1998)

Resistance to low pH was conducted with plate count method with media modifications. The media used was MRS broth which is set at pH 2,0 using HCl 37%. The refreshed culture in MRS broth that was incubated for 24 hours, was then inoculated in MRS broth as a control which is set at pH 2,0, then inoculated in MRS broth with pH of 2,0 and incubated in 37ºC for 0 and 4 hours. This corresponds to the length of the food in the stomach for 2-6 hours (Gropper and Groff, 2001). After incubation, total plate count was conducted with spread method and incubated at 37 °C for 48 hours. The cells that are resistant to low pH is the percentage of living cells after incubation at pH 2.0 for 0 and 4 hours. Resistance to low pH is calculated based on the difference of the number of colonies of log units that grow on the incubation time of 0 to 4 hours.

2. Resistance to Bile Salt (Modified by Moser dan Savage, 2001; Ngatirah *et al*., 2000)

The resistance to bile salt was conducted according to Ngatirah *et al*. (2000) but the concentration of bile salt used was 0.5% (Moser dan Savage, 2001). This concentration was chosen because it was equivalent with the physiological concentrations of bile salts in the duodenum. 1 ml culture of lactic acid bacteria incubated for 24 hours in MRS broth was 24 hours was included into 9 ml MRS broth containing 0.5% bile salts then incubated at 37°C for 0 and 4 hours. Total plate count was conducted with spread method and incubated at 37°C for 48 hours.

Resistance to bile salts is calculated based on the difference between the number of colonies of log units that grow on the incubation time of 0 to the culture that grew after incubated for 4 hours. The smaller the difference, the higher the resistance of lactic acid bacteria culture were tested against bile salts.

Analysis of Data

The data obtained from a series of tests are analyzed and presented descriptively and presented in tabular form, image or photo.

RESULTS AND DISCUSSION

Growth Capability of LAB At Low pH

From 20 isolates of LAB obtained from *kombucha* tea, 15 isolates has the ability to tolerate the acidity of the stomach which is characterized by the ability to survive for 4 hours at pH 2. Resistance to low pH is calculated based on the difference of the number of LAB grown in. Decrease in the number of lactic acid bacteria after incubated in an acid media for 4 hours can be seen in Figure 1.

In Figure 1 it can be seen that all of the LAB isolates grown in acid media decreased. A decrease in the total population of lactic acid bacteria ranges from 0.73 to 6.45 log cycles or 0.73 to 100%. The highest decrease was 100% in KMB 41, KMB 44, KRB 214 and KRB 215 while the other 14 isolates have a high resistance although decrease of population still occured. The highest resistance of LAB was by KMK31 in the amount of 88.65% with a decrease population of 0.77 log cfu/ml, followed by KRB 210 in the amount of 88.44% with a decrease population of 0.73 log cfu/ml, KMK 42 in the amount of 74.81% with a decrease population of 2.11 log cfu/ml, and KRS 22 in the amount of 65.48% with a decrease population of 2.88 log cfu/ml.

Growth media with high acidity (pH 2.0) has a very strong destructive nature against all isolates tested. It is shown from a decrease in the number of cells after incubation for 4 hours. Inhibition of acid on the growth of bacterial cells occurs through denaturation effect of enzymes that on the surface of cells, the damage of lipopolysaccharide and outer membrane and cytoplasmic pH decreases through the increase of membrane permeability to protons at a very large pH gradient (Puspawati, 2008).

Isolates that were slightly decreased under acidic conditions, is thought to have a mechanism of resistance to acids. Stomach acid (HCl), is a strong acid dissociated in medium and is capable of lowering the external pH but can not penetrate the cell membrane. Adaptation structure of the outer membrane is the mechanism of bacterial resistance to acids and are classified as strong acids. Adaptation can be in changes in the composition of fatty acids and membrane phospholipids. A similar trend is also shown from the research conducted by Jacobsen (1999), with acid resistance testing against 47 strains of lactic acid bacteria from various sources at pH 2.5. From these test results, only 29 strains of bacteria are able to survive at pH 2.5 and none of them was able to grow after incubation for 4 hours.

Lactic acid bacteria potential as probiotics should be resistant to stomach acid. According to (Chou dan Weimer, 1999), the time required for bacteria to survive in the stomach is about 90 minutes. Lactic acid bacteria isolate used as probiotics must be able to survive in the acidic conditions in the stomach for at least 90 minutes. The lower in the decrease of lactic acid bacteria population, the higher it becomes resistant to low pH.

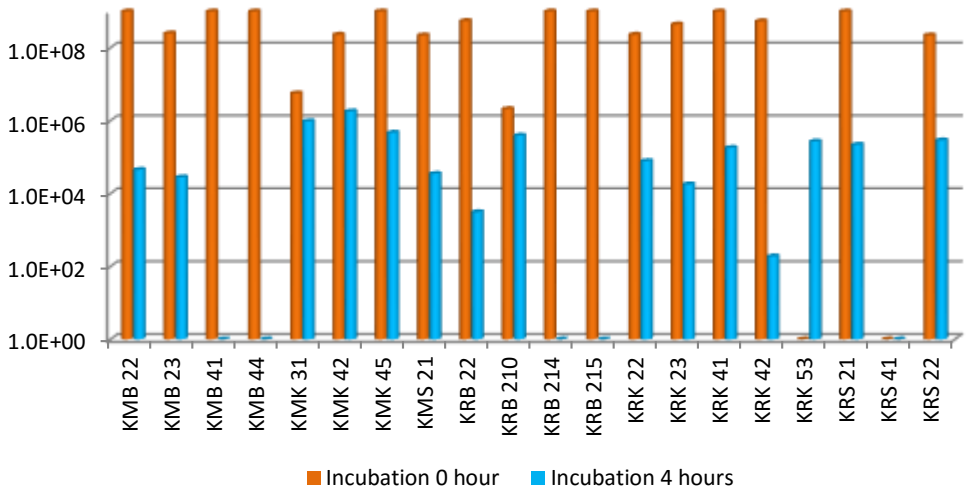


Figure 1. Decrease in number of lactic acid bacteria after incubated in MRSB at pH 2.0 for 4 hours

Resistance of LAB In Bile Salts

The ability to tolerate bile 0.5% is necessary for probiotic bacteria. The bacteria that managed to live after grown in MRSA media containing 0.5% bile salts otherwise is resistant to bile salts (Bridson, 1998). Lactic acid bacterial resistance to bile salts was conducted by calculating the difference between the number of colonies of log units that grow on the incubation time of 0 to 4 hours that can be seen in Figure 2.

Lactic acid bacterial resistance to bile salts is indicated by a decrease in total lactic acid bacteria after incubation with media containing 0.5% bile salts for 4 hours. Bacterial culture medium with bile salt concentration of 0.25%-0.5% is the effective minimal concentration for selecting strains resistant to bile salts. The results showed the level of resistance of lactic acid bacteria isolated from *kombucha* tea to bile salts is relatively high. From 20 isolates tested, 7 isolates shows a decrease of population after incubated in oxgall 0.5% for 4 hours, among others KMB 22, KMB 41, KMB 44, KMK 45, KRB 22, KRB 214 and KRB 215 with a decrease of population ranging from 0.07 - 6.0 log cycles. 12 isolates showed that there was an increase of population after being incubated for 4 hours in oxgall 0.5%.

Decrease in the population of lactic acid bacteria was caused by leakage of cells induced by bile salts but does not cause the cells to undergo lysis. In high concentrations, bile salts can cause leakage of intracellular material. Bile acid is toxic to living cells, therefore microbes in the digestive tract must have a defense mechanism to protect themselves from the toxic activity.

The fatty acids can reduce cell leakage caused by bile salts. Fatty acids contribute to improve the stability of the lipid membrane. Lipid is a compound that is predominantly found in the cell membrane

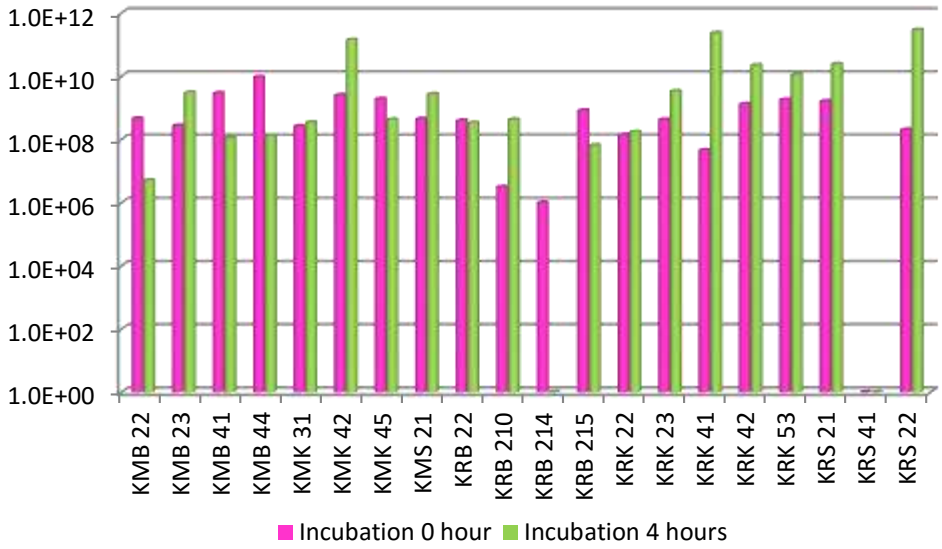


Figure 2. Decrease amount of lactic acid bacteria after incubated in MRSB in oxgall 0.5% for 4 hours

of gram-positive bacteria and are an essential part to keep the membrane structure **(**Kimoto *et al.* 2002). Bile salt tolerance is also thought to be caused by the role of polysaccharides as one of the components of the cell wall of gram-positive bacteria, but the mechanisms involved are unclear (Surono *et al.* 2000).

The morphology of the lactic acid bacteria obtained are coccus and basil, with gram stain positive. Generally, the morphology of lactic acid bacteria are coccus and basil. LAB colony can be seen in Figure 3.

CONCLUSIONS

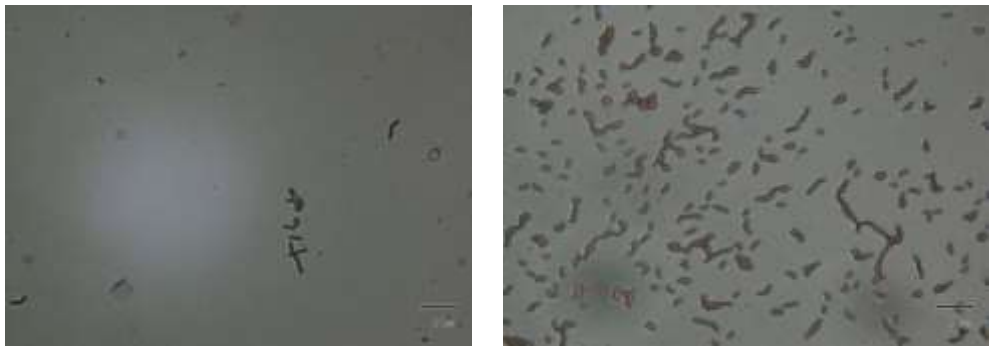
From the results it can be concluded that:

Lactic acid bacteria isolated from kombucha tea has a relatively high resistance to low pH (pH 2.0) except for KMB 41, KMB 44, KRB 214 and KRB 215.

Four isolates of LAB has a relatively high resistance to low pH (pH 2.0) among them are KMK 31, KMK 42, KRB 210, KRB 210 with a resistance of 88.65%, 74.81%, 88.44%, 5.48%.

Lactic acid bacteria isolated from kombucha tea has a relatively high resistance even experienced an increase of population after grown in media containing bile salt (oxgaleml 0.5%) isolates except for KMB 41, KMB 44, KRB 214 and KRB 215.

The lactic acid bacteria isolate which has the highest resistance to bile salt (oxgall 0.5%) are KMK 42 with an increase population of 1.75 log cycle, KRB 210 with an increase population of 2.13 log cycle, KRK 41 with an increase population of 3.81 log cycle and KRS 22 with an increase population of 3.16 log cycle.



a. KRB 210 isolate b. KMK 45 isolate

Figure 3. Gram staining photos of isolate KRB 210 and KMK 45 isolated from *kombucha* tea

Suggestion

Further testing needs to be conducted to determine the ability of other probiotic characteristics so that the characteristics of *kombucha* tea isolates can be completely known and could be developed as prime probiotic isolates.

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