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SHORT COMMUNICATION

Probiotic Potential of Lactic Acid Bacteria from Yellow Bamboo Shoot Fermentation using 2.5% and 5% Brine at Room Temperature

LINDAYANI*, LAKSMI HARTAJANIE, AND MONIKA PALUPI MURNIATI

UNIKA Soegijapranata, Jalan Pawiyatan Luhur IV/1, Semarang 50234, Indonesia

¹ Yellow bamboo shoot is a popular food material especially in Semarang because it is used as filling for *lumpia* (authentic food of Semarang). Beside used as filling, yellow bamboo shoot commonly known by Indonesian people. Considering bamboo shoot often processed into fermented traditional food, yellow bamboo shoot also potential to be examined as source of fermented food producing lactic acid bacteria. Lactic acid bacteria still become the most beneficial microorganisms associated with gastrointestinal system and moreover for obesity diet. The aim of this research is to study the probiotic potential of lactic acid bacteria produced from yellow bamboo shoot fermentation in 2.5% and 5% brine under room temperature (30 °C). From isolation, 22 single colonies obtained from 2.5% brine and 27 isolates obtained from 5% brine. The morphology and physiology analysis resulted in *Lactobacillus* and *Streptococcus* genus. All isolates were tested subsequently for probiotic potential. Based on the result, more than 50% identified isolates have probiotic potential.

Key words: lactic acid bacteria, probiotic, yellow bamboo shoot

² Rebung bambu kuning merupakan bahan makanan yang populer khususnya di Semarang karena digunakan sebagai pengisi untuk *lumpia* (makanan asli Semarang). Selain digunakan sebagai pengisi, rebung bambu kuning umum dikenal masyarakat Indonesia. Diketahui bahwa rebung umumnya diproses sebagai makanan fermentasi secara tradisional, rebung bambu kuning berpotensi diteliti sebagai sumber makanan fermentasi yang menghasilkan bakteri asam laktat. Bakteri asam laktat menjadi mikroorganisme yang menguntungkan berasosiasi dengan sistem pencernaan dan juga berguna untuk diet bagi orang yang kegemukan. Tujuan dari penelitian adalah untuk mempelajari potensi probiotik bakteri asam laktat yang dihasilkan dari fermentasi rebung bambu kuning dalam 2,5% dan 5% larutan garam pada 30 °C. Berdasarkan hasil isolasi, diperoleh 22 koloni tunggal dari fermentasi 2,5% larutan garam dan 27 koloni tunggal dari 5% larutan garam. Hasil analisa morfologi dan fisiologi diperoleh genus *Lactobacillus* dan *Streptococcus*. Semua isolasi dilakukan uji untuk mengetahui potensi probiotik. Berdasarkan hasil ujinya diketahui 50% isolasi mempunyai potensi probiotik.

Kata kunci: bakteri asam laktat, probiotik, rebung bambu kuning

Lactic acid bacteria (LAB) are group of beneficial microorganisms which important in food industry *i.e.* food fermentation, thus can extend the shelf life of food product (Othman *et al.* 2017; Lindayani and Hartayanie 2013). LAB fermentation produces lactic acid and smaller amount of acid *i.e.* acetic acid, ethanol, and CO₂ which inhibit spoilage and pathogenic bacteria. The example is antimicrobial activity of LAB isolated from beef (Al-Allaf *et al.* 2009), fermented milk (Savadogo *et al.* 2004), and traditional fermented beverage from Ethiopia (Tadasse *et al.* 2005).

The benefit of LAB in food industry has promoted further exploration into bacteria strain from various sources like fruit and vegetable fermentation (Nuraida

2015; Rahayu and Margino 1997). LAB has contributed in fermented food development especially fermented food with probiotics (Lindayani and Hartayanie 2013). The following genera considered the principal LAB *i.e.* *Lactobacillus*, *Leuconostoc*, *Streptococcus Pediococcus*.

The growth of LAB is greatly influenced by intrinsic and extrinsic factors. Different fermentation temperature, salt concentration, and area condition may allow for differences of LAB despite the same source or material used (Usmiyati and Marwati 2007).

³ Vegetable and fruit fermentation usually use salting method without addition of starter culture. Beside to extend shelf life, salting also affect the flavor formed by microorganism which occurs during fermentation, especially LAB (Rahayu 2003). LAB produced lactic acid as metabolite causing acidity to raw material (food). This condition gives advantage as low pH

*Corresponding author: Phone: +62-24-8441555, Email: lindayani@unika.ac.id

inhibits nearly all food spoilage microorganisms. According to Magala *et al.* 2015; Josephsen and Jespersen *cit.* Hui *et al.* (2004), LAB produces lactic acid and other antimicrobial substance which can inhibit the growth of spoilage bacteria.

Probiotic is living microorganisms that, when administered in adequate amounts confer health benefit on the host such as maintaining the balance of gut microbiota *i.e.* *Lactobacillus acidophilus* and protein. Probiotics produce lactic acid, acetic acid, hydrogen peroxide, lactoperoxidase, lipopolysaccharides, and other antimicrobial such as bacteriocins. Bacteriocin has ability to inhibit pathogenic bacteria with close relation to the producing strain.

Bamboo shoot is young bamboo bud that comes out of the ground. This young bud generally edible, thus included in group of vegetable, *i.e.* betung bamboo shoot (*Dendrocalamus asper*), temen bamboo shoot (*Gigantochloa verticillata*), yellow bamboo shoot (*Dendrocalamus litiflorus*), dan green bamboo shoot (*Bambusa aldhami*) (Andoko 2003). Raw bamboo shoot has 90% water content per gram material (Rai 2007).

Fermentation of bamboo shoot occurs spontaneously. The process carried on by adding salt solution (brine). Salt solution has functions to draw the liquid inside the bamboo shoot out through osmosis and to inhibit pathogenic bacteria, allowing only LAB to grow. The final product of fermentation often called yellow bamboo shoot pickle.

Salt effects the growth of LAB. In addition, salt has important role in fermentation process, where during fermentation, only LAB that grow. In a short fermentation, the salt addition is preferably 2.5-10% to allow intermediate to fast fermentation rate. Low salt concentration (<2.5%) may cause proteolytic bacteria to grow. Too high salt concentration (>10%) may result on wrinkled end fermentation product and may allow halophilic bacteria to grow or even no fermentation occurs.

In Indonesia, research about LAB is required scientifically because there is still lack of data's about LAB which isolated from crop fermentation, whereas it is known that the source of crop is abundant. In this research, selection of bamboo shoot has come from consideration that bamboo shoot is one of food material which get less attention and mostly used only for filling traditional food like *lumpia* in Semarang. Either edible or non-edible bamboo shoot are available in great amount and LAB which obtained from bamboo

shoot fermentation has a bigger opportunity to be developed to industrial scale to support fermentation product which can contributes better quality of human living.

This research was done at Microbiology Laboratory, Faculty of Agricultural Technology, Soegijapranata Catholic University, Semarang. Materials and chemicals used *i.e.* yellow bamboo shoot which obtained from Semarang, MRS broth and agar (Merck), alcohol 95%, NaOH solution, HCl solution, Gram staining solution (violet crystal, Lugol's iodine solution, alcohol / ethanol 95%, safranin), hydrogen peroxide (H₂O₂ solution) 3%, sterile aquadest, bile salt (Zigma-Aldrich) and bacteria culture (*Staphylococcus aureus*, *Eschericia coli*).

Equipment used *i.e.* petridish, reaction tubes, microscope, object glass, ose, hockey stick glass, Gram staining tools, spectrophotometer (Shimadzu), incubator, pipette, micropipette, autoclave, sterile filter discs, and Durham tubes.

Bamboo shoot fermentation was done for 7 d. Based on isolation and morphological test, 22 isolates were obtained from 2.5% fermentation under room temperature (code C) and 27 isolates from 5% fermentation under room temperature (code D). All bacteria sample were purified subsequently.

Each colony which has clear zone was tested subsequently to morphological test. Based on morphological test, all identified isolates give negative result on catalase test and non-motile (Fig 1 and 2).

Based on gas formation test, some isolates produced gas; some isolates produced no gas (no growth) (Fig 3).

According to Tadesse *et al.* (2005), lactic acid bacteria have characteristic *i.e.* rod or cocci, Gram positive, some producing CO₂ (heterofermentative), some not producing CO₂ (homofermentative).

Physiological analyses were tested including growth at different NaCl concentration (6.5% and 18% of NaCl), growth at different pH (4.4 and 9.6), and growth at different temperature (10 °C, 45 °C, and 50 °C). The result shown that all isolates code C were not growth at different NaCl concentration, whereas for isolates code D, only one isolate able to grow. At different pH, in pH 4.4 medium found ten isolates from code C were grown, whereas only six isolates were grown from isolate code D. In pH 9.6 medium, two isolates were grown for code C whereas for D code only one isolate was grown. Based on growth at 10 °C, 45 °C, and 50 °C were found that all isolates were able to grow at 10 °C incubation temperature, except one

isolate from code C which not grow. Similarly at 45 °C, all isolates were able to grow. But, at 50 °C, some isolates were not growing. Based on morphological and physiological analyses, lactic acid bacteria were categorized as *Lactobacillus* and *Streptococcus*. *Lactobacillus* bacteria generally found in plants, vegetables, dairy-based product, meat product, inside gastrointestinal tract of human and animal (Ray and Bhunia 2008; Karovičová and Kohajdová 2005).

All isolates (22 code C and 27 code D) were tested subsequently to probiotic potential *i.e.* tolerance to acid (pH 3 and 7 at 0, 1.5 and 3 h incubation) and bile salt

(0.3% and 0.5% concentrate at 0, 2, and 4 h incubation), and antimicrobial test against *Staphylococcus aureus* and *Eschericia coli*.

From result of bile salt tolerance test, it is found that for code C, 12 isolates were not able to grow in 0.3% bile salt medium at 0, 2, 4 h incubation, while in 0.5% bile salt medium at 0, 2, 4 h incubation 13 isolates were not able to grow. For code D, 5 isolates were not able to grow in 0.3% bile salt medium at 0, 2, 4 h incubation, while in 0.5% bile salt medium at 0, 2, 4 h incubation 11 isolates were not able to grow. From result of acid tolerance test, isolates code C and D shown different

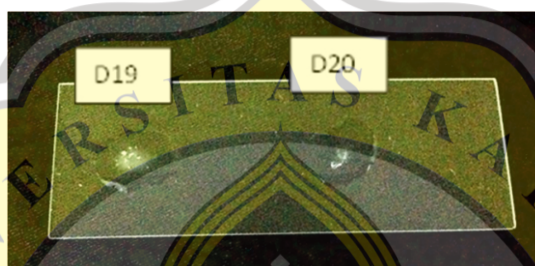


Fig 1 Catalase test of lactic acid bacteria fermented in 5% brine under room temperature.

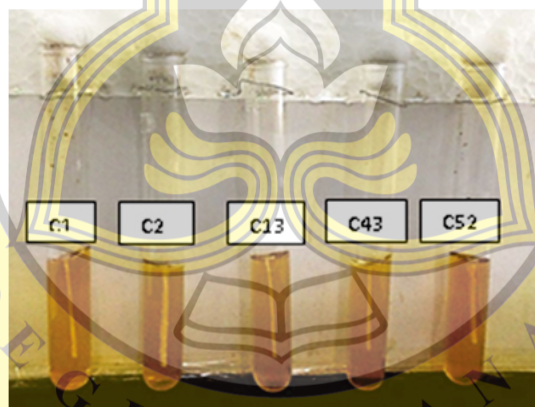


Fig 2 Motility test of lactic acid bacteria. The growth in stabbing area shows that the isolates are non-motile.

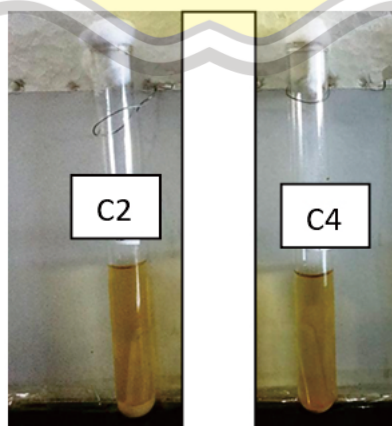


Fig 3 Isolate shows no gas production (homofermentative).

response. Isolates code C in pH 3 medium at 0, 1.5, 3 h incubation shown four isolates were not able to grow, while in pH 7 medium at 0, 1.5, 3 h incubation found 3 isolates were not able to grow. Isolates code D in pH 7 medium at all incubation times were able to grow and in pH 3 medium at all incubation times shown 2 isolates were not able to grow.

Based on antimicrobial test against *Staphylococcus aureus* and *Escherichia coli*, the result shown four isolates code C able to inhibit growth of *Staphylococcus aureus* and *Escherichia coli* while 21 isolates from code D were able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*.

According to Saarela *et al.* (2000), probiotics should have characteristics *i.e.* tolerance to bile salt, able to survive in human gastrointestinal system, has antagonistic properties against pathogenic bacteria, have antimutagenic and anticarcinogenic properties. Screening bacteria with probiotic characteristic has been performed by ability to survive at low pH, tolerance to bile salt and antibacterial activity against pathogen (*E. coli* and *S. aureus*).

Lactic acid bacteria should have ability to survive at low pH because lactic acid bacteria will enter human gastrointestinal tract which has low pH. Almost all isolates were able to survive at pH 3 and 7. According to research reported by Jay (2000) *cit.* Mavhungu (2005); Battcock and Azam-Ali (1998) which stated that almost all bacteria include *Lactobacillus* genus, able to grow at neutral pH.

Ability to survive of lactic acid bacteria is effected by bile salt concentration. Higher the concentrate of bile salt, the ability to survive of lactic acid bacteria is lower. Bile salt causes destruction to bacterial cell membrane, where the main components of cell membrane are lipid and fatty acid. Based on research done by Klayraung *et al.* (2008), *Lactobacillus* genus which isolated from fermented pork, fermented fish, fermented tea, and garlic pickle are able to survive at 0.3%-1% bile salt environment.

In overall, isolates code D have more inhibition ability against two indicator pathogens (*E. coli* and *S. aureus*). Antimicrobial activities by lactic acid bacteria is caused by the ability of lactic acid bacteria producing acid which inhibit pathogenic bacteria, or because another substance which called bacteriocins which produced by that bacteria (Josephsen and Jespersen *cit.* Hui *et al.* 2004).

To conclude, pure isolates from yellow bamboo shoot pickle in brine 2.5% and 5% in room temperature are 22 and 27 isolates, respectively. According to

morphological and physiological, all isolates have been identified as *Lactobacillus* and *Streptococcus*. Lactic acid bacteria which obtained from yellow bamboo shoot pickle have probiotic potential. Therefore, yellow bamboo shoot, has an opportunity to be probiotic bacteria source which can contributes to better quality of human living.

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