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Probiotic Activity Of Lactic Acid Bacteria Isolated From Fermented Palm Oil Sap.

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ABSTRACT

The aim of the study was to investigate the acid tolerance, bile salt tolerance and antibiotic resistance of lactic acid bacteria (LAB) isolated from fermented palm oil sap. The result showed that only one of six isolates had microbial counts remain greater than 10^6 at pH 2 for the 4th hour period with viability approximately 72%, and also there is only one strain showed a viability rate with the percentage at the 4th hour is 60% on bile salt tolerance. Based on the antibiotic susceptibility test, all the isolate are resistant against vancomycin and chloramphenicol. This indicates the uncontrollably high gene transfer frequency of the microbes in the fermented product in Indonesia

Keywords: lactic acid bacteria, palm oil sap, antibiotic resistance, bile salt, acid

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INTRODUCTION

Lactic Acid Bacteria (LAB) are a group of Gram-positive bacteria, non-spore forming, coccus or rods in morphology and produce lactic acid as the main end product derived from carbohydrate fermentation. In this group, Lactobacillus species are the most utilized microorganism due to probiotic properties [1]. Probiotics are non-pathogenic microorganisms which, if consumed in adequate quantities, will have the beneficial effect on the health of the host particularly the intestine. The mammalian intestine including human, is colonized by more than 100 trillions of the microorganism called microbiota which has an important role in health [2]. Recently, many probiotic products have been developed in order to improve the organism's health because of their ability to adaptation in two different environments, in an extra environment and the human intestine [3]. The selection of probiotic bacteria include several criteria, including safety, viability, resistance to acid and bile salts, adherence to gut epithelial tissue, ability to colonize the gastrointestinal tract, production of antimicrobial substances, ability to stimulate a host immune response and the ability to influence metabolic activities such as vitamin production and lactose reduction [4]. The opposite of probiotic is antibiotic. In many countries, antibiotics are available without prescription also and are mostly used inadequately in sub-therapeutic doses[5]. The probiotics normally do not carry transferable antibiotic resistance genes, since they are chromosomally encoded. Some probiotic strains with intrinsic antibiotic resistance could be useful for restoring the gut microbiota after antibiotic treatment. The aim of the present study was to determine the antimicrobial activity, the bile salt tolerance, acid resistance and antibiotic sensitivity of lactic acid bacteria isolated from fermented palm sap.

MATERIAL AND METHODS

Isolation of Lactic Acid Bacteria from Fermented Palm Oil Sap

1 mL of fermented palm sap was mixed to 9 ml sterile MRS broth in a test tube and incubated at 37°C for 18-24 h under anaerobic conditions. This was followed by serial dilution process. 0,1 mL of 10^{-7} dilution was spread to the MRS agar surface in the petri dish and incubated for 48 hours at 37°C under anaerobic condition. A total of 6 single colonies of LAB were randomly selected and subcultured using MRS agar media

Acid Tolerance

Acid tolerance test was investigated according to Kabore *et al* (2012) [6]. LAB was grown into MRS broth at 37°C for 18-24 hours, 1 ml aliquot of LAB was cultured into MRS broth which has been adjusted to pH 3 . the mixture was incubated at 37°C for 1, 2 and 3 hours. At every hour, the sample was diluted and spread onto MRS agar media and incubated for 37°C for 48 hours. The survival rate of LAB was calculated by percentage.

Bile Salt Tolerance.

Bile salt tolerance of LAB was investigated using MRS broth containing 0,3% bile oxgall. A total of 0,1 ml LAB cell suspension were cultured for 18 hours. The mixture was incubated at 37°C for 1,2, 3 and 4 hours. At every hour, 0,1 ml aliquots were diluted and spread onto the surface of MRS agar and incubated. The viability of bacterial colonies was calculated and the survival percentage of LAB was calculated by percentage.

Antibiotic sensitivity

Antibiotic sensitivity of LAB was investigated according to the method of Han *et al* (2011) [7] with slight modification. LAB was cultured into MRS broth for 24 hours at 37°C. After 24 hours, using sterile swab cotton, LAB suspension was spread to the surface of MRS agar. A total of 5 antibiotics were used in this test including vancomycin (30 µg), penicillin (10 U), tetracycline (30 µg), chloramphenicol (30 µg), gentamicin (10µg). The petri dish was incubated at 37°C for 24 hours, then the inhibition zone in measured including the diameter of the disc. The result is sensitive if the inhibitory diameter is ≥ 21 mm, intermediates are 16-20 mm and resistance is ≤ 15 mm

RESULT AND DISCUSSION

Lactic Acid Bacteria from Palm Oil Sap

After 48 hours incubation at 37°C, the total colony of LAB from fermented palm oil sap were $1,4 \times 10^7$ CFU/ml. the colonies were large (>1,5mm) and, bright creamy opaque. The similar result was reported by Shetty *et al* (2017), which reported Lab were the predominant species until 7th hour of incubation of palm sap from *Cocos nucifera*. The total colony of LAB was $8-12 \times 10^7$ CFU/ml). by the end of the 13th hour of fermentation of the LAB count decreased to $2,5 \times 10^7$ CFU/ml [8]. Syukur *et al* (2017) also reported that the total colony of LAB isolated from fermentation buffalo milk were $8,4 \times 10^9$ CFU/ml [9]. One property that expected to exhibit by LAB is the minimum number of colony forming unit. Kechagia *et al* (2012) reported that information about the minimum effective concentration is still insufficient, it is generally accepted that probiotics products should have minimum concentration of 10^6 CFU/ml or gram and that a total of some 10^8 to 10^9 probiotic microorganisms should be consumed daily for the probiotic effect to be transferred to the consumer [10].

Acid Tolerance of LAB isolated from fermented palm oil sap

A total of 6 LAB isolate from fermented palm oil sap were inoculated into sterile MRS broth at pH 2,5 incubated and the viable counts checked every hour for 4 hours. Only one of six isolates had microbial counts remain greater than 10^6 at pH 2 for the 4th hour period with the viability of approximately 72%.

Damayanti *et al* (2014) reported that LAB grouped in Lactobacillaceae had high viability percentages (79,07%-121,17%). The viability of LAB in low pH condition is affected by pH and strain variations [11]. Njoki *et al* (2015) also reported that all the tested isolates of LAB (15 isolates) strain from Mnazi had residual microbial count greater than 10^7 CFU/ml after 3 h of incubation, with majority of them had a survival rate of 77-90% suggesting they are able to tolerate well stomach condition [12]. The first host factors that may affect commercial probiotics are the high acidity in the proventriculus and ventriculus and the high concentration of bile components in the proximal intestine. Being tolerant to the acidic condition is an important criterion to be considered during the selection of potential probiotic isolates to assure their viability. Probiotic bacteria need to survive passage through the stomach where the pH can be as low as 1,5 to 2,0, and stay alive for 4 hours or more [13].

Bile Salt Tolerance

Tolerance to bile salts is a prerequisite for colonization and the metabolic activity of bacteria in the small intestine of the host [14]. after 4 hour of incubation in MRS medium supplemented with 0,3% (w/v) bile salts, only one strain showed a viability rate with percentage at the 4th hour is 60%.

Tudor and Zamfir (2012) reported LAB isolated from fermented vegetables showed a viability rate of at least 10^6 CFU/ml after 2 h of incubation in MRS medium supplemented with 0,3% bile salt [14]. Shehata *et al* (2016) reported 9 of 142 isolates of LAB isolated from dairy and non-dairy sources showed a wide spectrum of antimicrobial activity as well as good bile salt with the surviving percentage were 71,3% until 85%. The ability of probiotic strains to detoxify bile salt by producing bile salt hydrolase (BSH) enzyme activity has often been included among the criteria for probiotic strain selection [15]. Based on these result it is assumed that isolate EHS1 was a BSH producing lactic acid bacteria.

Antibiotic Susceptibility of LAB

Antibiotic susceptibility pattern of selected LAB isolates was observed by using the Kirby-Bauer disc diffusion method. the diameter of the disc used was 7,5mm. The result was shown in table.1

Based on the result, all of the isolates is resistance with vancomycin and chloramphenicol. The similar result was reported by Gad *et al* (2014) who investigated antibiotic resistance in lactic acid bacteria isolated from some pharmaceutical and dairy products. Lactobacillus strains were highly resistant to vancomycin (40,6%) [16]. Sukmarini *et al* (2014) who identify the antibiotic resistance genes from LAB in Indonesia fermented foods, reported that as many as 54 isolates from 120 LAB isolates are resistant against

Chloramphenicol [17]. In the study of Erginkaya et al (2017) it was determined that antibiotic resistance lactic acid bacteria were isolated from the fermented dairy product. They found that 58% of lactobacillus are resistant against vancomycin.

Fermented foods may be important vehicles for enormous amounts of living bacteria to enter the human body. These bacteria may carry transferable antibiotic resistance genes which could be transferred to commensal or pathogenic bacteria. Based on the result of our study, the LAB are resistant against vancomycin and chloramphenicol, these LAB are uncontrollably form and this situation is a risk factor in the spread of antibiotic resistance [18].

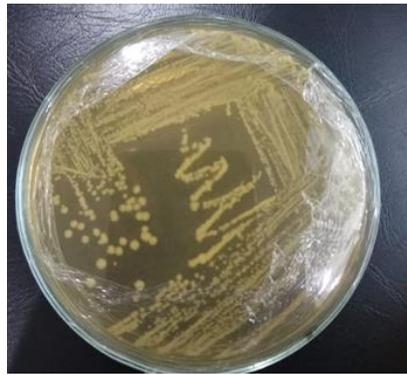


Figure 1: The growth of LAB isolated from fermented palm oil sap on MRS agar surface

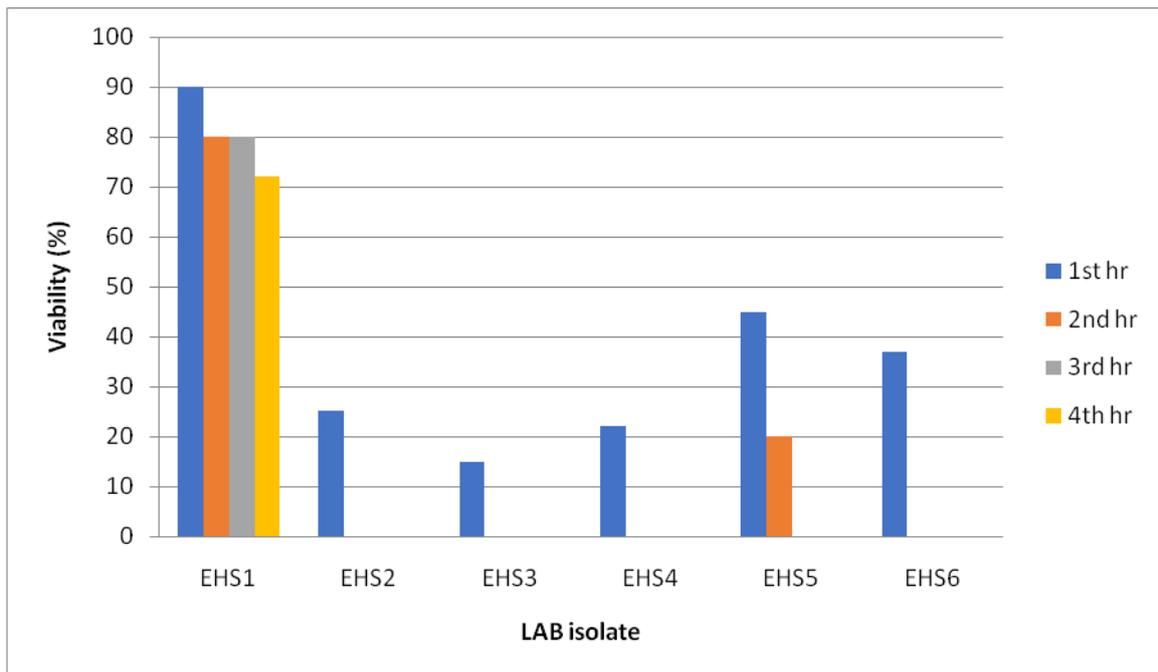


Figure 2: Viability percentage of LAB isolated from fermented palm oil sap in the acidic environment

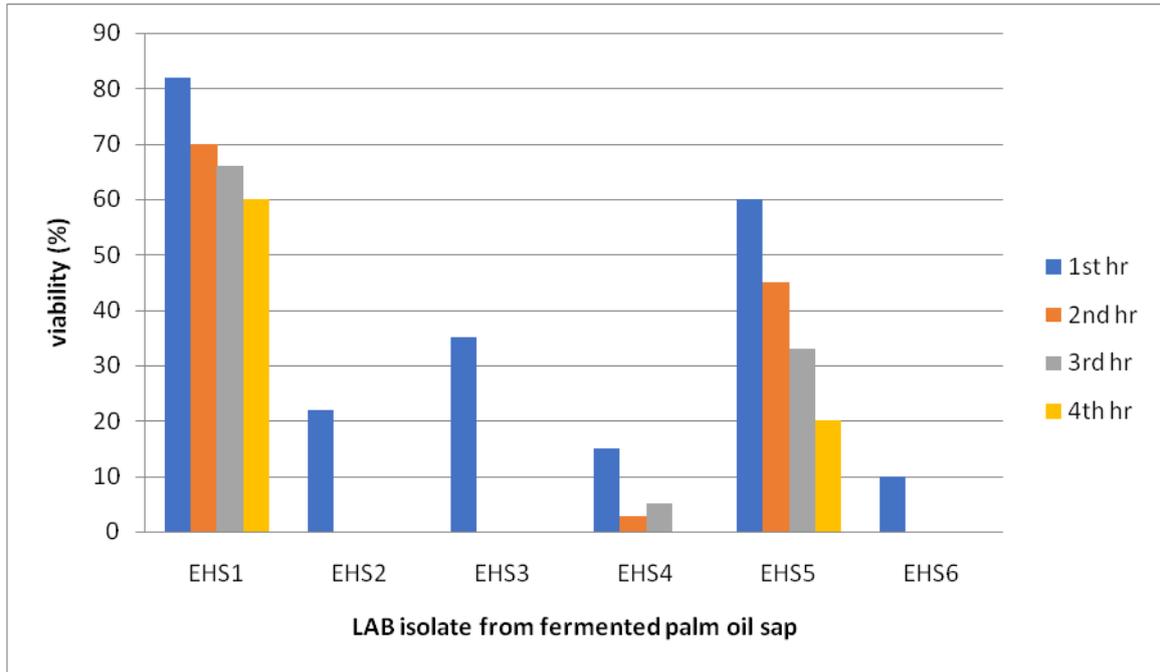


Figure 3: Viability percentage of LAB isolated from fermented palm oil sap in MRS broth supplemented with 0,3% bile salt

Table 1: The susceptibility of LAB isolated from fermented palm oil sap using 5 antibiotics

Isolate	The Diameter of inhibition (mm)				
	vancomycin (30 µg)	penicillin (10 U)	tetracycline (30 µg)	chloramphenicol (30 µg)	gentamicin (10µg)
EHS1	7,5	26,1	23	7,5	10,6
EHS2	7,5	7,5	7,5	7,5	7,5
EHS3	7,5	21,5	14	7,5	15
EHS4	7,5	7,5	7,5	7,5	7,5
EHS5	7,5	29	16,2	7,5	11,1
EHS6	7,5	25,3	21,8	7,5	11,1

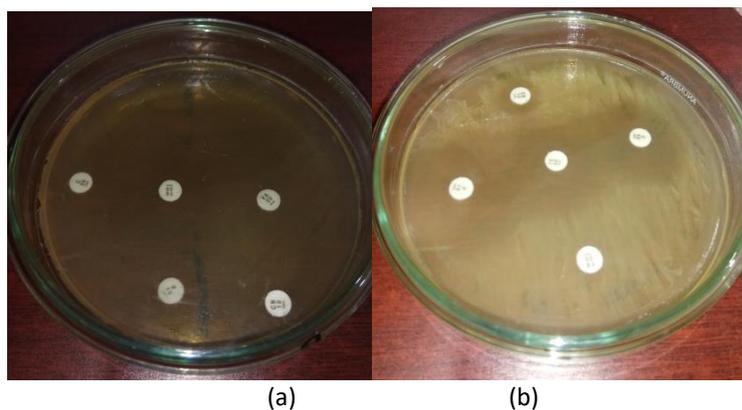


Figure 4: antibiotic sensitivity and resistance of lactic acid bacteria; (a) isolate EHS2; (b) isolate EHS1

CONCLUSION

In conclusion, the present study showed that LAB isolated from fermented palm oil sap have potency as probiotic due to tolerance against the acidic environment and bile salt. The present study also showed the

antibiotic resistance of some LAB. This indicates the high gene transfer frequency of the microbes in the fermented product in Indonesia.

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