Jurnal Sains & Matematika (JSM)
Volume 13, Nomor 3, Juli 2005

ISSN 0854 - 0675
Artikel Penelitian: 121-124

Isolation and Screening Of Lactic Acid Bacteria from Fermented Foods

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ABSTRACT---Biopreservative bear less risk compared to chemical preservative. Efforts to find microorganisms, which produce antibiotic against wide range of pathogens, have been pointed out to lactic acid bacteria. The fermented food is a source of various bacteria, especially lactic acid bacteria. Isolation of acid-producing bacteria from traditional fermented foods including Dadih, Tempoyak, Sawi asin, Candied fruits and fresh mangoes was carried out. Dilution plate was used in isolation process and screening for antibiotic properties was done against 2 pathogens, *Bacillus cereus* and *Staphylococcus aureus*. From this research it was found 5 lactic acid bacteria isolates that were *Lactobacillus casei, L. plantarum, L. fermentum, L. coryneformis* and *Streptococcus thermophyllus*. All of the isolates had antibiotic properties with *L. fermentum* showed the biggest zone inhibition against 2 pathogens tested.
Key word: lactic acid bacteria, biopreservative, fermented foods

INTRODUCTION

Foods that have been processed could be spoilt during storage in certain period of time, even though they have been designated free from harmful microorganisms. This condition has pushed food producers to frequently use high dose of dangerous chemical preservative in order to longer the shelf life of the products. The use of chemical preservative beyond their proper dosage will risk the health of the consumers who buy the foods preserved with chemicals.

Even though the use of chemical preservative is permitted by the official authority, but the consumers have started to think critically to choose alternative foods that do not contain chemical preservative. Refrigerated foods are one of the chosen foods with consideration that these foods are more natural and fresh compared to chemically preserved foods. Besides that, these foods also have flavor, texture and quality better than chemically preserved foods. Despite these advantages, refrigerated foods have weakness that is the storage time is relatively short. This feebleness is caused by contamination and growth of spoiling psychrotrophic microorganisms during processing and storage of the foods.

With the background stated above, it is needed an effort to find out biopreservative agents which can extend the storage time of the processed foods. Lactic acid bacteria are one of the candidates expected to answer this challenge. The use of lactic acid bacteria culture base on the consideration that these bacteria are able to breakdown substrate into acid and lower the pH of the foods, hence preventing the growth of spoiling and pathogen bacteria (Wong, 1988). In addition, lactic acid bacteria have antibiotic property which is hoped capable of inhibiting the growth of deleterious psychotrophic bacteria.

Lactic acid bacteria are microorganisms frequently used in food industries. The features of these bacteria are they can grow in low pH and non-pathogen. These features are becoming important in processing fermented foods using lactic acid bacteria, such as sauerkraut, pickle, cheese, and yogurt. The characteristics of these bacteria are Gram positive, rod or coccus in shape, motile or non-motile, sometimes forming colony like chain. They are anaerobe but few strains are facultative aerobe, negative catalase, little in pigment production, fermenting sugar into energy. Lactic acid bacteria produce lactic acid, acetyl acid, diacetyl and CO_2 (Lee, 1995 and Donald, 1990).

Based on metabolic pathways, lactic acid bacteria are distinguished into 2 groups that are homofermentative and heterofermentative. Moat (1995) stated that homofermentative bacteria carry out glucose metabolism via Embden Meyerhof Parnas (EMP) pathway producing lactic as a main product. Whereas heterofermentative bacteria use Hexose Mono Phosphate (HMP) pathway changing fructose into acetyl phosphate, acetal dehide and CO_2. However, there is possibility that both of the pathways (EMP and HMP) might be passed by lactic acid bacteria.

Another difference between homofermentative and heteroferementative is the endproduct of fermentation process that is effected by aldolase enzyme. This enzyme is one of the most important enzymes involved in glycolysis. Homofermentative group involves aldolase enzyme, whereas heterofermentative group requires transketolase enzyme (Moat,

1995). Genera of bacteria included in lactic acid bacteria are *Leuconostoc, Streptococcus* and *Lactobacillus* (Atlas, 1989). *Lactobacillus* is one of the groups frequently used in food preservation such as vegetable fermentation and milk fermentation (Buckle *et al.*, 1985).

The aims of the study were to isolate and identify lactic acid bacteria from traditionally fermented foods and fruit, and to test the antibacterial activity of the isolates against pathogens.

MATERIALS AND METHODS

Isolation and identification of lactic acid bacteria

Samples used were: dadih, tempoyak, pickles, salty cabbage, candied fruits and fresh mango. de Mann, Ross and Sagarosa Agar (MRSA) was used for isolation and testing of antibacterial activity of isolated lactic acid bacteria.. A 25 grams of each sample was diluted into 225 ml of 0.85% NaCl. The sample then smashed using Stomacher for 2 minutes with medium speed and diluted to a serial dilution up to 10^6. A 1 ml of each dilution was put into a plate and poured with 9 ml MRSA media. The plate was incubated for 24 – 48 hours at 37°C. The colonies emerged which showed different shape and color in the plate were cultured to another new plates contained the same media using streak method. This step was done until pure culture was obtained.

Identification of the isolates was done through morphological examination of both microscopic and macroscopic characteristics. Macroscopic examination consisted of shape and color of colony, whereas microscopic examination was performed through Gram stain. In addition to morphological examination, several biochemical and physiological characteristics were also examined. These included production of CO_2 and glucose, the growth at different temperature, ammonia production, the growth at 6.5% NaCl, Litmus milk reaction, catalase test, MR-VP reaction, reaction on OF basal media and carbohydrates utilization test.

Antibacterial Assay using Well Diffusion Method

This method was used to find out antibacterial activity of the isolates found against two tested bacteria, namely *Staphylococcus aureus* and *Bacillus cereus*. A 24 hours-old tested bacteria at 10^7 CFU/ml was inoculated into the plate and poured with liquid MRSA media. The media was then allowed to solidify. Three holes/wells were made in the plate and each hole was filled with 60 µl of the isolate. The plate was incubated for 24 – 48 hours at 37°C. The clear zone around the hole was examined and measured.

RESULT AND DISCUSSION

Isolation and identification of lactic acid bacteria

There were 20 pure culture obtained from the isolation process. However, based on the microscopic and macroscopic characteristics identification these isolates only consisted of five bacteria, namely *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus coryneformis* and *Streptococcus thermophyllus.* The isolates emerged from the samples used indicated that population of lactic acid bacteria existed in the traditionally fermented foods. The existence of these bacteria was involuntary, as the producer did not use pure culture in the making of the fermented foods. However, the effect of lactic acid bacteria on fermented foods was quite important. The foods chosen as sample generally showed shelf life longer and usually more nutritious than their untreated state.

In-vitro antibacterial activity of lactic acid bacteria

Antibacterial assay was done in order to find out whether or not the isolates could inhibit the growth of pathogenic bacteria. Inhibitory activity was shown with the formation of clear zone around the well on the plate. According to Vuys and Vandamme *in* Zamfir *et al.*, (1999) the inhibitory effect occurred due to production of lactic acid and other inhibitory substances such as hydrogen peroxide, ethanol, diacetyl, CO_2 and bacteriocin. These compounds could inhibit the growth of unwanted bacteria for example *Bacillus, Clostridium Staphylococcus* and *Listeria* on the foods.

The inhibitory activity against bacteria tested appeared in the first day of incubation, where the clear zone was formed. However the clear zone tended to narrow in second and third day, as shown in Table 1 where *L. plantarum* shows the highest activity among the isolates

tested against *Staphylococcus aureus*. This was quite intriguing because *L. plantarum* belonged to the group of homofermentative lactic acid bacteria which during its incubation time produced large amount of lactic acid. The reduction of clear zone might be caused by the amount of inoculum given was insufficient to produce inhibitory compounds needed to inhibit pathogens used. Hence, when the isolates were in contact with pathogens the inhibitory activity decreased.

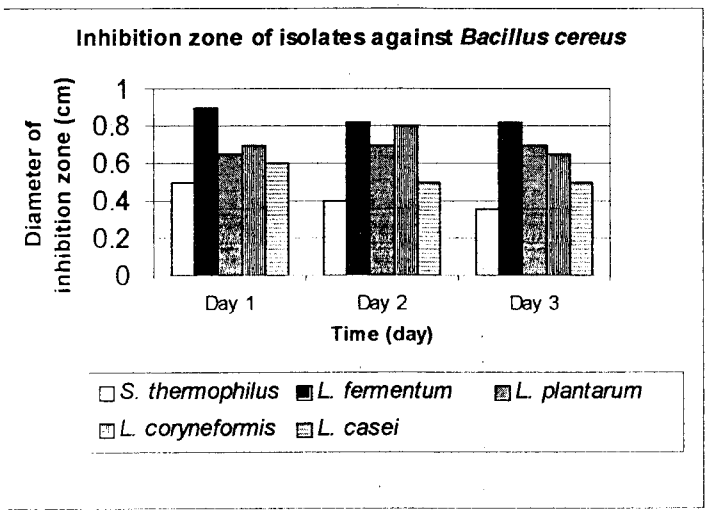

Figure 1 The inhibitory activity of lactic acid bacteria isolates against *Bacillus cereus*

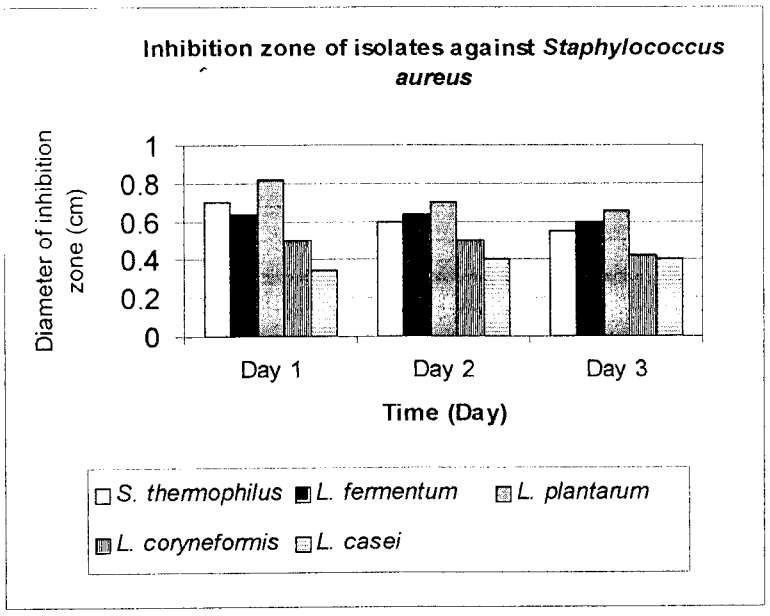

Figure 2. The inhibitory activity of lactic bacteria isolates against S. aureus

In vitro antibacterial activity of the isolates found against *Bacillus cereus* is shown in Table 2, which indicates that *L. fermentum* has the highest inhibitory activity. This could be caused by characteristics of *L. fermentum* which belonged to the group of heterofermentative lactic acid bacteria. This group produced acetic acid and CO_2 in addition to lactic acid which could contribute to its inhibitory effect against pathogen tested.

CONCLUSION

Five isolates of lactic acid bacteria were isolated from traditionally fermented foods these were *Lactobacillus casei, L. plantarum, L. fermentum, L. coryneformis* and *Streptococcus thermophyllus.* All isolates had antibacterial activity where *L. plantarum* had the highest activity against *Staphylococcus aureus* and *L. fermentum* had the highest activity against *Bacillus cereus.*

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