

Malaysian Isolates of Lactic Acid Bacteria with Antibacterial Activity against Gram-Positive and Gram-Negative Pathogenic Bacteria

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Abstract

Contamination of foodstuff with foodborne and pathogenic bacteria are global issue and it is serious hazard for

the health of the human. Lactic acid bacteria are well known for their health properties and their antimicrobial activity against spoilage and pathogenic bacteria. In this study, three isolates *Lactobacillus fermentum* Te007, *Pediococcus pentosaceus* Te010, *L. pentosus* G004 isolated from Malaysian fermented foods and fruits such as (tempeh, tempoyak, guava and banana) were evaluated for their antibacterial activity and antibiotic resistant against Gram-positive and Gram-negative bacteria by dual agar overlay method. The three isolates inhibited the growth of indicator bacteria and the activity was varied between weak and strong. All the isolates were resistant to the antibiotic nalidixic acid and vancomycin. The tested bacteria can be added to food as antibacterial agents to prevent the growth of harmful microorganisms.

Keywords: Tempeh, Lactic acid bacteria, Antimicrobial activity, Antibiotic resistance, Fermented foods

1. Introduction

The food safety is important concern in the processed foods industry because of the contamination with food borne pathogenic bacteria. The consumers demand for minimal processed food with less chemical additives (Cleveland *et al.*, 2001). These reasons have led to the increase of interest to introduce the biopreservatives to food to replace chemicals with natural preservation agent (José *et al.*, 2007). The foodborne and pathogenic bacteria, especially anaerobes and facultative anaerobes, even when they are present in low numbers they can grow during storage at low temperatures as in the refrigerators. These pathogens can multiply and cause risk to the safety of raw, processed and bakery products. Biopreservation is the extension of shelf life and food safety by the use of natural or controlled microbiota and/or their antimicrobial compounds (Stiles, 1996). Among the biopreservatives is lactic acid bacteria (LAB). They are known to produce variety of antibacterial **substances** as reported by several researchers (Moreno *et al.*, 2000; Mallesha *et al.*, 2010; Akpinar *et al.*, 2011; Muhialdin & Hassan, 2011a). LAB are safe and they have the status of general recognized as safe (GRAS), and have an important role in the preservation of foods and fermented products (Cintas *et al.*, 2001). The inhibition activity of LAB to the growth of pathogenic bacteria is most likely due to the production of organic acids and bacteriocin (De Vos, 1993; Klaenhammer, 1993).

There are more than a few reports about the antimicrobial activity of LAB. A total of 16 strains (5 *Enterococcus faecium*, 5 *Enterococcus mundtii*, 4 *Pediococcus pentosaceus*, 1 *L. coryniformis* and 1 *Lactococcus garvieae*) were reported to have inhibition activity to the growth of *Listeria innocua*. Bacteriocin-like inhibitory substances were also reported to maintain the inhibition activity against non-pathogenic and pathogenic food-associated and human pathogenic bacteria (Corsetti *et al.*, 2005). *L. pentosus* TV35b found to have inhibitory activity against the growth of *Clostridium sporogenes*, *Cl. tyrobutyricum*, and *Listeria innocua*, among others, the active compound produced was **produced** a bacteriocin-like peptide (pentocin TV35b) (Okkers *et al.*, 1999). Moreno *et al.*, (2002) reported that LAB isolated from fermented food Tempeh produced bacteriocins that inhibited the growth of Gram-positive indicators, including *Listeria monocytogenes*. Liasi *et al.*, (2009) reported that Malaysia isolates (*L. casei* LA17, *L. plantarum* LA22 and *L. paracasei* LA02) isolated from the fermented fish Budu inhibited the growth of (*B. cereus*, *S. aureus*, *Salmonella enterica*, *Listeria monocytogenes*, *E. coli* and *Lactococcus lactis*). The objective of this study is to evaluate the antimicrobial activity of lactic acid bacteria isolated from Malaysian fermented foods and fruits and their antibiotic susceptibility.

2. Materials and Methods

2.1 Cultures and isolates

L. fermentum Te007, *Pediococcus pentosaceus* Te010, *L. pentosus* G004 were isolated from Malaysian fermented fruits and foods and evaluated for their antibacterial activity against (*Bacillus subtilis*, *Serratia marcescens*, *Enterobacter aerogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Shigella sonnei*, *Klebsiella pneumonia* and *Salmonella* Typhimurium) obtained from the Microbiology Laboratory, Faculty of Food Science, University Putra Malaysia. The pathogenic bacteria were chosen to represent potential food borne bacteria. They were grown on nutrient broth (NB, Oxoid) at 30 °C for 24 h (Table 1).

2.2 Growth inhibition of pathogenic bacteria by LAB

The inhibition activities of the isolates were determined by using the dual agar overlay method as described by Magnusson & Schnurer (2001) with some modification. LAB isolates were inoculated in 2-cm lines on MRS agar plates and were grown at 30 °C for 24 h in anaerobic jars. The plates were then overlaid with 10 ml of nutrient agar containing 10⁵ CFU/ml of each bacterium separately. The zone of inhibition was measured after 24 h of aerobic incubation at 30 °C; the inhibition was measured by depending on the size of the inhibition zone. The scale was used is: + weak inhibition, ++ moderate inhibition and +++ strong inhibition. The test was done in

duplicate.

2.3 Antibiotic resistant of lactic acid bacteria

LAB isolates were tested for their resistant against several antibiotics using disk diffusion method (Herreros *et al.* 2004) with MRS agar. The antibiotics tested were vancomycin (5µm), nalidixic acid (30 µm), gentamycin (10 µm), streptomycin (10 µm), tetracycline (30 µm), penicillin G (10 µm) and chloramphenicol (30 µm) (Sigma). The antibiotic disc were placed on each plate and incubated at 37°C for 24 hours in an anaerobic jar. The results were taken by detection the inhibition zone around the antibiotic discs. The test was done in replicate.

3. Results

3.1 Growth inhibition of pathogenic bacteria by LAB

The three isolates of LAB (*L. fermentum* Te007, *P. pentosaceus* Te010, *L. pentosus* G004) showed antibacterial activity against tested strains of Gram positive and Gram negative bacteria. The results showed very strong inhibition activity from the isolate *L. pentosus* G004 and *L. fermentum* Te007 and moderated activity from the isolate *P. pentosaceus* Te010 (Table 2). The isolate *L. pentosus* G004 had very strong activity against all the tested bacteria except (*Salmonella* Typhimurium and *Bacillus subtilis*) which the isolate had moderated inhibition activity.

In contrast, with the isolate *L. fermentum* Te007 that showed very strong activity against (*Bacillus subtilis*, *Serratia marcescens*, *Escherichia coli* and *Staphylococcus aureus*) and moderated activity against (*Enterobacter aerogenes*, *Staphylococcus epidermidis*, *Shigella sonnei*, *Klebsiella pneumonia* and *Salmonella* Typhimurium). The isolate *P. pentosaceus* Te010 showed fair and good inhibition activity against all the examined bacteria. The three LAB isolates had moderated activity against *Salmonella* Typhimurium but no isolate showed very strong activity against it. In addition, *Bacillus subtilis* was sensitive for the isolate Te007 and the sensitivity was less for the other two LAB isolates.

3.2 Antibiotic resistant test of LAB isolates

All LAB isolates were resistant to nalidixic acid and vancomycin. Additionally, isolate *L. pentosus* G004 was resistant to Gentamycin, Streptomycin, Tetracycline and Penicillin G. Isolate *L. fermentum* Te007 was sensitive to Gentamycin, Tetracycline and Penicillin G activity to gentamycin. Isolate *P. pentosaceus* Te010 showed resistance against nalidixic acid and vancomycin but sensitive to the other examined antibiotics. All LAB isolates were sensitive to Chloramphenicol (Table 3).

4. Discussion

The LAB isolates inhibited the growth of all tested bacteria including the pathogenic strains in different spectrum range of activities and the activity range was between weak to strong depends on the LAB strain. The highest activity was from the isolate *L. pentosus* G004 which show high activity against the tested microbes, followed by the isolate *L. fermentum* Te007 and *P. pentosaceus* Te010, respectively. Moreno *et al.*, (2002) reported that LAB isolated from fermented food Tempeh produced bacteriocins that inhibited the growth of Gram-positive indicators, including *Listeria monocytogenes*.

S. aureus, *B. subtilis*, *S. typhimurium* and *E. coli* are pathogens that have been involved in outbreaks of food-borne disease in the several foodstuffs (Lindqvist *et al.*, 2001; Matarante *et al.*, 2004). *K. pneumonia*, *S. epidermidis* *Shigella sonnei* and *Serratia marcescens* are human pathogens and involve in many serious infectious diseases (Wang *et al.*, 1998; Ammor *et al.*, 2006). The concern of food safety is on increase and this comes with big responsibility of finding new, natural and inexpensive methods such as the use of the biopreservatives from safe microbes such as LAB. There are many reports described the success of the LAB isolates to inhibit the growth of pathogenic and foodborne bacteria *in vivo* and *in vitro* (Herreros *et al.* 2004; Ammor *et al.*, 2006; Al-Allaf *et al.*, 2009; Akpinar *et al.*, 2011).

In this study the selected LAB isolates showed good inhibition activity against indicator strains of the pathogenic bacteria and this promote the use of these isolates as antimicrobial agent that can be added to food to prevent the growth of spoilage and foodborne bacteria and prevent the production of the different toxins in foodstuff by these bacteria. The inhibition was fast after 24 h; we could observe the inhibition zone around the two lines of the LAB isolates on the MRS agar plates. Liasi *et al.*, (2009) reported Malaysia isolates (*L. casei* LA17, *L. plantarum* LA22 and *L. paracasei* LA02) to have inhibition activity against (*B. cereus*, *S. aureus*, *Salmonella enterica*, *Listeria monocytogenes*, *E. coli* and *Lactococcus lactis*), The LAB was isolated from the fermented fish Budu.

In this experiment, the selected isolates were resistant to antibiotic vancomycin and nalidixic acid. Vancomycin

resistance is of major concern because it is one of the antibiotics that broadly efficacious against clinical infections caused by multidrug-resistant pathogens (Johnson *et al.*, 1990; Woodford *et al.*, 1995). It is very important for LAB that added to food to be not carry transmissible antibiotic resistance genes, there are reports which indicate that LAB from fermented products may act as a vehicle of antimicrobial-resistance genes that could be transferred to pathogens, either in the food matrix or in the gastrointestinal tract (Mathur and Singh, 2005). This can lead to the development of new antibiotic-resistant pathogens and that is why those strains of LAB undesirable to be added to foodstuff (Morelli and Wright, 1997; Salminen *et al.*, 1998; Saarela *et al.*, 2000). In this study, all the isolates showed resistance to vancomycin and nalidixic acid, and the isolate *L. pentosus* G004 was resistant to the seven tested antibiotic except of Chloramphenicol. This study demonstrates that these isolates can be added to food as conservative's agents against the foodborne pathogenic bacteria and to improve the human's health especially the isolate *L. pentosus* G004.

5. Conclusion

Lactic acid bacteria isolated from different Malaysian environment inhibited the growth of (*Bacillus subtilis*, *Serratia marcescens*, *Enterobacter aerogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Shigella sonnei*, *Klebsiella pneumoniae*, and *Salmonella Typhimurium*). The three isolates had inhibition activity against tested microorganisms and the spectrum range was varied between fair and strong. The isolates were antibiotic resistant against vancomycin and nalidixic acid. LAB isolates from Malaysian fermented foods and fruits have inhibition activity against foodborne and pathogenic bacteria and it have potential to be use as food preservatives because of their ability to eliminate the growth of spoilage microorganisms.

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Table 1. Bacterial strains and media of growth used in this study

Strain	Media	Incubation temperature	Origin or reference
<i>Bacillus subtilis</i> 21332	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
<i>Serratia marcescens</i> 13880	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
<i>Enterobacter aerogenes</i> 13048	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
<i>Escherichia coli</i> 25922	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
<i>Staphylococcus aureus</i> 25923	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
<i>Staphylococcus epidermidis</i> 12228	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
<i>Shigella sonnei</i> 29930	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
<i>Klebsiella pneumoniae</i> 13883	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
<i>Salmonella Typhimurium</i> 13311	Nutrient broth	30 °C	ATCC (American Type Culture Collections)
<i>L. fermentum</i> Te007	MRS agar	37 °C	Muhialdin <i>et al.</i> , 2011b
<i>Pediococcus pentosaceus</i> Te010	MRS agar	37 °C	Muhialdin <i>et al.</i> , 2011b
<i>L. pentosus</i> G004	MRS agar	37 °C	Muhialdin <i>et al.</i> , 2011b

Table 2. Growth inhibition of Gram positive and Gram negative bacteria after 24 h incubation at 30°C by dual agar overlay method

Tested organism	Inhibition activity		
	<i>L. pentosus</i> G004	<i>L. fermentum</i> Te007	<i>P. pentosaceus</i> Te010
<i>Bacillus subtilis</i>	++	+++	++
<i>Serratia marcescens</i>	+++	+++	++
<i>Bacillus subtilis</i>	+++	++	+
<i>Escherichia coli</i>	+++	+++	++
<i>Staphylococcus aureus</i>	+++	+++	++
<i>Staphylococcus epidermidis</i>	+++	++	+
<i>Shigella sonnei</i>	+++	++	+
<i>Klebsiella pneumoniae</i>	+++	++	+
<i>Salmonella Typhimurium</i>	++	++	++

Table 3. Antibacterial activity of selected antibiotics against lactic acid bacteria measured by diameter of inhibition zone (mm) around the discs

Antibiotics	LAB		
	<i>L. pentosus</i> G004	<i>L. fermentum</i> Te007	<i>P. pentosaceus</i> Te010
Nalidixic acid	0	0	0
Gentamycin	0	5	13
Streptomycin	0	0	11
Tetracycline	0	11	9
Vancomycin	0	0	0
Penicillin G	0	7	8
Chloramphenicol	19	9	13



A

B

C

Figure 1. Clear zone of growth inhibition of *Staphylococcus epidermidis* formed around the streak lines of lactic acid bacteria (LAB) (Te007) incubated at 30 °C for 24 h by dual agar overlay method A. Te007, B. G004 and C. Te010