



Isolation of Lactic Acid Bacteria from Fermented Food Products for Probiotic Properties and Their Antagonistic Effects on Gastrointestinal Bacterial Pathogens

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Abstract

The aim of the present study was to isolate lactic acid bacteria from fermented food products for a probiotic property. The result showed that 2 isolates of lactic acid bacteria, namely FF1 and BS1, were isolated from fermented fish and bamboo shoots, respectively. The morphological characterization and biochemical tests for lactic acid bacteria identification indicated that the 2 isolates were Gram-positive, rod bacteria. Their catalase and oxidase test were also negative. The isolates were investigated for the ability of fermentation so that the isolates FF1 and BS1 were classified as homofermentative and heterofermentative bacteria, respectively. The inhibitory activity against gastrointestinal bacterial pathogens of both isolates was examined. The isolate FF1 inhibited Enteropathogenic *Escherichia coli* DMST 30546 and *Pseudomonas aeruginosa*. On the other hand, the isolate BS1 had antimicrobial activity against Enteropathogenic *E. coli* DMST 30546, *P. aeruginosa*, *Salmonella* Typhimurium and *Shigella sonnei* with clear zone 25.00-44.66 mm that significantly higher than gentamicin ($p < 0.05$). The result provided that the isolate FF1 and BS1 exhibited the preliminary probiotic property that is the inhibition of pathogen.

Keywords: *Lactic acid bacteria, Probiotic, Fermented food product*

1. Introduction

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit to the host (Hill et al., 2014). These microorganisms control the balance of intestinal microflora by inhibiting pathogens (Sybesma, Kort, & Lee, 2015). Probiotics adhere to intestinal cells and produce organic acids as inhibitory substances (Mahmoudi et al., 2016). Lactic acid bacteria are the most commonly studied and most widely used probiotic bacteria (Haghshenas et al., 2014). They are a group of non-spore-forming Gram-positive bacteria that produce lactic acid as the main end product among the fermentation of carbohydrates (Giraffa, Chanishvili, & Widyastuti, 2010). These bacteria have been consumed in terms of diverse food supplements and food preservation for thousands of years due to their probiotic properties and fermentation activities, and are “generally regarded as safe” (GRAS) (Ren et al., 2014). Also, lactic acid bacteria are a focal point of intensive universal research for their fundamental part in most fermented food as well as for their capacity to create different antimicrobial compounds advancing probiotic properties, reduction of serum cholesterol, stabilization of gut microflora, alleviation of lactose intolerance and stimulation of the immune system (Khedid et al., 2009). Many studies have reported that the best matrices to deliver probiotics are dairy fermented products, such as fermented milk and yogurt (Pisano et al., 2014). On the other hand, nowadays there is a need for novel and nondairy probiotics and it has been found that traditional fermented foods may constitute a good working base for the development of probiotic-type functional foods (Argyri et al., 2013).

Traditional fermented foods are consumed by a large population in Thailand and are produced by natural microbiota through a spontaneous fermentation process. Fermented fish and bamboo shoots are Thai traditional fermented products, fermented mainly by various lactic acid bacteria. There are previous reports indicate that lactic acid bacteria from fermented products showed probiotic properties (Lee et al., 2016; Angmo, et al., 2016; Khan & Kang, 2016; Oguntoyinbo & Nabad, 2016). Both products are possible as a source of novel probiotic strains with immense potential of various health benefits.

Intestinal pathogenic bacteria are one of the most main causes of food poisoning and diarrhea. The protection against infections with pathogenic microorganisms is one of the probiotic properties that make



humans or animals healthier (Lim & Im, 2009). There are also many studies that characterize lactic acid bacteria with antimicrobial activity.

In this study, the researchers were interested in the isolation of lactic acid bacteria from fermented fish and bamboo shoots. In addition, we assessed some probiotic potential that is antibacterial activity against gastrointestinal bacterial pathogens.

2. Objectives

To select potential probiotic strains of lactic acid bacteria from fermented fish and bamboo shoots.

3. Materials and Methods

3.1 Isolation of lactic acid bacteria from fermented food products

Two fermented food products were purchased from markets, Muang district, Pathum Thani, Thailand. These products included fermented fish and bamboo shoots. Twenty-five grams of each product was added into 0.1% (w/v) peptone solution and dilute to obtain the suitable dilution. Each dilution was seeded on Man Rogosa and Sharpe (MRS) agar (Difco, USA) containing 0.04% (w/v) bromocresol purple (Sigma-Aldrich, USA) plate. After the incubation anaerobically at 37 °C for 48 h, the yellow colonies were selected from each plate. The selected colonies were carried out using morphological and biochemical methods. Gram staining, catalase test, oxidase tests, and gas production from glucose were performed with isolated strains by standard microbiological techniques (Handa & Sharma, 2016).

3.2 Inhibition of bacterial gastrointestinal pathogen

To examine the antimicrobial activity of the selected isolates against Enteropathogenic *E. coli* DMST 30546, *P. aeruginosa*, *S. Typhimurium* and *S. sonnei*, the first strain was purchased from Department of Medical Sciences, Thailand and other strains were clinically isolated by the laboratory of Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. A spot-on-lawn method was used. A sample (5 µl) of an overnight culture of the strain was spotted on MRS agar and incubated anaerobically at 37 °C for 48 h. After incubation, the petri dishes were overlaid with Mueller Hinton agar (1% (w/v) agar; Difco, USA) previously inoculated with 10⁶ CFU of the indicator strain and incubated in aerobic condition at 37 °C for 18 h. After incubation, the inhibition zones were measured. Gentamicin disc (10 µg) was used as positive control. The test was performed in triplicate (Santos et al., 2016).

4. Results and Discussion

Lactic acid bacteria are isolated and characterized the probiotic properties before they are claimed as probiotic strain. Several mechanisms by which probiotics mediate their health benefits on the host have been suggested, and can be divided into three categories; (i) certain probiotics have antimicrobial activity and can exclude or inhibit pathogens; (ii) probiotic bacteria can enhance the intestinal epithelial barrier; and (iii) probiotic bacteria are believed to modulate the host immune response. The mechanisms of health-promoting effects of probiotic bacteria have proven difficult to elucidate in detail, and traditionally most attention has been given to their antipathogenic properties (Jensen et al., 2012).

In this study, to isolate lactic acid bacteria from 2 fermented food products, 2 isolates including FF1 and BS1 were Gram-positive, rod bacteria. The results showed that both isolates were catalase negative and oxidase negative. The isolate FF1 was homofermentative bacteria while BS1 was classified as heterofermentative bacteria. These indicated that the characteristics of the isolates correlated with lactic acid bacteria. The isolate FF1 showed antimicrobial activity against Enteropathogenic *E. coli* DMST 30546 and *P. aeruginosa*. In addition, the isolate BS1 significantly inhibited all tested pathogens better than gentamicin (Table 1). These results correlated with much research. Edalati et al. (2019) presented that 32 isolates were all Gram-positive bacteria and had antagonistic properties on *Staphylococcus aureus*. In another study, 16 isolates of lactic acid bacteria were identified as gram-positive, rod-shaped. Biochemically strains showed catalase and oxidase negative and positive for glucose fermentation (Tallapragada et al., 2018). The strain LAP2 isolated from Hentak, a fermented fish product of North-East India was gram-positive, rod-shaped, and had potential to ferment glucose. This strain was also found to be



potent bacterium due to the broad growth inhibitory zone against *Shigella flexneri*, *Micrococcus luteus*, *Yersinia enterocolitica* and *Proteus vulgaris* (Aarti et al., 2017). Furthermore, Vefon et al. (2017) showed that 17 strains showed typical characteristics of lactic acid bacteria: Gram-positive, catalase-negative, non-motile, non-spore forming rods and cocci.

Table 1 Inhibition of gastrointestinal bacterial pathogens by lactic acid bacteria isolated from fermented food products

Isolate	Inhibition zone (mm) \pm S.D.			
	Enteropathogenic <i>E. coli</i> DMST 30546	<i>P. aeruginosa</i>	<i>S. Typhimurium</i>	<i>S. sonnei</i>
FF1	16.66 \pm 2.88	15.33 \pm 0.57	0.00 \pm 0.00	0.00 \pm 0.00
BS1	35.00 \pm 0.57	44.66 \pm 0.57	25.00 \pm 0.00	28.00 \pm 2.64
Gentamicin (10 μ g)	23.00 \pm 1.00	17.00 \pm 0.00	8.66 \pm 0.57	8.66 \pm 0.57

5. Conclusion

In overall, we successfully isolated lactic acid bacteria with potential probiotic properties. The isolates included FF1 and BS1. These isolates were Gram-positive, rod bacteria with catalase and oxidase negative. For gas production from glucose, the isolates FF1 and BS1 were homofermentative and heterofermentative bacteria, respectively. The isolate FF1 inhibited some tested pathogens. On the other hand, BS1 showed significant antibacterial activity against all tested pathogens better than gentamicin ($p < 0.05$). These indicated that the isolates were probiotic candidate strains due to their antibacterial activity. However, identification of genus and species and other probiotic properties including susceptibility to antibiotics, production of inhibitory substances, survival ability in the gastrointestinal tract, aggregation activity, and adhesion capacity should be examined in the future study.

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7. References

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