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Preliminary Study of Antimicrobial Activity against *Escherichia coli* and Probiotic Properties of Lactic Acid Bacteria Isolated from Thailand Fermented Foods

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***Abstract*—**The lactic acid bacteria (LAB) were isolated from 10 samples of fermented foods (Sa-tor-dong and Bodo) in South locality of Thailand. The 23 isolates of lactic acid bacteria were selected, which were exhibited a clear zone and growth on MRS agar supplemented with $CaCO_3$. All of lactic acid bacteria were tested on morphological and biochemical. The result showed that all isolates were Gram's positive, non-spore forming but only 10 isolates displayed catalase negative. The 10 isolates including BD1 .1, BD 1.2, BD 2.1, BD2.2, BD 2.3, BD 3.1, BD 4.1, BD 5.2, ST 4.1 and ST 5.2 were selected for inhibition activity determination. Only 2 strains (ST 4.1 and BD 2.3) showed inhibition zone on agar, when using *Escherichia coli* sp. as target strain. The ST 4.1 showed highest inhibition zone on agar, which was selected for probiotic property testing. The ST4.1 isolate could grow in MRS broth containing a high concentration of sodium chloride 6%, bile salts 7%, pH 4-10 and vary temperature at 15-45°C.

***Keywords*—**Lactic acid bacteria, Probiotic, Antimicrobial.

I. Introduction

LACTIC ACID BACTERIA (LAB) are Gram positive, non-sporing, catalase negative, acid tolerant and acid producer, lactic acid (the major end product), acetic acid. The strains of bacteria LAB consist of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*. LAB are most important in fermented food because the lactic acid and other organic acids produced by these bacteria as natural preservatives. Lactic acid bacteria (LAB) have been used as starter cultures in various fermented food such as fermented dairies, fermented fish, fermented vegetable and fermented meat products. LAB are important in the fermentation process by rapid acidification of raw materials through the production of organic acids [1].

The characteristics of LAB are (a) their ability to produce antimicrobial compounds (bacteriocins, organic acids) for growth inhibition of harmful bacteria, (b) their ability to resist high concentrations of salts (in food), acids, and bile salts (in gastrointestinal tract), which is beneficial to health, and (c) their inability to produce amino acid decarboxylase (no biogenic amine accumulation) [2], [3]. The lactic acid bacteria (LAB) are proved to function as probiotics, which are benefit to host health, when ingested in sufficient quantities. The colonization of the gut by probiotic bacteria prevents growth of harmful bacteria by competition exclusion and by the production of organic acid and antimicrobial compounds. The acid and bile tolerance as well are two fundamental properties that indicate the ability of probiotic microorganism to survive the passage though the upper gastrointestinal tract, particularly acidic condition in the stomach and the presence of bile in the small intestine [4].

Bodo is a traditional food in the Southern of Thailand. It is made from fermented fish, salts and water with the natural microflora fermentation. Sa-tor-dong (*Parkia speciosa*) is widely consumed in the Southern of Thailand as well. Sa-tor-dong was preserved by fermentation process. Both products having the lactic acid bacteria are starter cultures like various the fermented foods. LAB have probiotic properties such as high tolerance at low pH and bile salts considered as important criteria. The probiotic culture includes the ability to adhere the intestinal epithelium cell and the ability to inhibit the pathogenic bacteria [5]. Therefore, the aim of this research is to isolate lactic acid bacteria from local fermented food (Bodo and Sa-tor-dong) and also to determine their probiotic property.

II. Materials and Methods

A. Isolation of LAB

Two type of commercial local fermented food, "Bodo" (fermented fish) and "Sa-tor-dong" (fermented *Parkia speciosa*) were bought (Fig. 1). Each of sample (10 g) was separately blended with 100 ml 0.85% NaCl solution in a 250ml Erlenmeyer flask. Then aliquots of the culture from each of the flasks were diluted serially to 10^6 times and 0.1 ml was spread on MRS agar plates, which added 0.5% (w/v) $CaCO_3$. The plates were incubated for 24-48 hour at 37°C. Calcium carbonate was used an indicator for acid-producing strains when interact with acid then a clear zone was observed [6]. The colonies exhibited clear zone on the plate were picked and streaked on fresh MRS agar plates. Morphological determination, the isolates were

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tested for catalase activity by dropping 3% hydrogen peroxide solution on the cells. Immediate formation of bubbles indicated the presence of catalase in the cells. Only those isolates displayed catalase-negative were continued to Gram-stain test.

(a) Bodo (b) Sa-tor-dong

Fig. 1 The samples of fermented food

B. Screening for Antimicrobial Activity

The isolates were grown in 5 ml of MRS broth for 18 hours at 30°C. Then, the cells were removed by centrifuged at 9,000 g for 10 minutes at 4°C. The supernatant was adjusted to pH 5.0 with a 3M NaOH solution and then filter-sterilized (0.22-µm pore size). The solution thus obtained, designated as Cell Free Supernatant (CFS), was stored at 4°C and –20°C.

Antimicrobial activity was determined by Agar well method and using *Escherichia coli* as target strain. Agar test plates of target strain were prepared. 100 ml of nutrient broth (NB) inoculated with the target strain was incubated at 37°C for 24 hour. Mixed well (0.5 ml) of the culture of target strain was added to 50ml nutrient agar (NA) before agar forming, mixed well and poured on plate. The NA containing target strain was allowed to set and then used a sterile cork borer for hold making.

The sample culture was dropped in each hole 200 µl, then incubation at 37°C for 24 hour. The diameter of the clear zones of inhibition was measured.

C. Probiotic Properties: Bile Salt, Sodium Chloride, Temperature and pH Tolerance

Suitable characteristics of LAB are ability to high concentrations of salts (in food), acids and bile salts (in gastrointestinal tract), which is beneficial to health. Bile salts concentration of 0, 1.0, 3.0, 5.0 and 7.0% (w/v) were prepared for Bile salt tolerance test. Sodium chloride concentration of 0, 2, 4, 6, 8 and 10 % (w/v) was used for sodium chloride tolerance test. The highest concentrations of bile salts and sodium chloride that the isolates were able to grow were recorded. The temperature tolerance was incubated at 15, 25, 37, 45 and 55°C. pH tolerance was adjusted to pH 2-10 and incubated at 37°C for 24 hours.

III. Results and Discussion

A. Isolation of LAB

The isolation of LAB from Bodo and Sa-tor-dong fermented. It was found that 23 isolates exhibited a clear zone and growth on MRS agar supplemented with $CaCO_3$ as shown in Table I. Calcium carbonate was used an indicator for acid-producing strains when interact with acid then a clear zone was observed [6].

However, 23 isolates were identified as LAB using the criteria of being Gram-positive and catalase negative. Ten isolates were cocci (8 isolates) and rods (2 isolates) shape bacteria. The 10 isolates were BD1 .1, BD 1.2, BD 2.1, BD2.2, BD 2.3, BD 3.1, BD 4.1, BD 5.2, ST 4. 1and ST 5.2 as shown in Table II.

TABLE I
Microorganisms Isolated from Bodo and Sa-Tor-Dong

Sample	Source	Isolated	Isolated Name
Bodo 1	Housewife group, Pattani	2	BD1.1
			BD1.2
Bodo 2	Housewife group, Tanyong Mat, Narathiwat	3	BD2.1
			BD2.2
			BD2.3
Bodo 3	Fresh Market, Yaring Pattanee	1	BD3.1
Bodo 4	Housewife group, Saiburi, Pattanee	1	BD4.1
Bodo 5	Housewife group, Pattanee	3	BD5.1
			BD5.2
			BD5.3
Sa-tor-dong 1	Housewife group, Yaha, Yala	3	ST1.1
			ST1.2
			ST1.3
Sa-tor-dong 2	Fresh Market, Yaha ,Yala	3	ST2.1
			ST2.2
			ST2.3
Sa-tor-dong 3	Housewife group, Raman, Yala	3	ST3.1
			ST3.2
			ST3.3
Sa-tor-dong 4	Housewife group, Bannagsta, Yala	1	ST4.1
Sa-tor-dong 5	Fresh Market , Tanyong Mat, Narathiwat	3	ST5.1
			ST5.2
			ST5.3

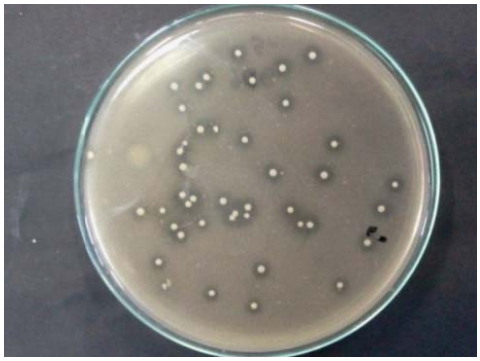

Fig. 2 LAB colony on MRS agar supplemented with $CaCO_3$

TABLE II
CHARACTERISTICS OF THE LAB ISOLATED

Isolate Name	Source of Isolates	Cell Morphology	Gram	Catalase test
ST 4.1	Sa-tor-dong	Cocci	+	-
ST 5.2	Sa-tor-dong	Rod	+	-
BD 1.1	Bodo	Cocci	+	-
BD 1.2	Bodo	Cocci	+	-
BD 2.1	Bodo	Cocci	+	-
BD 2.2	Bodo	Cocci	+	-
BD 2.3	Bodo	Rod	+	-
BD 3.1	Bodo	Cocci	+	-
BD 4.1	Bodo	Cocci	+	-
BD 1.1	Bodo	Cocci	+	-

B. Screening for Antimicrobial Activity

The antibacterial activity of 10 LAB isolates toward *Escherichia coli* was firstly determined by an agar well test. It was found that 2 isolates exhibited antibacterial activity. Two strains (ST 4.1, BD 2.3) showed inhibition zones diameters 4.25 and 2.0 mm, respectively. (Table III). *Escherichia coli* is Gram-negative bacteria with a thinner layer (10%of cell wall). Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space. The antibacterial action of lactic acid is largely, but not totally, assigned to its ability in the undissociated form to penetrate the cytoplasmic membrane [7]. Lactic acid is able to cause sub-lethal injury to *E. coli* [8]. Moreover, the result similar properties have also been assigned to acetic acid [9]. The ST 4.1 strain had inhibition zone larger than BD 2.3. Therefore, ST 4.1 was selected for some characterizes testing.

TABLE III
INHIBITION ZONE DIAMETER OF LAB ISOLATED ON *ESCHERICHIA* SP. AS INDICATOR

Isolated Number	Inhibition Zone (mm)
ST 4.1	4.25
BD 2.3	2.00

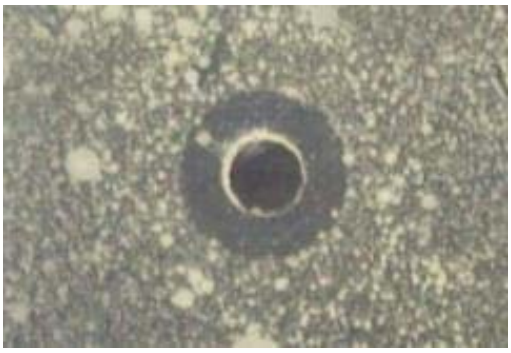

Fig. 3 Inhibition zone of isolated ST 4.1 on *Escherichia* sp.

C. Bile Salt, Sodium Chloride, Temperature and pH Tolerance

Regarding to the inhibition zone of isolated ST 4.1 was larger than BD 2.3. Therefore, ST 4.1 was selected for probiotic properties test. Isolated ST 4.1 was able to grow in bile salt concentration up to 7% (w/v) and also in concentration of sodium chloride up to 6.0% (w/v). Moreover, isolated ST 4.1 was able to grow at 15–45°C and pH 4-10. Our result related to Ahmad et al., 2007 which reported that five strains of the *Lactobacillus* sp. grew at 15–45°C, FM isolated was the most tolerant to high NaCl concentration up to 7.5% [10].

Moreover, isolated ST 4.1 was able to grow in bile salt concentration up to 7% (w/v). The bile salt concentration up to 7% (w/v) was suitable properties for starter cultures as they may tolerate to bile condition in duodenum. From this result shown high tolerance at low pH and bile salts considered as important selection criteria for probiotic. Bile salt hydrolase was an important enzyme of intestinal bacteria. Bile salt hydrolase activity may relate to ability of bacteria to survive and multiply in gastrointestinal tracts [11].

Therefore, lactic acid bacteria isolates with probiotic properties may be used as starter culture in fermented food. Consumption of food with live probiotic bacteria was beneficial to human health. Probiotic bacteria may mediate various health effects such as the decrease of cancer risk, improvement of the clinical outcome in many intestinal disease targets, and improvement of immune and mucosal barrier function [12].

IV. CONCLUSION

The isolate ST 4.1, found in local fermented food from southern of Thailand,that exhibited antimicrobial activity against *Escherichia coli*. Moreover, isolate ST 4.1 also displayed the effective probiotic properties including bile salt, sodium chloride and pH tolerance. Isolate ST 4.1 could potentially be used as fermented food starter cultures and also could provide significant health benefits and enhance safety of the products as natural antimicrobial agent.

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