Isolation and Susceptibility to Antibiotics of Lactic Acid Bacteria from Fermented Beef Product (*Mum*)

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Abstract: Important properties of beneficial microbes, e.g. lactic acid bacteria (LAB), characteristics and antimicrobial susceptibility of the microbes in fermented food products were concerned in several countries. The aim of this study was to determine the antimicrobial susceptibility of LAB strain isolated from Thai traditional fermented beef product, *mum*. The concentration of this LAB was also studied. Antimicrobial susceptibility and resistance of 14 representative LAB strains to 8 antibiotics were investigated using the disk diffusion method. The antibiotic used for the tests included Penicillin, Ampicillin, Erythromycin, Tetracycline, Vancomycin, Streptomycin, Sulfamethoxazole-trimethoprim and Metronidazole. The average concentration of LAB, of which the product was fermented at room temperature for 2 days, was 2.5×10^{11} CFU/g of product. The inhibition zone diameters of all antibiotics were between 0 mm and 30 mm for all the LAB strains isolated and tested. All 14 LAB isolates were resistant to Vancomycin, Streptomycin, Sulfamethoxazole-trimethoprim and Metronidazole. The LAB strains were highly resistant to Tetracycline (11 isolates), to Penicillin (10 isolates) but showed low resistant to Ampicillin (4 isolates). All of 14 LAB isolates were sensitive to Erythromycin. Only 3 LAB isolates were sensitive to all 4 antibiotics (Penicillin, Ampicillin, Erythromycin and Tetracycline), which were commonly used in the treatment of bacterial infections for human and animals. These 3 LAB isolates could possibly be used as starter cultures in this fermented product.

Key words: Antimicrobial susceptibility, lactic acid bacteria, fermented beef product, *mum*.

1. Introduction

Thai traditional fermented foods are produced by natural fermentation from various foods included fishery product (*nam-pla*, *ka-pi*, *bu-du*), fermented fish (*pla-ra*, *pla-som*, *pla-chao*, *som-fak*, *pla-chom*), meat products (*nham*, *Sai-krog-prieo*, *mum*) and plant products (*naw-mai-dong*, *Phak-gard-dong*, *Miang*, *Khao-mak*, *khanom-jeen*) [1]. *Mum* is found in the north eastern part of Thailand. It is produced from shredded pork or beef meat with ground roasted gelatinous rice (*khaao khuaa*), salt and garlic. *Mum* is fermented at room temperature for 2 days. In Thailand,

lactic acid bacteria (LAB) are a large group of beneficial bacteria that produce lactic acid as an end product of the food fermentation. Currently, there is concern about the possible spread of antibiotic resistant from antibiotics used for inhibition of pathogenic microorganisms or from beneficial microbes occurred naturally in foods. LAB from fermented products may act as a reservoir of antibiotic resistance genes that could be transferred to pathogenic bacteria or normal flora in the gastrointestinal tract of human and animals [2]. Antibiotic resistance of LAB are of two characteristics: (i) natural or intrinsic resistance, being nontransmissible; (ii) acquired resistance, usually caused from bacterial mutation or may carry plasmid encoding of antibiotic resistance genes and potentially

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transmissible to other bacteria [3]. There is little information about the antibiotic susceptibility in commensally bacteria such as LAB isolated from fermented food products and no data exist about antibiotic susceptibility in LAB of fermented beef products, *mum*. The present studies were to isolate the LAB and to investigate their antibiotic susceptibility profiles isolated from the *mum* products.

2. Materials and Methods

2.1 Sample Collection and Isolation of Lactic Acid Bacteria (LAB)

In June 2010, twenty samples of fermented beef products, *mum* have been collected from local markets (along the road) in the area of Aumphur Pol, Khon Kaen Province. *Mum* was made from beef meat. Ten kilograms of shredded meat were grinded and then garlic, salts and ground gelatinous rice were added. It was fermented at room temperature for 2 days. Ingredient proportions of the *mum* product are shown in Table 1. Samples were kept in air-tight plastic bags and were also kept at 4 °C. To find the volume of lactic acid bacteria, the samples were divided into portions of 5 grams that were dissolved into 45 milliliters of solvent Maximum Recovery Diluents (MRD) (Oxoid Inc., Hampshire, UK) and agitated for 30 s. Serial 10-fold dilutions from the homogenate were made according to ISO-6887-1 [4] and plated in De Man Rogosa and Sharpe (MRS) agar [5] with modification by added 0.4% (w/v) CaCO₃ using the pour plate method. The incubation was carried out aerobically for 2 d at 37 °C as described by ISO-15214 [6]. Bacterial colonies were enumerated by produced acid which clear zone surrounding from the plate that grew 30-300 colonies. Bacterial concentrations calculated (volume

of bacterial colonies × dilution factor) and expressed as colony forming unit per gram of sample (CFU/g). The different morphologies of bacterial colonies from each sample were picked up and stored in a modified freezing medium (Trypticase Soy Broth (Criterion™), 0.6% yeast extract and 20% glycerol) at -70 °C for further test.

Cell morphologies of bacteria were checked using Gram staining. The isolates were tested a catalase reaction. Rod and coccal cells of bacteria showed catalase negative and gram positive were characterized as LAB [7] and they were selected for further test.

2.2 Antimicrobial Susceptibility Test

The representative LAB isolates were further tested for antimicrobial susceptibility by the disk diffusion method [8]. Eight antibiotics commonly used in treating human or animal infections [9] and provided different antibiotics classes were chosen for the test. They were penicillin G (10 µg), ampicillin (10 µg), erythromycin (15 µg), tetracycline (30 µg), vancomycin (30 µg), streptomycin (10 µg), sulfamethoxazole-trimethoprim (25 µg) and metronidazole (50 µg). All antibiotic disks (diameter = 6 mm) were obtained from Oxoid (Oxoid, England).

The antimicrobial susceptibility test was similar to those as described in Ref. [10]. Briefly, each LAB isolate was inoculated with 10⁸ CFU (turbidity of 0.5 Mac Farland standards) at 37 °C in MRS broth and incubated anaerobically for 18 h. Culture solution was dipped using sterile cotton swap and swabbed in three directions on Mueller-Hinton agar plates. All antibiotic disks were seeded in the plates and incubated anaerobically at 37 °C for 48 h. The diameters of antibiotic inhibition zones were measured using a ruler under a colony counter apparatus (Gallenkamp, England) and expressed in millimeters which included diameter of antibiotic disk. Antimicrobial susceptibility interpreted according to the cut-off levels proposed in Ref. [10] with strains considered resistant if inhibition zone diameters were

Table 1 Ingredients used in *mum* processing.

Ingredients	Total weight
Beef minced	10 kilograms
Garlic	1 kilogram
Salts	100 grams
Ground roasted gelatinous rice	100 grams

equal to or smaller than 19 mm for penicillin G and ampicillin, 14 mm for vancomycin and tetracycline, and 13 mm for erythromycin. Equal to or smaller than 8 mm of inhibition zone diameters for streptomycin, sulfamethoxazole-trimethoprim and metronidazole were considered as resistant, according to the cut-off levels with minimal modifications of Ref. [11]. All antibiotics were tested in duplicate.

3. Results and Discussion

3.1 Lactic Acid Bacteria Isolation

Lactic acid bacteria were successfully isolated from the fermented beef products, *mum* using a selective medium of MRS agar and modification by the added 0.4% (w/v) CaCO₃. They showed clear zone appearance surrounding bacterial colonies. These clear zone colonies were because of the reaction of acid production from them. However, these colonies were confirmed as LAB using cell morphologies, Gram staining and catalase reaction. There were researchers who had attempted to develop the selective medium for lactobacilli [12]. In this study, the selective medium (MRS agar) was developed easily to isolate the LAB. Characteristics of 20 bacteria isolates and concentration of 14 representative LAB strains are shown in Table 2 and Table 3, respectively.

3.2 Antimicrobial Susceptibility

Results of antimicrobial susceptibility are shown in Table 4. All LAB isolates in this study showed resistance to vancomycin, sulfamethoxazole-trimethoprim, metronidazole and aminoglycoside antibiotics (streptomycin). These LAB strains presented intrinsic mechanisms of resistance for these antibiotics. All LAB strains in this study were resistant to metronidazole as similar report of Ref. [2]. This antibiotic resistance was usually intrinsic character. Although LAB strains are species-dependent intrinsic resistance to sulfamethoxazole-trimethoprim [13], all LAB strains

Table 2 Characteristics of 20 bacteria isolates isolated from the fermented beef products, *mum*.

Bacteria isolates	Cell form	Gram staining	Catalase
M1	Rods	+	-
M2	Rods	+	-
M3	Rods	+	-
M4	Rods	+	-
M5	Rods	+	-
M6	Rods	+	-
M7	Rods	+	-
M8*	Rods	-	-
M9	Rods	+	-
M10*	Rods	+	+
M11	Rods	+	-
M12	Rods	+	-
M13*	Cocci	+	+
M14	Rods	+	-
M15*	Rods	-	-
M16*	Rods	+	+
M17	Rods	+	-
M18	Rods	+	-
M19	Rods	+	-
M20*	Cocci	+	+

+: Positive reaction; -: Negative reaction; *: Isolates were excluded from the basic properties of LAB.

Table 3 Concentration of 14 representative LAB strains isolated from the fermented beef product, *mum*.

Number of sample	Average number of colony ¹ at various dilutions			Average LAB count (CFU/g)
	10 ⁻⁸	10 ⁻⁹	10 ⁻¹⁰	
14	nc ²	310	25	2.5 × 10 ¹¹

¹ count with triplicate plates.

² numerous colonies.

in our study showed intrinsic resistance to sulfamethoxazole-trimethoprim which they may have some antagonistic components such as *p*-aminobenzoic acid and thymidine [14]. All LAB strains in this study showed intrinsic resistance to vancomycin, these strains were due to the presence of D-alanine ligase and this enzyme could be inactivated vancomycin activity [15]. Acquired antibiotic resistances in some LAB strains in this study showed resistant to penicillin, ampicillin and tetracycline. These strains may receive the antibiotic resistance that spread out in bacteria from bacterial treatments of animal infections. This study showed low resistance to

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Table 4 Interpretative zone diameter (mm) with eight antibiotics of 14 representatives LAB strains selected from fermented beef product, *mum*.

Bacteria strains	Antibiotics							
	P (10 µg)	AMP (10 µg)	E (15 µg)	TE (30 µg)	VA (30 µg)	S (10 µg)	SXT (25 µg)	MTZ (50 µg)
M1	17.5 (R)	21 (MS)	23 (S)	13 (R)	0 (R)	7 (R)	0 (R)	0 (R)
M2	17 (R)	17 (R)	23 (S)	12 (R)	0 (R)	0 (R)	0 (R)	0 (R)
M3	30 (S)	30 (S)	26 (S)	17 (S)	0 (R)	0 (R)	0 (R)	0 (R)
M4	22 (S)	17 (R)	25 (S)	8 (R)	0 (R)	0 (R)	0 (R)	0 (R)
M5	17 (R)	28 (S)	24 (S)	14 (R)	0 (R)	0 (R)	0 (R)	0 (R)
M6	17.5 (R)	20 (MS)	25.5 (S)	8 (R)	0 (R)	8 (R)	0 (R)	0 (R)
M7	28 (S)	28 (S)	25 (S)	18 (S)	0 (R)	0 (R)	0 (R)	0 (R)
M9	17 (R)	28 (S)	23 (S)	14 (R)	0 (R)	0 (R)	0 (R)	0 (R)
M11	18 (R)	17 (R)	23 (S)	13 (R)	0 (R)	8 (R)	0 (R)	0 (R)
M12	29 (S)	29 (S)	26 (S)	17 (S)	0 (R)	0 (R)	0 (R)	0 (R)
M14	18 (R)	16 (R)	23 (S)	13 (R)	9 (R)	8 (R)	0 (R)	0 (R)
M17	17.5 (R)	21 (MS)	23 (S)	14 (R)	8 (R)	7 (R)	0 (R)	0 (R)
M18	19 (R)	23 (MS)	24 (S)	14 (R)	8 (R)	7 (R)	0 (R)	0 (R)
M19	19 (R)	24 (MS)	24 (S)	14 (R)	9 (R)	8 (R)	0 (R)	0 (R)

Susceptibility expressed as (R), resistant; (MS), moderately susceptible; (S), sensitive; susceptible.

P = penicillin; AMP = ampicillin; E = erythromycin; TE = tetracycline; VA = vancomycin;

S = streptomycin; SXT = sulfamethoxazole-trimethoprim; MTZ = metronidazole.

penicillin (71.43%) than the study of Savadogo et al. [16] who reported that LAB strains isolated from Faso fermented milk showed 78.94% of penicillin resistances. *Lactobacillus* spp. isolated from chicken feces has been reported at 36.37% of tetracycline resistances [17]. In this study, LAB isolated from *mum* showed highly resistant to tetracycline (78.57%). The selected 3 LAB isolates (M3, M7, M12) were intrinsic resistance to vancomycin, sulfamethoxazole-trimethoprim, metronidazole and streptomycin but they were sensitive to all 4 antibiotics (Penicillin, Ampicillin, Erythromycin and Tetracycline), which were commonly used in the treatment of bacterial-infections for human and animals. Therefore, these 3 LAB isolates could be safely applied and could possibly be used as starter cultures in this fermented product.

4. Conclusion

Although lactic acid bacteria are a large group of bacteria isolated from traditional fermented beef product (*mum*) in Thailand, there are only some strains that are sensitive to antibiotics commonly used

in animals. The selection of these bacterial strains used for the starter culture in food fermentation is an alternative that will give consumers confidence and not to eat products containing lactic acid bacteria to be the reservoirs transferred an antibiotic resistance genes to other bacteria. Further research, should be tested for sensitivity to the antibiotics in the other traditional Thai fermented foods that from animal origins including *pla-som*, *pla-chom* and *nham*.

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