SCREENING OF BACTERIOCIN-PRODUCING BACTERIA ASSOCIATED IN TRADITIONAL THAI FERMENTED BEEF (MUM)

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Introduction

Lactic acid bacteria (LAB) are widely used as starter cultures for reliable and consistent acid production in various fermented foods. The inhibition of other microorganisms may also occur by the formation of various compounds, which are produced during fermentation. Among the variety of the inhibitory compounds synthesized by these LAB, bacteriocins have received much attention in the past decade. Mum is a kind of traditional thai fermented meat, which is normally made from minced beef, raw cow spleen and cow liver, cooked together with salt, garlic and cooked rice, mixed well and stuffed into cow's intestine. The product is left to ferment at room temperature for 3 days. The most important microorganisms during the spontaneous fermentation of this product belong to the LAB genera *Lactobacillus plantarum* and *Pediococcus cerevisiae* (Somathiti and Surapantapisit, 1987). Numerous reports about bacteriocin-producing LAB isolated from another Thai fermented meat products, such as Nham (Swetwiwathana, 2005), have applied these LAB as starter cultures to harm various pathogens during fermentation. The objective of the study was to isolate bacteriocin-producing LAB with potential to use as starter cultures to increase the microbiological safety of Mum. The brief characterisation of bacteriocins, such as inhibitory spectrum, identification using carbohydrate fermentation kit and microscopic identification, are also reported in this paper.

Materials and Methods

Isolation of bacteriocin-producing bacteria from Mum: In order to search for bacteriocinogenic bacteria, a total of 100 strains were randomly isolated from 10 samples of Mum sold in Khonkaen province by spread plate technique on MRS agar + 0.5 % calcium carbonate and incubated under micro-aerobic condition (candle jar) at 30°C for 48 h. Each colony with a clear zone was selected for the detection of antagonistic activity. All strains were precultured overnight in MRS broth (Oxoid) at 30°C before using to screen for bacteriocin production (De Man *et al.*, 1960).

Bacteriocin screening medium: A special bacteriocin screening medium (BSM), which was developed on the basis of MRS medium, was used as the bacteriocin screening medium for all isolates (Tichaczek *et al.*, 1992).

Determination of antagonistic activity: The direct method was performed as described by Fleming *et al.*, (1985). Evaluation of bacteriocin-producing strains was studied using the methods described by Tichaczek *et al.*, (1992) and Ennahar *et al.*, (1999) with 6 indicators (Table 1). Antimicrobial producers were examined after 24 hours for zones of inhibition. The most potent strains, which showed the best inhibitory spectrum to all 6 tested indicators, were selected for confirmation of their bacteriocins production in MRS broth with various indicators as described by Ennahar *et al.*, (1999) (Table 3) and compared to known bacteriocin-producer strains such as pediocin PA-1 producer strain of *P. pentosaceus* TISTR 536 (Swetwiwathana, 2005).

Identification of suspect bacteriocin-producing strains: Suspect bacteriocin-producing isolates were identified based on carbohydrate fermentation patterns using API 50 CHL kit test (bioMorieux Vitek, Inc., Hazelwood, Mo.). Cell morphology of each isolate was studied using a Gram stain. A catalase test for each strain was performed as recommended by Schillinger and Luecke, (1989).

Results and Discussion

Eight of 100 strains were found to produce antagonistic compounds against several indicators (Table 1). Only 2 strains M 13-5 and M 20-4 showed bactericidal board spectrum on all 6 tested indicators. Among these potent strains, M 13-5 was the only strain that exerted the best bactericidal board spectrum on *Lis. innocua*. Thus, these 2 aforementioned strains were selected for further characterisation. From the results of the catalase test, cell morphology and carbohydrate fermentation using API 50 CHL kit test of 2 selected isolates (Table 2), it can be concluded that there are at least 2 groups of suspected bacteriocin-producers of LAB isolated from Mum. One is M 13-5 which showed 99.8 % of identity to *P. pentosaceus* , and the other is M 20-4 which showed 99.9 % of identity to *Lb. plantarum* in API 50 CH database. These 2 groups of isolates, however, are currently being identified by 16S rDNA sequencing method.

In order to confirm the 'bacteriocin' definition of the product of these 2 isolates, inhibitory spectrum profiles of the antagonistic products were compared to the spectrum of a known pediocin PA-1 producer (Table 3). Most results were similar to the known pediocin PA-1 producer, therefore it can be assumed that M 13-5 is *P. pentosaceus* a group of bacteriocin-producers whose product is related to pediocin. The products from the other group of *Lb. plantarum* (M 20-4) exhibited a more narrowed spectrum than M 13-5 and might be an antilisterial bacteriocins. Both of these isolated LAB, are currently been investigated further by 16S rDNA sequences, for strain confirmation by bacteriocin

purification and identification. Further studies are being conducted to improve the quality and safety of these products in Mum production.

Table 1: Preliminary screening results of antagonistic substances produced by 8 of 100 strains isolated from Mum against 6 indicators using direct method.

Indicator strain	M 13-2	M 13-5	M 15-3	M 15-4	M 15-8	M 16-6	M 17-5	M 20-4
Lb. sakei (JCM1157^T)	+	+	+	+	+	-	+	+
Lb. plantarum ATCC14917^T	+	+	-	-	+	-	+	+
Lis. innocua ATCC33090^T	+	++	+	+	+	+	+	+
Staph. aureus ATCC12600	-	+	+	-	-	+	-	+
E. coli JM 109	+	+	+	+	+	+	+	+
Ent. faecalis JCM 5803 ^{T}	+	+	-	+	+	+	+	+

- = no inhibition (0-0,9 mm), + = inhibition zone 1-5 mm., ++ = inhibition zone 6-10 mm
ATCC, American Type Culture Collection, Rockville, Md; JCM, Japanese Culture of Microorganisms, Japan; JM, commercial strain from Toyobo, Osaka, Japan

Table 3: Antimicrobial spectrum of BLIS from isolated LAB from Mum.

Indicator[a]	TISTR 536	M 13-5	M 20-4
P. pentosaceus JCM 5885	0	0	0
Lb. plantarum ATCC 14917^T	800	800	200
Lb. sakei subsp. *sakei* JCM 1157^T	3,200	3,200	800
Lc. lactis subsp. *lactis* ATCC 19435 ^{T}	0	0	0
Lc. lactis subsp. *lactis* JCM 7638	0	0	0
Lc. lactis subsp. *cremoris* TUA 1344L	800	800	0
Leuconostoc mesenteroides JCM 6124^T	400	400	0
Micrococcus luteus IFO 12708	800	800	0
Listeria innocua ATCC 33090 ^{T}	12,800	25,600	400
Enterococcus faecalis JCM 5803 ^{T}	1,600	1,600	100
Staph. aureus ATCC 12600	0	0	0
Bacillus circulans JCM 2504^T	0	0	0
B. coagulans JCM 2257^T	0	0	0
B subtilis JCM 1465 ^{T}	0	0	0
Escherichia coli JM 109	0	0	0
TISTR536	0	0	0
A 5	0	0	0
M 13	0	0	0

[a] ATCC, American Type Culture Collection, Rockville, Md; JCM, Japanese Culture of Microorganisms, Japan; JM, commercial strain from Toyobo, Osaka, Japan; TUA, Tokyu University of Agriculture, Japan; IFO, Institute for Fermentation, Osaka, Japan; TISTR 536, the pediocin PA-1 producer strain (*P. pentosaceus*) [8]

Table 2: Catalase test, morphology and carbo-hydrate fermentation (API 50 CHL) of the most potent strains.

Test	M 13-5	M 20-4
Catalase	-	-
Cell morphology	tetrad cocci	long rod
Gram stain	+	+
API 50CHL (percentage of identity)	*P. Pentosaceus* (99.8 %)	*Lb. plantarum* (99.9 %)

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