Identification and partial characterization of pediocin PA-1 producing *Pediococcus pentosaceus* associated in traditional Thai fermented beef (Mum)

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Introduction

Mum is a kind of traditional thai fermented meat, which is normally made of minced beef, raw spleen and liver from cow, cooked salt, garlic and cooked rice, mixed well and stuffed in cow's intestine. The product is left to ferment at the 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* (Piayura et al., 2006). According to numerous reports on bacteriocin-producing LAB isolated from another Thai fermented meat products such as Nham (Swetwiwathana, 2005), which were applied these LAB as starter cultures to harm various pathogens during fermentation, were reported. Thus, the isolation of bacteriocin-producing LAB with potential to use as starter for increasing the microbiological safety of Mum was studied (Piayura et al., 2006). The paper reported that M 13-5 was only the strain that exerted the best bactericidal board spectrum on *Lis. innocua*. By the preliminary study of using API 50 CHL carbohydrate fermentation kit test, M 13-5 showed 99.8 % of identity to *P. pentosaceus* in API 50 CH database. With the coincidence of most results to the known pediocin PA-1 producer , it is assured that prior identify as *P. pentosaceus* of M 13-5 was a group of bacteriocin-producers and its produces are related to pediocin.

According to the report on antagonistic substances produced from LAB strain M 13-5 and its antagonistic spectrum related to pediocin, thus, this study was conducted to identify the strain of by using 16S rDNA sequence analyses. In addition, identification of bacteriocins using PCR analysis and DNA sequencing of bacteriocin from this strain was also reported in the study.

Materials and Methods

Identification of M 13-5 by 16S rDNA sequence analyses : Partial phenotypic characterization of the strain was performed by firstly preparing overnight cultured of M13-5 in MRS broth. 2 ml was harvested by centrifugation. The cells were then resuspended in 80 µl of TE buffer (50 mM Tris, 50 mM EDTA, pH 8). Lysis was initiated by the addition of 5 mg/ml lysozyme. After incubation at 37° C for 1 h, the mixture was further provided with MagExtracter-Genome (TOYOBO) as specified by manufacturer. 16S rDNA gene was applified from genomic DNA using primer 8UA (5'- AGAGTTTGATCCTGGCTCAG –3') and 1510B (5'- GTGAAGCTTACG GCTACCTTGTTACGACTT –3') based on primers described by Martinez-Murcia et al. (1995). Reaction [50 µl contained 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$ and 0.1% Triton X-100, TOYOBO Co. Ltd.), 200 µmol of each deoxynucleotide triphosphate (TOYOBO Co. Ltd.), 20 pmol of primer and 1 U of Taq polymerase (TOYOBO Co. Ltd.)] was set up in a tube containing 200 ng of template DNA and genomic DNA amplification was then performed using Astec program temp control system PC-800. The program was 3 min at 94° C for 1 cycle followed by 30 cycles of 94° C at 30 sec, 55° C at 30 sec and 72° C at 1 min. The additional step for extending incomplete products was performed at 72° C for 5 min. PCR product was then purified by QIAquick PCR Purification kit (QIAGEN, Germany). The purified product was sent for DNA sequencing by a commercial DNA sequencing company (Macrogen, Seoul, Korea). The results from DNA sequencing were analyzed using GENETYX-WIN software (GENETYX, Tokyo, Japan). Database searches were performed using BLAST of the National Center for Biotechnology (NCBI, http://www. ncbi.nlm.nih. gov/ BLAST/).

Bacteriocins purification : The cell-free supernatant of 1 liters culture incubated at 30° C of M 13-5 was purified by a four step procedures as described by Ennahar *et al.* (1999). The final sample containing the purified bacteriocins was dried by Speed-Vac rotary evaporator (Savant Instruments) and stored at –20°C for molecular mass determination.

Mass spectrometric : The molecular masses of purified bacteriocins were determined using a Accu TOF spectrometer, model JMS-T100LC (Agilent Technologies, Germany).

PCR analysis and DNA sequencing of bacteriocin genes from bacteriocin-producing M 13-5 : The total DNA of M 13-5 was isolated by using the method described by Anderson and McKay (1983). Pediocin PA-1 primers designed and synthesized (Hokkaido System Science Co. Ltd., Hokkaido, Japan) for PCR amplification are Pedi-1F (5'–GAGTGGGAACTAGAATAAGCGCGTA–3') and Pedi-1R (5' –TTACTCTTATTCATAAAATCACCCC –3'). DNA analyses and sequence alignments were carried out using GENETYX-WIN software (GENETYX,

Tokyo, Japan). Database searches were performed using BLAST of the National Center for Biotechnology (NCBI, http://www.ncbi.nlm.nih.gov/BLAST/). Alignment analysis of peptides was performed using ClustalW of the DNA Data Bank of Japan (DDBJ, http://www.ddbj.nig.ac.jp/search/clustalw-e.html).

Results and Discussion

Identification of M 13-5 by 16S rDNA sequence analyses : The result of about 1500 bp. 16S rDNA sequences from M 13-5 showed 98 % identity to *P. pentosaceus* (Fig.1). This 16S rDNA sequence result was concurred to the earlier results of carbohydrate fermentation kit test of API 50 CHL (Swetwiwathana et al., 2006), which informed that M 13-5 was related to *P. pentosaceus* (99.8 % of identity).

gi [82393805] gb [DQ267152.1]
***Pediococcus pentosaceus* 16S ribosomal RNA gene. Partial sequence Length = 1547**

Score = 1697 bits (856), Expect = 0.0
Identities = 901/912 (98 %),
Gaps = 3/912 (0 %)
Strand = Plus/Plus

Figure 1 : Partial 16S rDNA sequence of bacteriocin-producing strain M 13-5

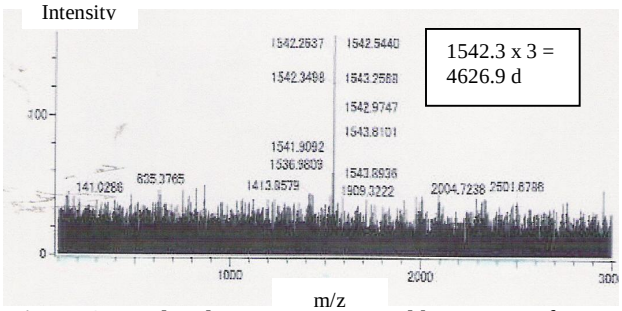

Figure 2 : Molecular mass of purified bacteriocin fraction from M 13-5 by Accu-TOF spectrometer

The molecular mass of purified bacteriocin about 4,626.9 d from M 13-5 (Fig. 2) was implied the similarity to pediocin PA-1 (4,623 d) produced by *P. acidilactici* PAC1.0 (Marugg et al., 1992) and *Lb. plantarum* WHE 92 (Ennahar et al., 1996). By these results, we are realized that this *P. pentosaceus* strain M 13-5 must be a pediocin PA-1 producer strain isolated from Mum.

PCR analysis and DNA sequencing of bacteriocin genes from bacteriocin-producing M 13-5 : In order to prove that the bacteriocins produced by *P. pentosaceus* M 13-5 was pediocin PA-1, PCR analysis using the known sequences of the pediocin structural genes was performed. The expected 300 bp fragments containing the structural genes of pediocin PA-1 of M 13-5 strain were amplified and then sequenced (Fig. 3). The results indicated that the sequence from M 13-5 strain was 100% identical to that of pediocin PA-1. Thus, it is concluded that the isolated strain of *P. pentosaceus* M 13-5 from thai traditional fermented beef (Mum) is a pediocin PA-1 producer. This strain can be used as a starter culture for microbiological improvement of this type and related thai traditional fermented meat production.

Identities = 61/61 (100%), Positives = 61/61 (100%) Score = 138 bits (347), Expect = 7e-32
Query : KKIEKLTEKEMANIIGGKYYGNGVTCGKHSCSVDWGKATTCIINNGAMAWATGGHQGNHK
: KKIEKLTEKEMANIIGGKYYGNGVTCGKHSCSVDWGKATTCIINNGAMAWATGGHQGNHK
Subject : KKIEKLTEKEMANIIGGKYYGNGVTCGKHSCSVDWGKATTCIINNGAMAWATGGHQGNHK

Figure 3 : Nucleotide sequence and deduced amino acid sequence of the pediocin PA-1 gene isolated from *P. pentosaceus* M13-5.

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