A novel pediocin-like bacteriocin produced by *Lactobacillus plantarum* KMITL-QU 54 isolated from traditional thai fermented meat-rice sausage (Sai-krog Isan)

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Abstract

A total of 150 strains of lactic acid bacteria (LAB) were randomly isolated from the 15 samples of traditional thai fermented meat-rice sausage (Sai-krog Isan) sold in Bangkok and screened for bacteriocin-producing strains. Only the LAB strain KMITL-QU 54, which implied to produce bacteriocin and displayed the strongest antilisterial effect as pediocin, was later identified at the DNA level as *Lactobacillus plantarum*. Molecular mass of the purification to homogeneity of the bacteriocin produced by this strain showed that it was 4,689 Da, which was about 60 Da higher than pediocin AcH/PA-1 (4,628 Da) produced by known pediocin producers of *Pediococcus acidilactici* H and *Lb. plantarum* WHE 92. The study of pediocin producing structural gene of this pediocin-like producing strain revealed that *Lb. plantarum* KMITL-QU 54 exhibited 96% and 94% of the structural genes identical to pediocin family of Coagulin (CoaA) produced by *Bacillus coagulans* I_4 and pediocin AcH produced by *Pediococcus acidilactici* H, respectively. The molecular weight calculation of the pediocin-like structural sequence genes from KMITL-QU 54 informed that its molecular weight was about 4,691 Da and more related to the prior purified bacteriocin (4,689 Da) than coagulin (4,615 Da) and Pediocin AcH/PA-1 (4,628 Da). By these results, hence, we realize that *Lb. plantarum* KMITL-QU 54 might be produced a novel pediocin-like bacteriocin and the strain could be applied as starter for microbiological quality enhancement during this traditional thai fermented meat product fermentation.

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 produces during fermentation. Among the variety of these inhibitory compounds synthesized by these LAB, bacteriocins have been received much attention in the past decade (Ennahar et al., 1996; Gaenzle et al., 1997; Swetwiwathana, 2005). Thus, an attempt on finding the most potent bacteriocin-producing LAB strains from fermented meat-rice sausage (Sai-krog Isan) and use of these potent bacteriocin producing strains as starter cultures in order to improve the quality and safety during the fermentation of this product had been studied and reported (Swetwiwathana et al., 2007). Only 2 strains of *Lactobacillus plantarum* RS-49 and RS-54 had been reported to produce bacteriocin which were shown the same spectrum and activity against the tested indicators. But the spectrum of their bacteriocins was different from the known bacteriocin of nisin A and pediocin PA-1 which produced from *Lactococcus lactis* NCDO and *Pediococcus pentosaceus* TISTR 536, respectively. Hence, the further characterization of bacteriocin produced from RS-54 had been studied and reported in this paper. The code of strain had been changed from RS-54 to be KMITL-QU 54 according to the collaborative works between King Mongkut's institute of Technology Ladkrabang (KMITL) – Thailand and Kyushu University (QU) – Japan.

Materials and methods

Bacteriocins purification : The cell-free supernatant of 1 liters culture incubated at 30^{o} C of KMITL-QU 54 was purified by a four step procedures as described by Ennahar *et al.* (1996). The final sample containing the purified bacteriocins was dried by Speed-Vac rotary evaporator (Savant Instruments) and stored at –20^{o}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 KMITL-QU 54 : The total DNA of KMITL-QU 54 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

Molecular mass of bacteriocin produced by KMITL-QU 54 : Bacteriocin produced by *Lb. plantarum* KMITL-QU 54 was showed the molecular mass at about 4,690 d (Fig. 1). This molecular mass of bacteriocin from KMITL-QU 54 revealed about 67 d different from the molecular mass of those pediocins produced by *P. pentosaceus* TISTR 536 (produced pediocin PA-1, molecular mass about 4,623 d – Swetwiwathana, 2005) and *Lb. plantarum* WHE 92, which was reported to produce pediocin AcH (molecular mass 4,623 d; Ennahar *et al.*, 1996).

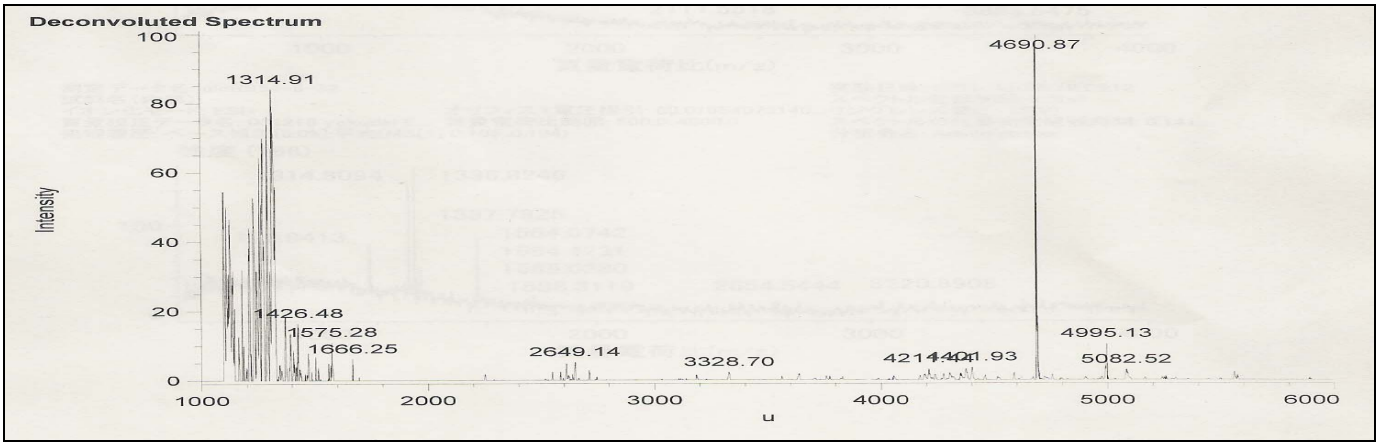

Figure 1. Molecualr mass of purified bacteriocin fraction from *Lb. plantarum* RS-54 .

The results of pediocin producing structural genes from KMITL-QU 54 (Fig. 2) informed that KMITL-QU 54 exhibited 96% of pediocin-like structural genes identical to CoaA (Coagulin) produced by *Bacillus coagulans* I_4 (Le Marrec *et al.*, 2000) and 94% of structural genes identical to pediocin AcH produced by *Pediococcus acidilactici*. The molecular weight calculation of this pediocin-like structural sequence genes from KMITL-QU 54 was later compared to both structural sequence genes from coagulin of *B. coagulans* I_4 and pediocin of *Pediococcus acidilactic* (Fig. 3). It was informed that the pediocin-like structural sequence genes of *Lb. plantarum* KMITL-QU 54 exhibited the related molecular weight to the purified bacteriocin (4,691.1 d). We are, hence, realized that the bacteriocin from KMITL-QU 54 might be a novel pediocin-like bacteriocin. This bacteriocin is under studying the best purification methods for further amino acid sequence step.

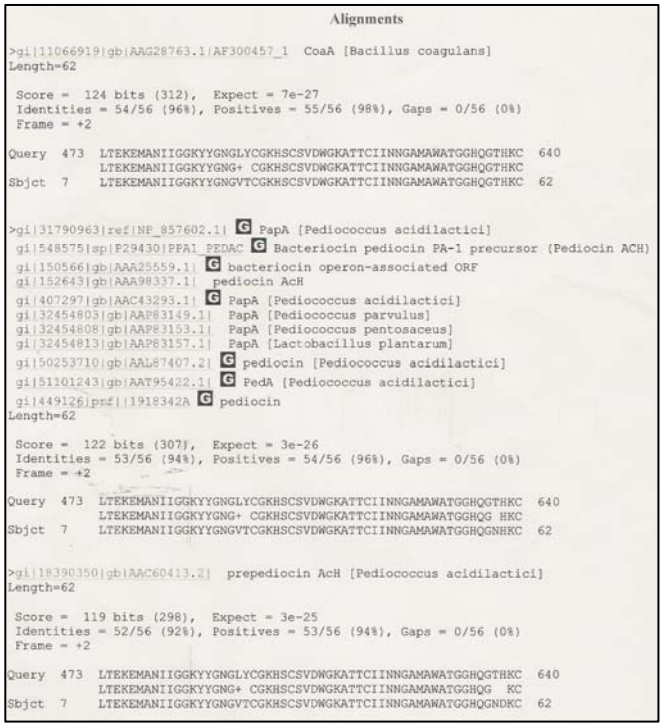

Figure 2. Pediocin-like structural genes of *Lb. plantarum* KMITL-QU 54.

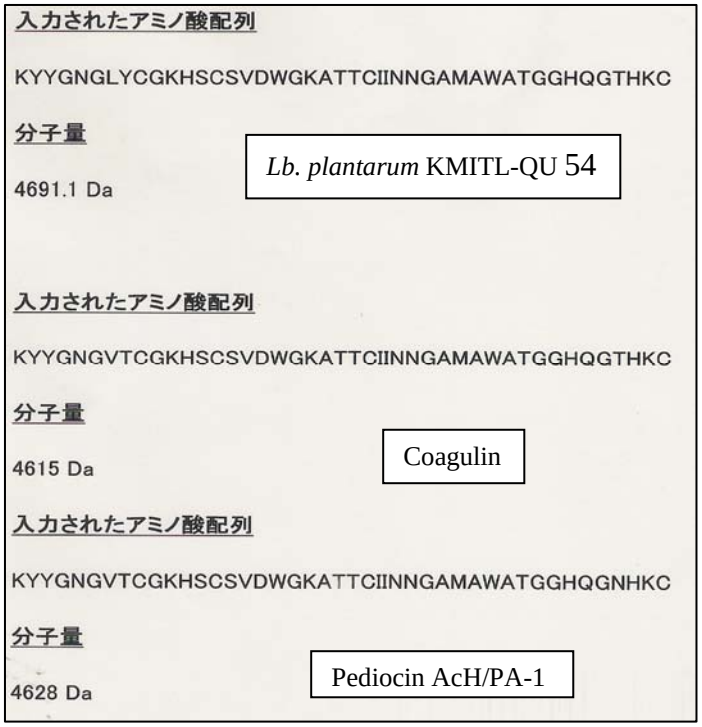

Figure 10. Comparison of molecular weight from pediocin-like structural sequence genes of *Lb. plantarum* KMITL-QU 54, coagulin and pediocin AcH/PA-1.

Conclusions

The study informs that *Lb. plantarum* KMITL-QU 54 might be produced a novel pediocin-like bacteriocin with MW about 4,691 d. Moreover, the strain could be applied as starter for microbiological quality enhancement during this traditional thai fermented meat product fermentation.

Acknowledgements

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