PE6.07 A novel bacteriocin from 2 peptides bacteriocin-producing weissella cibaria KMITL-QU 21 associated in traditional Thai fermented meat-rice sausage (Sai-krog Isan) 209.00

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***Abstract* – From our recently study on screening of bacteriocin-producing lactic acid bacteri (LAB) in traditional thai fermented meat-rice sausage (Sai-krog Isan) produced by one fermented meat industry in Bangkok. It revealed that only the LAB strain KMITL-QU 21 implied to produce bacteriocins and displayed the strongest effect on mostly gram-positive bacteria This strain was later identified at the 16s rDNA sequence level as *Weissella cibaria*. Bacteriocin activity produced by this strain was totally eliminated by exposure to high temperature (100° C) for 10 min at pH 7.0 and under autoclave condition (121° C) for 15 min at pH 3.0, 4.4 and 7.0. Reverse phase HPLC in the final purification step showed two active peptide peaks and mass ESI-MS analysis of each peptide showed their molecular wights to be 3,930 and 5,975 Da, respectively. N-terminal amino acid sequence analysis of both peptides was performed and implied that the amino acid sequence of the first peptide (MW 3,930 Da) was identical to a known bacteriocin of leucocin A-UAL 187, while the latter bacteriocin (MW 5,975 Da) yielded no amino acid, suggesting it to be blocked at N-terminus. According to it molecular weight, the latter one was suggested to be novel bacteriocin. This is also the first report of *W. cibaria* strain to produce leucocin A-UAL187 and 2 peptides bacteriocins. The further clarification of amino acid sequence and characterization of this latter bacteriocin as well as the applying as a biopreservative of these 2 peptides bacteriocins for traditional thai fermented meat are under studying. This is the first report of bacteriocin-producing *W. cibaria* strain associated in this traditional thai fermented meat rice sausage (Saikrog Isan).**

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Index Terms – Novel bacteriocin, 2 peptides bacteriocin, *Weissella cibaria*, fermented meat-rice sausage, Sai-krog Isan

I. INTRODUCTION

Sai-krog Isan, a traditional thai fermented meat-rice sausage, is normally made of minced pork, cooked rice, cooked salt, garlic and food additives, mixed well and stuffed tightly in edible casing. The product is left to ferment at the room temperature for 2-3 days. The most important microorganisms during the spontaneous fermentation of this product belong to the LAB genera Lactobacillus, Pediococcus and Micrococcus. According to numerous reports on using LAB and bacteriocin-producing LAB as starter cultures to harm various pathogens in fermented foods (1, 2) and traditional thai fermented meat such as Nham (3), thus, an attempt on finding the most potent bacteriocin-producing LAB strains from meat-rice sausage (Sai-krog Isan) and use of these potent strains as starter cultures in order to improve the quality and safety during the fermentation of this product was studied. Our earlier reported had informed that only one from 60 isolates of LAB from Sai-krog Isan designated as KMITL-QU 21 implied to produce bacteriocins and displayed the strongest effect on mostly gram-positive bacteria This strain was later identified at the 16s rDNA sequence level as *Weissella cibaria*. Thus, the partial characterization and identification of bacteriocins from *W. cibaria* KMITL-QU 21 were further studies and reported in this paper.

II. MATERIALS AND METHODS

1. Purification of bacteriocins produced by W. cibaria KMITL-QU 21

The cell-free supernatant of 1 liters culture incubated at 30° C of *W. cibaria KMITL-QU* 21 was purified by a four step procedures as described by Ennahar et al. (2). Inhibitory activity of each purified step was determined by using a surface spot method (SSM) against *Lactobacillus sakei* subsp. *sakei* JCM 1157 ^{T} as an indicator strain. 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.

2. Mass spectrometric and amino acid sequences of purified bacteriocins

The molecular masses of purified bacteriocins were determined using a Accu TOF spectrometer, model JMS-T100LC (Agilent Technologies, Germany). The N-terminal amino acid sequence of the purified bacteriocin was determined by Edman degradation using a PPSQ-21 gas-phase automatic protein sequencer (Shimadzu, Kyoto, Japan) as described by Iwatani et al. (4).

III. RESULTS AND DISCUSSION

Purification of bacteriocins produced by W. cibaria KMITL-QU 21

After the last purification step of reverse phase HPLC (RP-HPLC), the results (Fig. 1) revealed that there were 2 activities shown against an indicator of *Lb. sakei* subsp. *sakei* JCM 1157 ^{T} at 18 min for the first active fraction and between 27-30 min for the second active fractions. By these results, it was implied that the bacteriocins produced by *W. cibaria KMITL-QU* 21 might be 2-peptide bacteriocins. Thus, the purified fractions of these 2-peptide bacteriocins were further studied for their molecular mass and amino acid sequences.

Mass spectrometric and amino acid sequences of purified bacteriocins

Analysis of active purified fraction of bacteriocins from *W. cibaria KMITL-QU* 21 using a Accu TOF spectrometer revealed that the first active peptide exhibited molecular weight (MW) of 3,933 Da (Fig. 2) and the second active peptide showed MW around 5,975 Da (Fig. 3). Edman sequencing from the first 20 amino acids of the first active peptide and its molecular mass (Fig. 4) implied that this first active peptide was related to leucocin A-UAL187 (MW 3930.3 Da), which has been reported by Hastings et al. (5). The second active peptide of MW about 5,975 Da yielded no amino acid under Edman sequencing, suggesting it to be blocked at N-terminus. According to it molecular weight, which has not found in any report, this second active peptide was suggested to be novel bacteriocin. This is also the first report of *W. cibaria* strain KMITL-QU21 to produce leucocin A-UAL187 and 2 peptides bacteriocins.

IV. CONCLUSION

The study implied that *W. cibaria* strain KMITL-QU21 isolated from Thai traditional fermented meat-rice sausage (Sai-krog Isan) is a novel bacteriocin-producing strain to produce 2 peptides bacteriocins. One peptide is related to leucocin A-UAL187 with MW about 3933 Da. The other is expected to be a novel bacteriocin of N-terminus blocking group with MW about 5975 Da.

ACKNOWLEDGEMENTS

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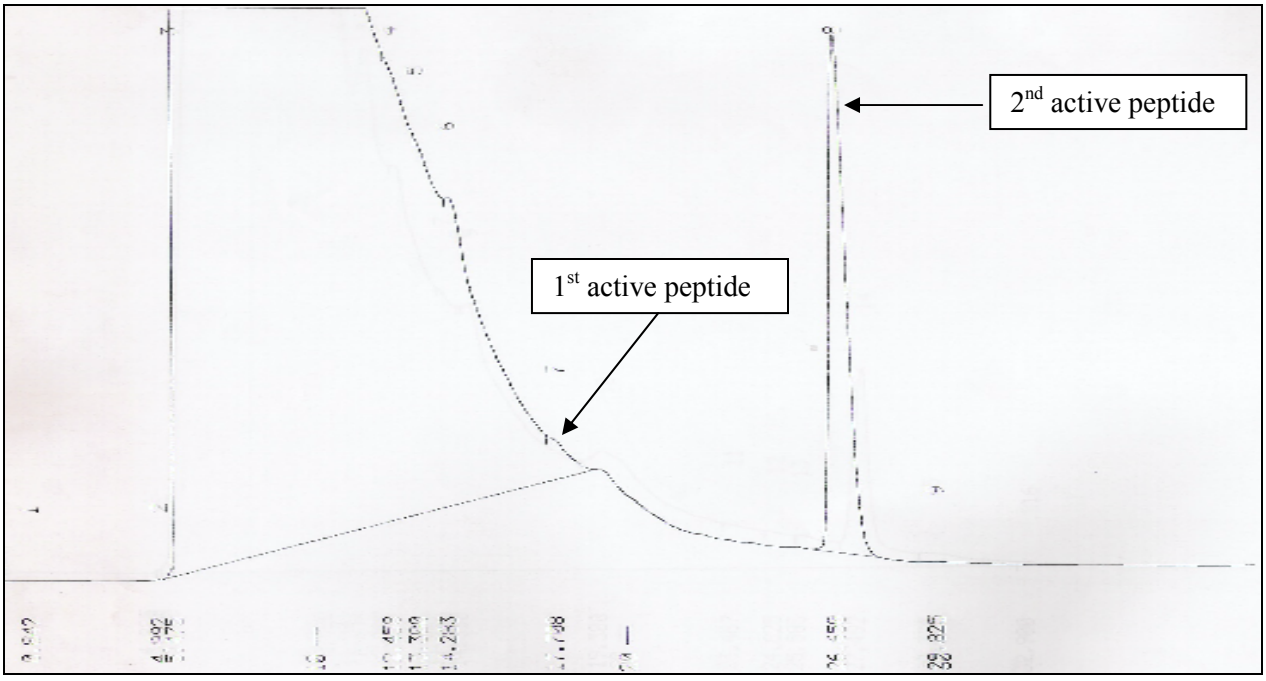

Fig. 1 Reverse-Phase Chromatography of Bacteriocins Produced by *W. cibaria* KMITL-QU21

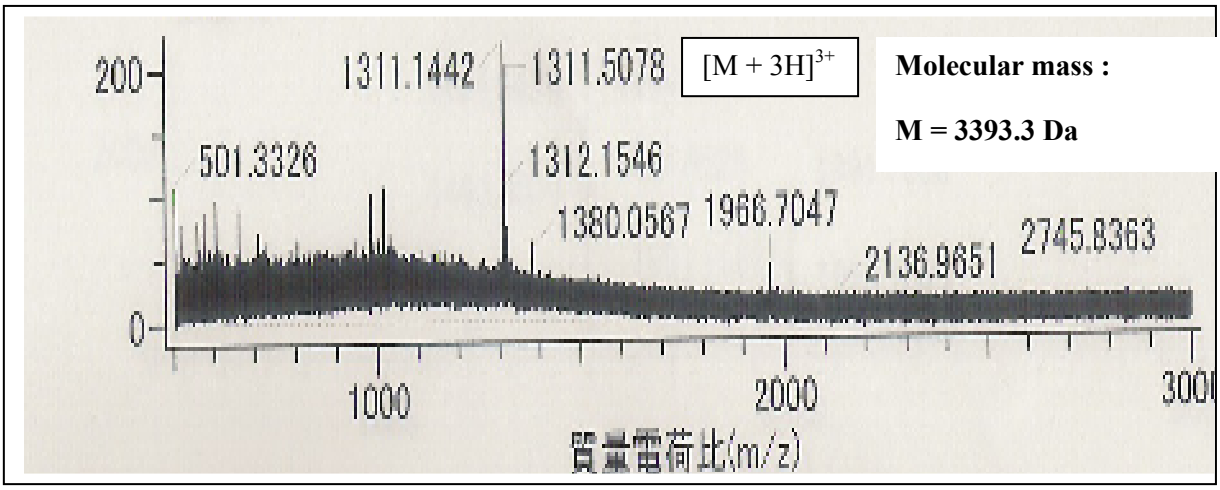

Fig. 2 ESI-TOF Mass Spectrum of Purified 1st Active Peptide

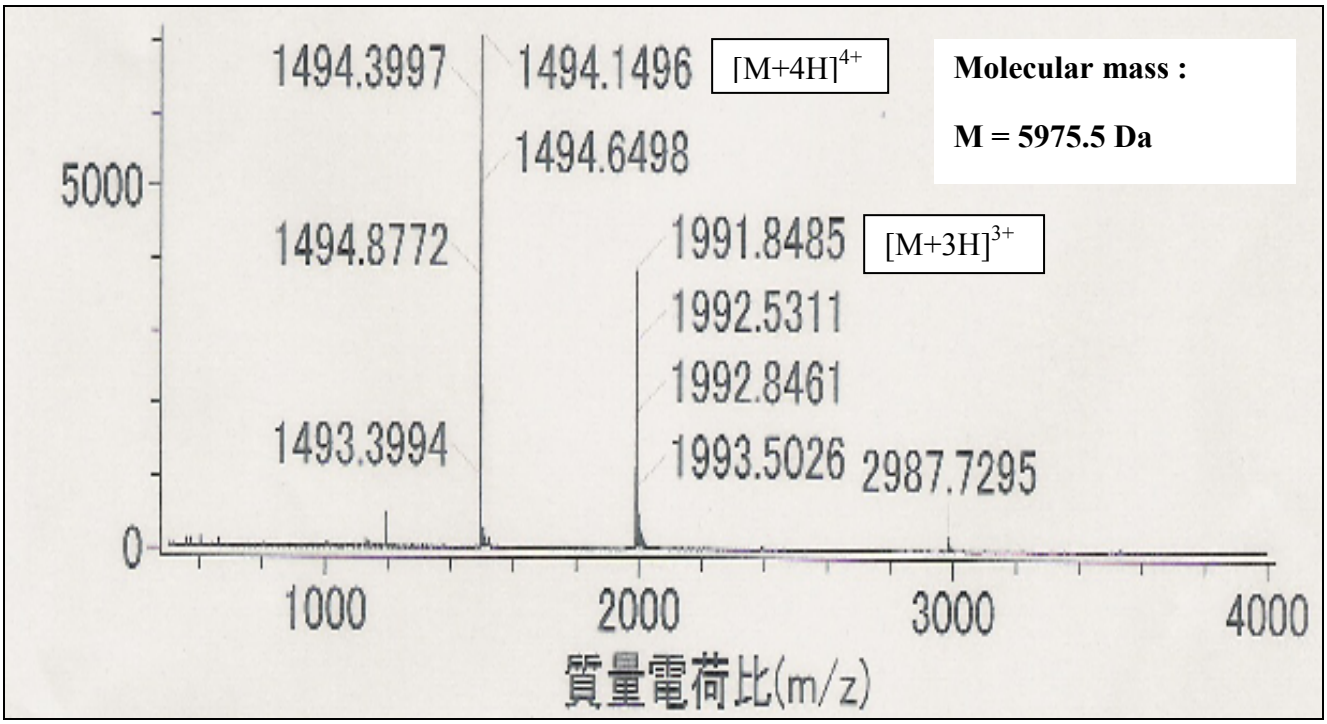

Fig. 3 ESI-TOF Mass Spectrum of Purified 2nd Active Peptide

	Putative amino acid sequence	Molecular mass
1st active peptide 3933 Da	**KYYGNGV XX TKSG XX VNWGE**	
Leucocin A-UAL187	**KYYGNGV HC TKSG CS VNWGE AFSAGV HRLANGGNGFW**	**3930.3 Da**

Fig. 4 Alignment of Partial Deduced Amino Acid Sequence of 1st Active Peptide Produced from *W. cibaria* KMITL-QU21 Compared to Known Alignment of Leucocin A-UAL187