P-02-33

Identification of bacteriocin-producing strain of *Lactobacillus plantarum* Ski2 from Thai traditional fermented meat-rice sausage (Sai-Krog Isan) (#535)

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Introduction

Sai-krog Isan (SI), Thai traditional fermented meat-rice sausage, is mostly consumed and sold in the whole country of Thailand. This product was mostly 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 lactic acid bacteria (LAB) genera Lactobacillus, Pediococcus and Micrococcus [1]. According to many reports on using LAB and bacteriocin-producing LAB as starter cultures to harm various pathogens in fermented foods [2, 3] and traditional Thai fermented meat such as Nham [4], thus, an attempt to find the most potent bacteriocin-producing LAB strains from SI and use of these potent strains as starter cultures in order to improve the quality and safety during the fermentation of SI was studied. From our earlier report, we found that the isolated Ski2 from SI revealed to produce bacteriocin [5]. The bacteriocin from this strain showed the different inhibition spectrum from nisin and pediocin PA-1 producers. Thus, this bacteriocin-producing Ski2 was further study to identify for the species identification by 16S rDNA sequences. Moreover, molecular weight (MW) of the purified bacteriocin from this strain was also investigated and reported in this paper.

Methods

1.Bacterial strain

Gram positive rod shape of Ski2, which was isolated from SI produced by S. Khonkaen [5], was stored at -80° C in MRS broth containing 15% (v/v) glycerol. Before use, the strain was cultivated twice for 24 h at 30° C in MRS broth.

2. Identification of the suspected bacteriocin-producingSki2 by 16S rDNA sequence

Partial phenotypic characterization of strain Ski2 was performed follow the procedures described by Ennahar et al. [3]. After overnight cultured of Ski2, the cells was centrifuged and lysis using lysozyme. The mixture was further provided with MagExtracter-Genome (TOYOBO) as specified by the manufacturer. 16S rDNA gene region of strain Ski2 was analyzed by PCR using primer 8UA and 1510B and PCR product was purified using a QIAquick PCR purification kit. Purified PCR product was used for DNA sequencing at Macrogen (Seoul, Korea). The obtained DNA sequences were analyzed using GENETYX-WIN software (GENETYX, Tokyo, Japan). Database searches were performed using BLAST of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/ BLAST/).

3. Liquid Chromatography/Mass Spectrometry (LC/MS) analysis

1-L of overnight cultured of Ski2 was done in MRS broth at 30 °C and crude bacteriocin in the cell-free supernatant from 18-h cultured was purified by a four step procedures as described by Ennahar et al. [3]. *Lactobacillus dextranicus* JCM 5887^T has been used as indicator strain to investigate the potent fraction of purified bacteriocin after reverse phase HPLC. The MW of the most potent purified bacteriocin from Ski2 was determined using LC/MS. The total ion chromatograms were taken in a mass range from *m/z* 500 to 3000. To detect and identify a bacteriocin from supernatant, a mass chromatogram was taken in a mass range from *m/z* 1000 to 3000. The data acquisition was performed using a JOEL MassCenter program.

Results

1. Identification of bacteriocin-producing Ski2 by 16S rDNA sequence

Ski2 which has informed from in our early report that its bacteriocin exhibited low spectrum and showed inhibitory effect only on 2 indicators of *Lb. dextranicus* and *B. coagulans* (no activity on *Lb. sakei* and *Listeria innocua*) [5]. The identification using partial 16S rDNA sequence under the NCBI blast program revealed that this strain was related to *Lb. plantarum* with 100% identity (Data not shown). Thus, the strain Ski2 revealed to be a safety strain to be used as starter culture to improve the quality and safety for the production of SI.

2. Liquid Chromatography/Mass Spectrometry (LC/MS) analysis

The purified peptides of bacteiocin from Ski 2 after reversed phase HPLC column was shown on Figure 1. The results revealed that the purified fractions of bacteriocin from Ski2 were started to show its inhibitory effect against *Lb. dextranicus* JCM 5887^T from fraction 19 until fraction 35. When compared the active fraction with the chromatogram results on Figure 1, we found that the purified peaks was shown on fraction 19-20 minutes after eluted with gradient acetonitrile. The peak was shown a bit higher up again when the purified bacteriocin was eluted with gradient acetonitrile after 30 minutes. Thus these 2 purified bacteriocin fractions were further identified for their MW using LC-MS.

The results from LC-MS revealed that both purified peptides showed the MW about 3,390 dalton for fraction 19 and 3,102 dalton for fraction 30 as shown in Figure 2 and Figure 3, respectively. From these MW results, thus,

Notes

we realize that this *Lb. plantarum* Ski 2isolated from SI should produce 2 peptide bacteriocins.

Conclusion

The results of this study informed that bacteriocin-producing LAB strain Ski2 isolated from SI is belonged to *Lb. plantarum.* The strain might be produced 2-peptide bacteriocins. This strain is possible to use as starter culture for improving the good quality and safety production of traditional Thai fermented meat products for this food industry.

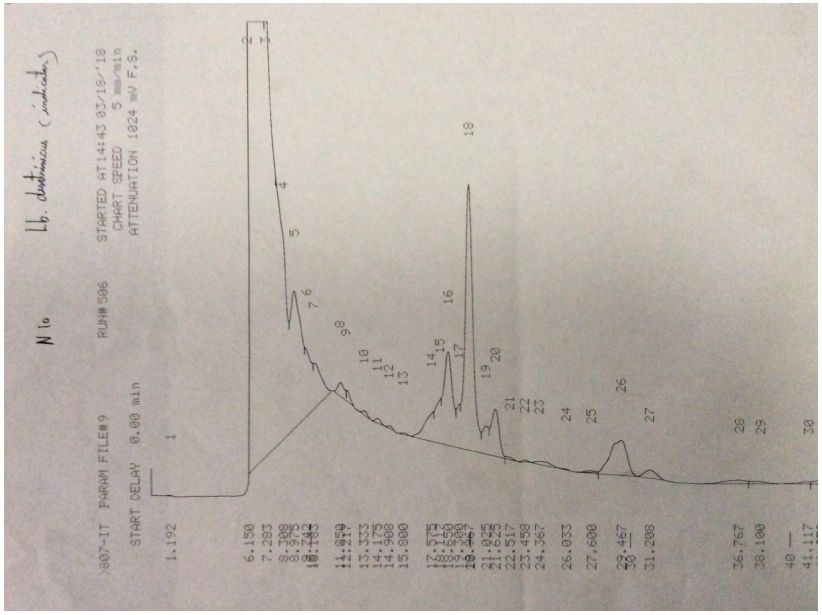

Figure 1
Chromatogram results of purified bacteriocins from Ski 2 after HPLC reversed phase purification step

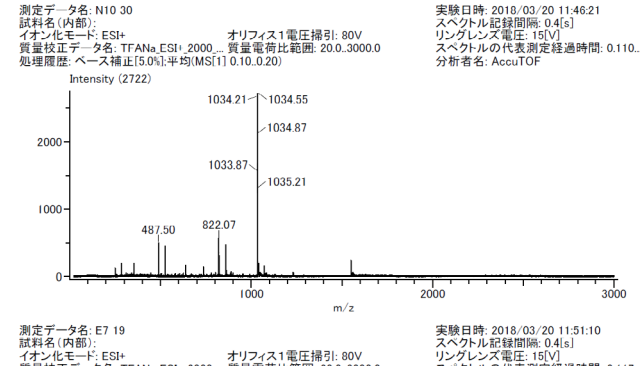

Figure 3
Molecular mass determination of purified bacteriocin freaction 30 from Ski 2 using LC-MS

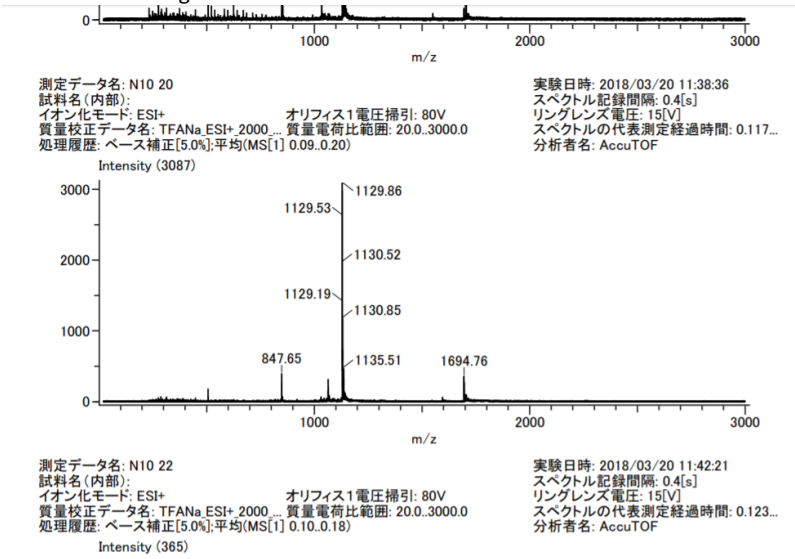

Figure 2
Molecular mass determination of purified bacteriocin freaction 19 from Ski 2 using LC-MS

Notes