Komkhae Pilasombut 2006: Purification and Characterization of Bacteriocins Produced by *Lactobacillus salivarius* K4 and K7 Isolated from Chicken Intestine. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program Thesis Advisor: Assistant Professor Thavajchai Sakpuaram, Ph.D.
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Three hundred isolates of lactic acid bacteria (LAB) were isolated from chicken intestines and screened for their abilities to produce bacteriocins. Among these isolates, two isolates, K4 and K7 were selected for further studies. Cell free culture supernatant (CFS) of K4 inhibited the growth of *Lactobacillus sakei* subsp. *sakei* JCM 1157^T, *Leuconostoc mesenteroides* subsp. *mesenteroides* JCM 6124^T, *Bacillus coagulans* JCM 2257^T, *Enterococcus faecalis* JCM 5803^T, *Listeria innocua* ATCC 33090^T and *Brochotrix campestris* NBRC 11547 ^{T} whereas CFS of K7 exhibited antibacterial activities against *Lb. sakei* subsp. *sakei* JCM 1157^T, *Leu. mesenteroides* subsp. *mesenteroides* JCM 6124^T and *B. coagulans* JCM 2257^T. Using *Lb. sakei* subsp. *sakei* JCM 1157^T as a target strain, biological and physical properties of CFS of K4 and K7 were studied. The results showed that the antibacterial activities of CFS of both isolates were destroyed by proteolytic enzymes, indicating that they have proteinaceous nature being bacteriocins. The bacteriocins were heat resistance at 121 °C for 15 min and wide pH range 3-10. Based on morphological, biochemical characteristics (API 50 CH kit) and 16S rDNA nucleotide sequence analysis, K4 and K7 were identified as *Lactobacillus salivarius,* and hence designated *Lb. salivarius* K4 and K7. *Lb. salivarius* K4 showed higher antibacterial activities and broader spectrum of bacteriocins activities than *Lb. salivarius* K7 did. Therefore, *Lb. salivarius* K4 was selected for the further purification of bacteriocins. The purification of bacteriocin from *Lb. salivarius* K4 was achieved two purified fractions and designed as FK12 and FK15. The partial N-terminal amino acid sequences of these two bacteriocins were determined by Edman degradation. To get a complete sequence of bacteriocins FK12 and FK15, specific primers were designed and structural genes were amplified by PCR. The PCR products were sequenced and the peptides sequences were deduced. The result showed that deduced peptide of FK12 was highly homology to presalivaricin B (96%) and bacteriocin-like prepeptide (94%) whereas the deduced peptide of FK15 showed only 64% similarity to abp 118 α. This suggested that bacteriocin FK15 is a novel and hence designed as Salvicin K. Molecular weight of FK12 and Salvicin K were determined by Electrospray-Ionization (ESI) Mass Spectrometry (MS) and showed 4436.46 and 4347.32 Da, respectively.

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