

Jiraporn Tangthong 2012: Cloning of *kac5* Gene Encoding Bacteriocin KAC5 from *Lactobacillus reuteri* KUB-AC5. Master of Science (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Associate Professor Sunee Nitisinprasert, D.Sc. 119 pages.

Chromosomal DNA of 1-2 kb from *Lactobacillus reuteri* KUB-AC5 producing bacteriocin like inhibition substance (BLIS) of 4.7 kD was cloned into BamHI site of pNZ307 and transformed to *Escherichia coli* DH5 $\alpha$ . Only 3 recombinant clones exhibited inhibition activities of 10 AU/ml against *Salmonella* Enteritidis S003. The recombinant clone ACE-C10, ACE-C46, and ACE-C182 containing the insert DNA of 1115, 1384 and 1581 bp showed % identity of 98, 88 and 59 to the sequence of gene *amp<sup>r</sup>*, a hypothetical protein of *Lactobacillus reuteri* MM4-1A, and hypothetical protein gene sequence of strain *Lactobacillus farciminis* KCTC3681, respectively. Since the cell free supernatant of clone ACE-C46 still displayed the inhibition activity against *Salmonella* Enteritidis S003 for 24 h while another two clones did for only 6 h, the clone ACE-C46 was therefore selected for further study. Its supernatant exhibited inhibition activities against 8 serotypes of *Salmonella*, Albany, Altona, Enteritidis, Infantis, Kedougou, Mbandake, Sandiego and Wandsworth. Two periplasmic protein, ACE46-I and ACE46-II of higher than 4 and 26 kD analyzed by SDS-Tricine PAGE displayed inhibition activity of 100 AU/ml by spot on lawn method. Based on the data of amino acid sequence from GenBank, these two proteins had no similarity to bacteriocin or BLIS previously studied. They both were proposed to be novel antimicrobial substances.

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Thesis Advisor's signature