Supranee Arai 2011: Characterization and Purification of Antimicrobial Substances from *Bacillus* strain B-1 from Fish Pond. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor. Chalor Limsuwan, Ph.D. 93 pages.

Bacillus B-1, was isolated from fish ponds in Kamphangsaen Fisheries Research Station, Faculty of Fisheries, Kasetsart University, Kampangsan campus, Nakornprathom Province, can produce the antimicrobial substance to inhibit *Streptococcus agalactiae*, then use to study the antimicrobial substance. Identification of the strain was performed by molecular genetic (16 S rDNA) basis. This strain was identified as *B. subtilis* strain B-1. The microbial inhibitory activity was determined by spot on lawn and agar well diffusion technique and the inhibitory activity of *B. subtilis* strain B-1 was shown to be effective against *Streptococcus agalactiae* ABRCS 01, *Aeromonas hydrophila* ABRCA 01, *Staphylococcus aureus* ATCC 12600, *Listeria innocua* ATCC 33090, *Micrococcus luteus* IFO 12708, *Bacillus circulans* JCM 2504 and *Bacillus coagulans* JCM 2257. The physical characterization of the antimicrobial substance that was produced by *B. subtilis* strains B-1 can inhibit many strains of the pathogenic bacteria especially *L. innocua* ATCC 33090 which yielded the highest sensitivity of indicator strain (320 AU ml^{-1}). The cell-free neutralized supernatant (CFNS) of *B. subtilis* B-1 were stabled at 100°C for 50 min (320 AU ml^{-1}) and maximum activity of 320 AU ml^{-1}were retained between pH 2.0-10.0 at 4 °C, and the activities decreased under pH 2.0-8.0 at 100°C for 60 min. The antimicrobial substances of *B. subtilis* B-1 were sensitive to the proteolytic properties of α-chymotrypsin, trypsin, proteinase K and protease, and exhibited stable qualities under 3, 6, 9, 12 and 15% sodium chloride (NaCl). Antimicrobial substances purification was carried out by amberlite adsorption, and reverse-phase high performance liquid chromatography. Molecular mass was determined by Matrix-Assisted Laser Desorption/Ionization-Time of Flight mass spectrometry (MALDI-TOF). The purification of antimicrobial substances by amberlite adsorption and reverse-phase chromatography resulted in only one single active peak at 30.221 min, which was designated B1-1. Molecular weight of this fraction by mass spectrometry was 3,398.05 Da then analyzed the antimicrobial substance by the 2D-PAGE technique that reported 5 spots after that cut them to LC-MS/MS. The result showed the third and the fifth spots related to mycosubtilin and lipopeptide respectively.

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