Executive Summary

A total of 75 bacterial strains producing proteinase were outside the cells screened from 50 samples of bat faeces in Wat Suwankuha cave, Takua Thung District, Phang Nga, Thailand, by agar plate assay. The results showed that strain PN51 (Phang Nga 51) gave the highest proteinase activity in the casein Folin-Ciocalteau assay. The 16S rDNA fragment of PN51 was amplified by PCR and its sequence was determined by an automatic DNA sequencer. The partial conserved region in 16S rDNA sequence of PN51 gave a significant similarity to 5 bacterial strains. Strain PN51 showed a homology of 100% correlation to that of *Bacillus* sp. CNJ904 PLO4. The evolutionary tree between strain PN51 and other bacteria showed that PN51 is in this respect close to *Bacterium* sp. 47083 (96.5%), *Bacillus* sp. CNJ815 PLO4 (90.2%), *Bacterium* JL-74 (90.2%), and *Bacillus* sp. P01 (90.2%).

The strain PN51 produced its highest amount of proteinase at pH 8, meaning that the strain is an alkalophile. Maximal proteinase production occurred at 35°C in a modified Lee's medium containing 1.0% peptone, 0.5% yeast extract, $CaCl_2$ 0.04% and $MgCl_2$ 0.02% with 0.5% starter at 180 rpm for 22 hr. Regarding the growth of strain PN51 in a 500-ml of NB, modified NB, or Lee's medium, the proteinase activity was much lower than a small volume, 5- or 50-ml, of each medium (data not shown). The enzyme was purified in a 3-step procedure involving ammonium sulfate precipitation, DEAE-cellulose DE52 and Mono Q FPLC. The results suggest that the last step, Mono Q column chromatography gave the lowest yield.

Molecular mass of the major band was estimated to be 35.0 kDa. The enzyme, which showed the highest activity at 50°C and pH 10.0, was stable in a pH range from 7 to 10 after treatment at 4°C for 20 hr and up to 60°C at pH 10 for 1 hr. The activity was strongly inhibited by phenylmethyl sulfonyl fluoride (PMSF), and chymostatin, but not by EDTA. The amino-terminal's 25 amino acids' sequence was NH_2-Y-V-P-N-D-P-A-Y-K-Q-Q-Y-A-P-Q-K-V-G-T-E-Q-A-W-D-T, which is up to about 70% identical with those of *Natrialba asiatica* halolysin precursor and *Natrialba magadii* halolysin-like extracellular serine protease, and about 60% identical with those of serine protease halolysin R4 from *Haloferax mediterranei.* Thus, the enzyme from the strain PN51 was thought to be a serine-type with chymotrypsin activity.

In general, proteinases from alkalophilic bacteria have high pH optima, broad pH activity spectra (pH 8-12), high temperature optima (60-65°C), and higher temperature stability. These properties of the alkaline proteinases are suitable for use in detergent industry. The strain PN51 could therefore be used in commercial alkaline protease production and the enzyme may be considered as a candidate additive for detergent industry, exploited for protein hydrolysates preparation, e.g. constituents of dietic and health product, flavoring agents, and applied for waste treatment from food processing factories and household activities.