CHAPTER V

CONCLUSION

Pla-ra (fermented fish) is produced from fish and contains a large proportion of salt. It is made mainly from fresh water fish and containing roasted rice, a source of carbohydrate and fermented for many months. The end product gave salty and sligthly sour taste, with a strong and characteristic flavor Pla-ra. The halotolerant bacteria present in the fermentation are involved in the proteolysis of the fish. In this study, a total of 65 samples were collected at the markets in Bangkok, Nakhon Nakyok, Loei, Nakhon Ratchasima, Kalasin, Chaiyaphum, Samutm Prakarn, Prachinburi, and Singburi. Forty-six halophilic bacteria were isolated and they showed proteoase activity on the halobacterium agar JCM. No.168 with 10 - 15% NaCl containing 1% skim milk. All were rod-shaped, gram-positve, catalase and oxidase positive bacteria. They were halophilic bacteria.Twelve strains with high activity on 15% NaCl agar plate were selected for the protease activity study. On the basis of morphological, cultural, physiological, biochemical and chemotaxonomic characteristics including DNA-DNA similarity, they were separated into 3 groups, 5 strains in Group I ; 6 in Group II, and 1 in Group III. The tested strains contained *meso*-diaminopimelic acid in the cell wall. Menaquinone with 7 isoprene units (MK-7) was a major component. DNA G + C content of the tested strains ranged from 36.2 to 49.6 mol %. The 16S rDNA sequence similarity of the representative strains were almost identical (99.7-99.8%) with *V. marismortui* and *V. halodenitrificans*. Therefore, Group I strains were identified as *V. marismortui*, Group II were *V. halodenitrificans*, and Group III were left unidentified.

The strains NB2-1 identified as *V. marismortui* was selected for the further protease optimization. The optimal conditions for protease production of strain NB2-1 was as following : modified halobacterium JCM No. 168 medium without casamino acid containg 15% (w/v) NaCl, pH 10 at 50° C for 3 days. At the optimal condition, strain NB2-1 produced protease at 0.0322 U/ml which was 1.27 times increase.

The optimum pH, temperature and NaCl concentration of the crude protease was pH 10, 50 °C and 5 % (w/v) NaCl. Thus the enzyme was slightly or moderately

thermophilic. The PMSF was found to inhibited the protease activity strongly, whereas the other protease inhibitors did not. The results suggested that the crude protease from NB2-1 is serine type protease.

In this study, a lot of halophilic isolates distributed in pla-ra. They were the moderately halophiles in which may play role in the fermentation of fish in high salt. It is still interesting to identify and characterize the enzyme activity of other isolates obtained in the study.