CHAPTER I

INTRODUCTION

Microbial protease are a well-studied group of hydrolases that catalyze the total hydrolysis of proteins. Besides their physiological importance, they constitute a class of enzymes of great application in commercial fields (Rao et al., 1998). Recently, protease account for approximately 40% of the total enzyme sales in various industrial market sectors, such as detergent, food, pharmaceutical, leather, diagnostics, waste management and silver recovery (Godfrey and west, 1996). These industrial processes are carried out under specific physical and chemical conditions which cannot always be adjusted to the optimal values required for the activity of the available enzymes. Although there are many microbial sources available for producing protease, only a few are recognized as commercial producers. For that reason, it would be of great importance to have available enzymes showing optimal activities at different values of salt concentrations and temperature. Halophiles are the most likely source of such enzymes, because not only are their enzymes salt-tolerant, but many are also thermotolerant (Sanchez-Porro, 2003).

The halophilic bacteria are salt-loving organisms that inhabit hypersaline environments. They include mainly prokaryotic and eukaryotic microorganisms with the capacity to balance the osmotic pressure of the environment and resist the denaturing efforts of salts. They may be classified according to their salt requirement: slight halophiles grow optimally at 2-5% (w/v) NaCl; moderate halophiles grow optimally at 5-20% (w/v) NaCl; and extreme halophiles grow optimally above 20-30% (w/v) NaCl (Kushner, 1985). They are well adapted to hypersaline environments and have a number of novel molecular characteristics, such as halophilic exoenzyme that function in high salt concentration and could be of commercial interest and used in biodegradation processes. They have the advantage that most species are able to grow in wide range of salinities, in contrast to the more strict requirement of salt presented by halobacteria. Thus, their euryhaline response would permit their use in processes in which the salt or

metallic ion concentrations are variable, and change from very low to almost salt saturation.

Although halophilic microorganisms have attracted much attention in recent years, most studies have been performed in halobacteria. However, moderately halophilic bacteria represent an excellent model of adaptation to frequent change in extracellular osmolality and constitute an interesting group of microorganisms from biotechnological point of view (Ventosa, 1998).

In recent years the isolation and characterization of protease enzyme produced by these microorganisms have acquired enormous interest. The running of industrial processes at high salt concentrations requires the availability of protease showing optimal activities at such elevated salinity. The addition of such enzyme in laundry and dishwashing detergents has been of great importance. The treatment of agricultural waste and waste from food processing industries constitutes other areas of interest for halophilic hydrolytic enzyme. For example, a protease-producing moderate halophiles was isolated in north Taiwan can be used for deproteinization of crustacean wastes in the preparation of chitin and used for waste treatment from sea food industrial (Yang et al., 2000). So the screening of protease-producing halophilic bacteria is an interesting.

Previously study, most of protease-producing halophilic bacteria were isolated from hypersaline enviroment such as desert, salt lakes, sediment of solar saltern, saline soils and thalassic while the protease-producing halophilic bacteria from traditional fermented food have not been extensively studied.

Pla-ra (fermented fish) is produced from fish and a large proportion of salt. It is made mainly from fresh water fish and containing roasted rice, a source of carbohydrate and fermented for 6 – 12 month. The end product gave salty (about 7.7 – 17.89% NaCl) and sligthly sour taste, with a strong and characteristic flavor of Pla-ra. According to Amano (1962), the fermentation of Pla-ra involes the combined effect of fish and microbial enzymes supplied in the form of starter cultures on fish flesh and entrails with added salt. These products are rich in various nutrient, particularly amino acid and peptides, and contain a high concentration of NaCl, which allow various halophilic

bacterias to thrive (Lopetcharat et al., 2001). Hence, Pla-ra is a good screening source for isolation of halophilic bacteria that show proteinase activity. There are several previous studies on proteinase from bacteria isolated from fish sauce, but few protease-producing bacteria from Pla-ra.

The main objectives of this presence study are as followed:

1. To screen the protease-producing halophilic bacteria from Pla-ra with highest protease activity.
2. To identify the protease-producing halophilic bacteria from Pla -ra.
3. To maximize the protease production through optimization of the modified media and cultivation conditions.
4. To study some properties of crude protease