CHAPTER I

INTRODUCTION

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, moderate halophiles and extreme halophiles (Kushner, 1985). They have the advantage that most species are able to grow in wide range of salinities, in contrast to the more strictly requirement of salt presented by halobacteria. Several halophilic bacteria are often found on salted food, such as salted fish, meat and other food products. Villar, Ruiz-Holgado and Sanchez (1985) found that *Pediococcus halophilus* was a dominant bacterium at the end of the curing process of anchovies. While *Halomonas salina* was isolated from fully cured wet and dry bachalao (dried salted codfish) that contained about 19% salt (Vihelmsson, Hafsteinwsson and Kristjansson 1996). *Pseudomonas beijerinckii* and *Halomonas halodenitrificans* were isolated from salted beans preserved in brine and meat curing brines, respectively. *Chromohalobacter japonicus* was isolated from Japanese salted food (Sanchez-Porro et al., 2007), *Halomonas alimentaria* isolated from jeotgal, a traditional Korean fermented seafood (Yoon et al., 2002).

Halophilic bacteria are well adapted to hypersaline environments and have a number of novel molecular characteristics, such as halophilic exoenzyme that function in high salt concentration. 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. The applications of halophilic bacteria were used in food, pharmaceutical industries and cosmetic products, production of enzymes, polymers, biotechnological remediation and biological control in agriculture have been reported by several authors (Oren, 2002; Ramos-

Cormenzana, 1990; and Ventosa et al., 1998b; Nicholson and Fathepure, 2004; Sadfi-Zouaoui et al., 2008)

Today 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. The industrial application of halophilic enzyme is very attractive, since these enzymes can function under extreme conditions where most normal enzyme cannot. For industrial use, a halophilic enzyme must be stable even without salt and active in the presence of high salt concentration. In addition, halophiles not only are their enzymes salt-tolerant, but many are also thermotolerant (Sanchez-Porro, 2003). So the screening of protease-producing halophilic bacteria from hypersaline environments is an interesting.

Pla-ra (fermented fish) is produced from fish, carbohydrates (roasted rice or rice bran) and a large proportion of salt. The production of pla-ra in Thailand was 20,000 - 40,000 tons/year and the value of pla-ra was about 800 million baht/year but the value of pla-ra for exporting was more than 20 million baht/ year (Anonymous, 2000). Pla-ra was popular among the people who live in every region of Thailand especially in the northern and northeastern parts of Thailand. Pla-ra was popular among ASEAN countries such as Myanma, Laos, Vietnam and Cambodia. (Poosereepab, 1996; Yamprayoon and Sukkho, 1999). According to Amano (1962), the fermentation of pla-ra involves the combined effect of fish and microbial enzymes supplied in the form of starter cultures on fish flesh and entrails with added salt. Hence, pla-ra is a good screening source for isolation of halophilic bacteria that show proteinase activity. In Thailand, *Halobacterium* sp., *Halococcus* sp., *Halobacillus* sp. SR5-3, *Filobacillus* sp. RF2-5, *Lentibacillus salicampi*, *Lentibacillus halophilus* sp.nov., and *L. juripiscarius* sp. nov were isolated from Thai fish sauce (Thongthai and Suntinanalert, 1991; Hiraga et al., 2005; Namwong et al., 2005, 2006;

Tanasupawat et al.,2006). *Piscibacillus salipiscarius* (Tanasupawat et al., 2007) and *Halobacterium piscisalsi* (Yachai et al., 2008) were isolated from fermented fish (pla-ra). The protease-producing halophilic bacterial strains, *Halobacillus* sp. SR5-3, *Filobacillus* sp. RF2-5 and *Virgibacillus* sp SK37 (Hiraga et al., 2005; Namwong et al., 2005, Sinsuwan, et al., 2008) have been extensive studied in fish sauce and *V. marismortui* (Chamroensaksri et al., 2008) in pla-ra. However, the protease-producing halophilic bacteria from pla-ra have not been extensively studied.

Research objectives

The main objectives of this present study are as followed:

1. To isolate and screen the halophilic bacteria from pla-ra.
2. To identify and characterize the isolates from pla-ra based on the phenotypic and chemotaxonomic characteristics including DNA-DNA relatedness and 16S rDNA sequencing.
3. To maximize the protease production of the selected isolate through optimization of the media and cultivation conditions.
4. To purify and characterize the protease from selected protease-producing isolate.