CHAPTER I
INTRODUCTION

Surfactants, or surface active agents, can be classified into two main groups: synthetic surfactants and biosurfactants. While synthetic surfactants are produced by organic chemical reactions, biosurfactants are produced by biological processes, being excreted extracellularly by microorganisms such as bacteria, fungi, and yeast. Compared to synthetic surfactants, biosurfactants have several advantages, including low toxicity, high biodegradability, low irritancy, and compatibility with human skin (Banat *et al.*, 2000; Cameotra and Makkar, 2004). Biosurfactants are able to retain their properties even under extreme conditions of pH, temperature, and salinity. Due to these superior characteristics, biosurfactants have potential uses in petrochemical, petroleum, food, cosmetics, and pharmaceutical industries (Desai and Banat, 1997). An increase in the concern about environmental protection leads to the development of cost-effective bioprocesses for the biosurfactant production (Mercadé *et al.*, 1993; Fox and Bala, 2000; Abalos *et al.*, 2001; Nitschke *et al.*, 2004; Wei *et al.*, 2005; Nitschke and Pastore, 2006).

Synthetic surfactants are usually categorized according to the nature of their polar head group; however, biosurfactants are commonly differentiated based on the types of biosurfactant-producing microbial species and the nature of their chemical structures. Most of biosurfactants are either nonionic or anionic, and only a few are cationic, such as those containing amine groups. Normally, the hydrophobic parts of the biosurfactant molecules contain long-chain fatty acids, hydroxyl fatty acids, or α-alkyl-β-hydroxy fatty acids, while the hydrophilic parts are carbohydrates, carboxylic acids, amino acids, cyclic peptides, phosphates, or alcohols (Mulligan *et al.*, 2001). The critical micelle concentrations (CMCs) of biosurfactants are found to be in the range of 1-200 mg/l, and their molecular weights generally range from 500 to 1500 amu (Kosaric *et al.*, 1987). Major classes of biosurfactants include glycolipids, phospholipids, lipopeptides and lipoproteins, and polymeric surfactants (Healy *et al.*, 1996).

Ones of the most common biosurfactants that have been isolated and studied are the glycolipids, which are composed of carbohydrates in a combination with long-chain aliphatic acids or hydroxyl aliphatic acids. From the point of view of surfactant properties, ones of the best examples of glycolipids are rhamnolipids produced by certain species of *Pseudomonas* (Cameotra and Makkar, 2004). In general, rhamnolipids, rhamnose-containing glycolipid biosurfactants, are excreted as a heterogeneous mixture of several homologues. With the use of modern analytic methods, including liquid chromatography (LC) and mass spectrometry (MS), the chemical structure of each homologue in the biosurfactant mixture is elucidated. Rhamnolipids can provide good physicochemical properties (Finnerty, 1994; Nitschke *et al.*, 2005) and biological activities (Abalos *et al.*, 2001; Benincasa *et al.*, 2004; Thanomsub *et al.*, 2006). In addition, these surface-active compounds can be produced from various types of low-cost substrates, and high production yields are achieved by controlling environmental factors and growth conditions. Therefore, rhamnolipids represent ones of the most effective biosurfactants for commercial and industrial exploitation. To enlarge the potential applications of rhamnolipids, knowledge on their characteristics is another important factor.

In this present study, *P. aeruginosa* SP4, isolated from petroleum-contaminated soil in Thailand, was used to produce a biosurfactant. The biosurfactant production was done by using a nutrient broth supplemented with palm oil as a carbon source. The biosurfactant was extracted, and the key components in the extracted biosurfactant were fractionated by using the high performance liquid chromatography technique. The chemical structure of each fraction was identified, and the physicochemical properties of the extracted biosurfactant were studied in a comparison with those of two synthetic surfactants. The aggregation behavior of the extracted biosurfactant was also studied, and the spontaneous vesicle formation of the biosurfactant was observed. To determine the potential use of the biosurfactant vesicle in delivery systems, the encapsulation efficiency of the biosurfactant vesicle was evaluated. To further investigate the potential applications in biomedical fields, the extracted biosurfactant was used to tailor the

surface characteristics of polymeric substrates via adsorption process. The growths of human dermal fibroblasts and keratinocytes on the surface-modified polymer films were subsequently observed.