

# Chapter I

## Introduction

### 1.1 Introduction

Gamma Poly Glutamic Acid ( $\gamma$ -PGA) is a naturally occurring poly amino acid that is synthesized by certain strains of *Bacillus* (Kubota *et al.*, 1993).  $\gamma$ -PGA is an unusual anionic polypeptide in which D-and/or L-glutamic acid units are polymerized via  $\gamma$ -amide linkages between  $\alpha$ -amino and  $\gamma$ -carboxylic groups (Kang *et al.*, 2007). Its  $\gamma$ -carboxylate side chains can be chemically modified to introduce various bioactive ligands, or to modulate the overall function of the polymer (Tachibana *et al.*, 2003; Shimokuri *et al.*, 2004).  $\gamma$ -PGA cannot be synthesized by polycondensation of glutamic acid, because intramolecular cyclization predominantly proceeds to form a stable five-membered lactam, pyroglutamic acid. Unlike general poly (amino acid)s,  $\gamma$ -PGA has unique characteristics on enzymatic degradation and immunogenicity.  $\gamma$ -PGA is resistance against many proteases because  $\gamma$ -linked glutamic acids are not easily recognized by common proteases (Obst and Steinbuchel, 2004).

$\gamma$ -PGA is a compound of mucilage produced by *Bacillus subtilis* in fermented soybeans and by several *Bacillus* species as an extracellular polymer.  $\gamma$ -PGA has molecular weights ranging from 100,000 to over 1,000,000 (Kubota *et al.*, 1993). Because  $\gamma$ -PGA is not a chemically synthesized product but exists naturally in foods,

it is generally regarded as safe. It is completely biodegradable and nontoxic to humans (Yoon *et al.*, 2000). Several studies have shown that  $\gamma$ -PGA is helpful in increasing intestinal calcium absorption as well as intensifying the strength and content of the femur (Lee *et al.*, 2006), enhances immune-stimulating activity and potentially improves antitumor activity (Kim *et al.*, 2007). For medical applications, the special chemical properties of the  $\gamma$ -PGA polymers helps to render drug water-soluble transport to tumor sites and control the release of drug over time.

Several bacterial strains secrete  $\gamma$ -PGA outside the cells; however, they need nutrient and long cultivation times for  $\gamma$ -PGA production. For the commercial production of  $\gamma$ -PGA, it is necessary to increase the productivity and decrease the nutrient supply.

This study aimed to isolate an efficient  $\gamma$ -PGA producing bacteria strain, able to produce large quantity of  $\gamma$ -PGA. The efficient  $\gamma$ -PGA producing isolate was identified using morphological, biochemical and phylogenetic studies. The obtained results was confirmed by Restriction Fragment Length Polymorphism (RFLP) analysis. The molecular weight of  $\gamma$ -PGA was determined by Sodium dodecyl sulfate poly acrylamide gel electrophoresis (SDS PAGE) electrophoresis.