2.1 INTRODUCTION

Many bacteria, including those in the soil, are capable of Polyhydroxybutyrate (PHB) production (Anderson et al., 1990). PHB was accumulated intracellular as reserve granules by many bacteria in harsh environmental conditions (Kim, 2000). PHB was first isolated and characterized by Lemoigne, 1926. Polyhydroxyalkanoates (PHAs) are biopolymers synthesized by bacteria as storage compounds for energy and carbon, normally under nutrient limiting conditions with excess carbon. PHAs are biodegradable thermoplastics that can be obtained from renewable resources such as sugars and vegetable oils (Sudesh et al., 2000 and Tsuge, 2002). They are water insoluble, non toxic, biocompatible, and have recently received attention because of their applications in the packaging industry, medicine, agriculture, and food industry. Currently, there plans are already to produce some PHAs on an industrial scale employing gram-negative bacteria such as Ralstonia eutropha and recombinant Escherichia coli (Schubert et al., 1988 and Valappil et al., 2007) because these bacteria show better growth and higher accumulation of PHAs than others, including gram positive bacteria. However, PHAs isolated from gram negative bacteria contain outer membrane lipopolysaccharide (LPS) endotoxins, which induce a strong immunogenic reaction in humans. Hence, these PHAs are undesirable, particularly for biomedical applications. Gram positive bacteria have thick outer membranes that do not contain LPS, and therefore, are potentially better sources of PHAs for use in biomedical applications (Chen, 2005). In addition, some studies have addressed gram positive bacteria as preferable candidates for host cells of PHA production. For example, members of the genus Rhodococcus which frequently resides in arid sites like deserts permits the cells to survive in stressful environments (Hernandez et al., 2008), and may therefore, adapt to the case of PHA production under stressful conditions. Corynebacterium glutamicum, which has long been used
for fermentation production of amino acids, has been investigated as a candidate for a safe and established platform for industrial PHA production (Jo et al., 2007).

A series of enzymes, synthetases or depolymerases, are implied in the biosynthesis and biodegradation of poly-β-hydroxybutyrates respectively and also of other polyhydroxyalkanoates (PHAs) (Abe et al., 2005). These biodegradable polyesters display a special interest due to their possible use as substitutes of common plastics because they are completely degraded by the microorganisms present in the environment and they can be produced from regenerable carbon sources (Pettinari, 2001 and Khanna et al., 2005). *Alicaligens eutrophus* H16 is a facultative autotroph and can grow rapidly in simple media, for PHB production it requires anaerobic conditions with CO₂ and N source (Schlegal et al., 1961). Molecular structure of PHB does not depend on the features of the strain and conditions of carbon nutrition of microorganisms producing PHB (Volva et al., 2000). Most of the bacteria which produce PHB are nitrogen-fixing microorganisms.

For the past decades, the production of PHA by bacteria and their PHA biosynthesis pathways have been extensively studied. However, there is scarce information about the diversity of PHA producing bacteria in Antarctic regions, the properties of these PHA and the enzymes involved in their biosynthesis. Only one species of *Pseudomonas* from Antarctic origin which produced PHB had been described (Ayub et al., 2004 and Lopez et al., 2009). Some studies on non-Antarctic bacteria had shown that the accumulation of reserve polymers such as PHA could help bacteria to withstand starvation and hostile environmental conditions (Matin et al., 1979; López et al., 1995; Wang and Bakken, 1998; Ruiz et al., 2001 and Pham et al., 2004). Therefore, the accumulation of PHA in Antarctic bacteria might also increase the survival capabilities of these bacteria in the extreme environments and poor nutrient availability had
suggested that PHA metabolism in an Antarctic isolate, *Pseudomonas* sp. 14-3, was an adaptation mechanism to withstand oxidative stress and drastic changes in temperature, which are common stress factors encountered in Antarctic environments (Ayub *et al.*, 2004 and Ayub *et al.*, 2009). More studies on PHA production by Antarctic bacteria would be useful to demonstrate the possible association between PHA accumulation and stress tolerance.

Gram positive bacteria have another potential advantage in terms of raw materials for PHA production. The gram-positive genera *Corynebacterium*, *Nocardia*, and *Rhodococcus* are capable of naturally synthesizing the commercially important copolymer poly (3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] from abundant and inexpensive carbon sources such as glucose (Haywood *et al.*, 1991 and Valappil *et al.*, 2007) in contrast, gram-negative bacteria need expensive structurally related substrates such as propionic acid, valeric acid, or other fatty acids with an odd number of carbon atoms to produce 3HV units (Valappil *et al.*, 2007). The relatively high expenditure involved is a major hindrance in PHA copolymer production. Hence, gram positive producers could considerably reduce the production cost. The best characterized gram-positive bacteria group and the first PHA producer from the genus Bacillus was identified as *Bacillus megaterium* in 1926 (Lemoigne, 1926). Till date, many species of PHA-producing *bacilli* have been isolated from various environments and characterized. Some of these are able to produce PHA copolymers from inexpensive and structurally unrelated carbon sources. *Bacillus* sp. 88 D isolated from a municipal sewage treatment plant is able to produce P (3HB-co-3HV) from glucose as a sole carbon source (Reddy *et al.*, 2009). *Bacillus cereus* SPV is able to use fructose, sucrose, and gluconate to produce P (3HB-co-4-hydroxybutyrate) [P (3HB-co-4HB)] and P (3HB-co-3HV-co-4HB) (Valappil *et al.*, 2008). *Bacillus* sp. INT005 isolated from a gas field soil produces P (3HB-co-3-
hydroxyhexanoate) [P (3HB-co- 3HHx)] from glucose (Tajima et al., 2003). The diversity of PHA products in the genus *Bacillus* are presumed to be due to class IV PHA synthase, which was recently identified as a new class and has relatively broad monomer specificity (Maeda et al., 2006). This indicates that the genus *Bacillus* could be used for the industrial production of PHA copolymer, which prompted us to isolate new copolymer producers with excellent cell growth and polymer accumulation from a unique environment.

The *Azotobacter* species fix the molecular nitrogen and have the capacity to accumulate poly-β-hydroxybutyrates when they are grown on different carbon sources, including sucrose media (Quagliano et al., 1994). The ability of the organism to survive in the adverse conditions depends on its capacity to develop or utilize different survival mechanisms. Therefore, this study was focused on the isolation, screening and characterization of polyhydroxybutyrate (PHB) producing soil bacteria and provides a basis for further investigation of PHB biosynthesis by members of the *Bacillus* group.