CHAPTER I

INTRODUCTION

Lignocellulose, the most abundant and renewable organic compounds in nature, comprises of average 40% cellulose, 33% hemicellulose and 23% lignin by dry weight (Sa-pereira *et al.*, 2002). The cellulose is composed of D-glucose units linked together to form linear chain via ß-1,4-glycosidic linkages (Salmon, 1997). Cellulolytic enzymes, cellulase, is a group of enzymes which hydrolyse ß-1,4-glycosidic linkages in cellulose. The cellulolytic enzymes is composed of at least tree different enzymes. Endoglucanase (1,4- ß-D-glucan-4-glucanohydrolase, E.C. 3.2.1.4) attack randomly internal linkages within the cellulose chain , creating free chain ends. Exoglucanases (1,4-ß-D-glucan cellobiohydrolase, E.C. 3.2.1.91) hydrolyse cellulose from the free chain ends creating mainly cellobiose as an end product. ß-glucosidase (E.C. 3.2.1.21) hydrolyse the small oligomer including cellobiose to produce glucose. The exoactivity is important for glucose liberation because accumulation of cellobiose strongly inhibits cellobiohydrolase. The cellulolytic enzymes act together in synergism. (Beguin and Aubert, 1994; Harjunpaa, 1998). Therefore, complete set of the enzymes is necessary to degrade native cellulose.

Xylan is the most abundant of the hemicellulose which is heteropolysaccharide having a linear backbone of ß-1,4-linked xylopyranose units that often have side chains of O-acetyl, arabinosyl and methylglucuronosyl substituents (Rawashdeh *et al.*, 2005). Complete hydrolysis of xylan requires the combined action of various enzymes such as endoxylanase (endo-1,4-ß-xylanase, 1,4-ß-D-xylan xylanohydrolase, E.C. 3.2.1.8), ß-xylosidase (1,4-ß-D-xylan xylohydrolase, E.C. 3.2.1.37), and several accessory enzymes to hydrolyse substituted xylan. The endoxylanase attacks internal xylosidic linkages on the backbone and the ß-xylosidase releases xylosyl residues by endwise attack of xylooligosaccharide (Wong *et al.*, 1988).

Due to increasing demands for more environmental friendly methods, the use of enzymes instead of chemicals has significantly increased during the past two decades. Cellulases have been used in several industries, for example, paper production, juice clarification, textile process, lignocellulosic ethanol production, extraction of valuable components from plant cells, and

improvement of nutritional values of animal feed, etc. (Bhat, 2000; Csiszar *et al.*, 2001; Haki and Rakshit, 2003; Lyne *et al.*, 2002; Vielle and Ziekus, 2001). While an examples of xylanase industrial application are pretreatment of forage crops and other lignocellulosic biomasses to improve nutrient utilization, flour improvement for bakery products, saccharificaton of hemicellulosic wastes (Gilbert and Hazlewood, 1993), pulp and fibre processing (Yang *et al.*, 1995), clarification of juices and wines, extraction of plant oils and coffee (Kulkarni and Shendye, 1999; Uma Maheswari and Chandra, 2000). A wide variety of bacteria, yeast and fungi are know to produce cellulase and xylanase.

Extracellular cellulase from several bacteria have been studied and characterized *e.g.* *Clostridium*, *Caldocellum*, and *Acidothermus* (Bergquist *et al.*, 1999), *Acetovibrio* (Ding *et al.*, 1999), *Ruminococcus* (Aurilia *et al.*, 2000), *Sinorhizobium* (Chen *et al.*, 2004) and *Cellulomonas*, *Micrococcus* and *Bacillus* (Immanuel *et al.*, 2006). Several strains of *Bacillus* species including *B. brevis*, *B. firmus*, *B. polymyxa*, *B. pumilus*, *B. subtillis*, *B. circulans* were reported as cellulase producing bacteria (Priest, 1997; Hakamada *et al.*, 2002), *Paenibacillus* sp. (Ogawa *et al.*, 2007).

Many bacteria are known to produce different type of xylanases. Enzymes vary between different organisms. Several extracellular xylanase from bacteria have been studied and characterized *e.g.*, *Bacillus firmus* (Tseng *et al.*, 2002), *B. thermantarcticus* (Lama *et al.*, 2004), *B. coagulans* (Wong *et al.*, 1988), *B. circulans* (Kyu *et al.*, 1994), *B. pumilua* (Duarte *et al.*, 2000), *B. subtilis* (Yuan *et al.*, 2005), and *B. polymyxa* (Sandhu and Kennedy, 1984). Recently the novel species of *Paenibacillus*, *P. xylanilyticus* (Rivas *et al.*, 2005) and *P. favisporus* (Valazquez *et al.*, 2004), *Microbacterium*, *M. xylanilyticum* (Kim *et al.*, 2005) and *M. ulmi* (Rivas *et al.*, 2004); *Cellulomonas xylanticus* (Rivas *et al.*, 2004) and *Ruminococus flavefaciens* (Cotta and Zeltwanger, 1995) were proposed as xylanase producer. However, such applications require cellulase and xylanase with particular properties, *e.g.* active under high temperature and/or alkaline condition. Bacterial cellulase and xylanase are generally higher thermostable than fungal cellulases. Most cellulase and xylanase from fungi have pH optima between 4 and 6 (Zhu *et al.*, 1982; Yazdi *et al.*, 1990), while bacterial cellulase and xylanase active at alkaline pH have been reported (Ruttersmith and Daniel, 1993; Blanco and Zueco, 1999). The climatic condition in Thailand is hot and humid which is highly conducive for microbial growth. Use of temperature at 40°C in the screening method gave a change to meet the new cellulase and xylanase producing bacteria. In addition, the relatively diverse soil types and natural high biodiversity of this region.

Therefore, soil and biofertilizer samples are interesting and challenging resources for a discovery of novel cellulase and xylanase producing bacteria. This work deals with isolating , screening and identification of cellulase- and xylanase-producing bacteria from soils and biofertilizers in Thailand.

The main objectives of this present study are as follows:

1. To isolate and screen cellulase- and xylanase-producing bacteria.

2. To identify and characterize the cellulase- and xylanase-producing bacteria isolated based on their phenotypic and chemotaxonomic characteristics including 16S rRNA gene sequencing.

3. To characterize cellulase and xylanase production of the selected isolates based on the effect of pH and temperature.