CHAPTER I

INTRODUCTION

The acetic acid bacteria (AAB) are classified to order α-*Proteobacteria* family *Acetobacteraceae* and they are classified into nine genera: *Acetobacter* (Beijerinck, 1898) *Gluconobacter* (Asai, 1964), *Acidomonas* (Urakami *et al.*, 1989), *Gluconacetobacter* (Yamada *et al.* 1997), *Asaia* (Yamada *et al.*, 2000), *Kozakia* (Lisdiyanti *et al.*, 2002), *Swaminathania* (Logonathan and Sudha. 2004), *Saccharibacter* (Jojima *et al.*, 2004) and *Neoasaia* (Yukphan *et al.*, 2005). They are Gram-negative, strictly aerobic bacteria and are commonly found in nature on various plants (flowers, herbs, fruits etc.). They have an ability to oxidize different kinds of alcohols and sugars into commercially important foods and chemical products (vinegar, kombucha, tea, sorbose, gluconic acid, etc.). Another characteristic of the AAB is the capability of producing considerable amounts of extracellular polysaccharides. However, the AAB can also spoil beer, juice, wine and fruits.

The strains in genus *Acetobacter* are frequently used for the industrial vinegar (Saeki *et al.*, 1997). There are classified into 14 species; *A. aceti*, *A. indonesiensis*, *A. cerevisiae*. *A. cibinongensis*, *A. pasteurianus*, *A. lovaniensis*, *A. orleanensis*, *A. estunensis*, *A. malorum*, *A. orientalis*, *A. peroxydans*, *A. pomorum*, *A. syzygii*, *A. tropicali* and *A. oeni* (Luis *et al.*, 2006)

The genus *Gluconobacter* is classified into 5 species; *G. oxydans*, (Henneberg, 1987) *G. cerinus*, (Yamada and Akita, 1984), *G. frateurii*, (Mason and Claus, 1989) *G. thailandicus* (Tanasupawat *et al.*, 2004) and *G. albidus*. (Yukphan *et al.*, 2005). *Gluconobacter* strains generally produce acid during growth on several carbohydrates and show a pronounced and efficient ketogenesis from polyhydroxyalcohols via a single-step oxidation. The latter property finds numerous applications in industry. They produce gluconic acid, 2-ketogluconic acid, 5-ketogluconic acid, L-sorbose, etc. (Adachi *et al.*, 2003).

AAB species have conventionally been identified by testing physiological and chemotaxonomic properties (Delay *et al.*, 1984). The identification of the AAB, using phenotypic characteristics, especially on the species level, is difficult and is not only inaccurate, but also very time-consuming. Therefore, the application of the molecular methods

based on restriction pattern and sequence analyses, could be a proper solution for a quick and accurate identification of these microorganisms.

Recently, the 16S-23S rDNA internal transcribed spacer region was genetic variation on 16S rDNA in the acetic acid bacteria and other microorganisms. The sequenced region for the 16S-23S rDNA ITS contained genes for $tRNA^{Ile}$ and $tRNA^{Ale}$ and the anti termination element (Trček and Teuber, 2002). In addition, the restriction fragment length polymorphism (RFLP) of 16S-23S rDNA intergenic spacer was not useful for identification of acetic acid bacteria at the species level although it might be a variation spacer on 16S rDNA similar with the other regions but as well as these details were useful for generic level identification(Trček and Teuber, 2002). At the specific level identification of acetic acid bacteria, the phenotypic characteristics were traditionally utilized. Therefore, we selected the 16S-23S rDNA ITS regions for identification of *Acetobacter* and *Gluconobacter* strains at the species level.

Acetic acid bacteria are well known as vinegar producers. They produce acetic acid from ethanol by two sequential catalytic reactions of membrane-bound alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH). Such oxidation reactions are called "oxidative fermentation" (Matsushita *et al.*, 1994). The recent hot summer has raised indoor temperatures beyond 30°C, event at night time, in many countries. This is a serious problem for vinegar fermentation and other fermentation industrials, since they have to pay increases energy costs to keep the fermentation at the optimum. Domestic vinegar fermentation by acetic acid bacteria is usually carried out at 30°C and strict, temperature control is necessary irrespective of whether the culture is static or submerged. A temperature increase of 2-3°C causes a serious deterioration in both the fermentation rate and fermentation efficiency. In submerge culture, a large amount of heat is generated during fermentation and thus cooling costs become rather expensive (Saeki *et al.*, 1997). Therefore, if favorable strains of acetic acid bacteria that can work optimally at 37-40°C were available, the cooling expensive would be reduced greatly.

The main objective of this presence study are as follows:

1. To isolate *Acetobacter* and *Gluconobacter* strains from flowers, fruits and other materials in Thailand.
2. To identify and characterize the *Acetobacter* and *Gluconobacter* strains based on the phenotypic and chemotaxonomic characteristics including 16S-23S rDNA restriction pattern analysis and 16S rDNA sequencing.
3. To screen the high acetic acid-producing thermotolerant *Acetobacter* strains
4. To determine the effects of the acetic acid production of the selected isolate based on cultivation conditions.