CHAPTER V

CONCLUSION

Total 181 strains of acetic acid bacteria were isolated from fruits, flower and other materials collected in Thailand. All isolates were Gram-negative, aerobic, catalase positive, oxidase-negative rod-shaped bacteria. They produced clear zones on the GEY-$CaCO_3$ agar plates and grew on the GEY broth at pH 3.5. On the basis of their phenotypic and chemotaxonomic characteristics, 16S-23S rDNA restriction pattern analysis and the phylogenetic analysis using 16S rDNA sequences, they were divided into 11 groups. Fifty-three isolates were identified as *A. pasteurianus*, 42 as *A. orientalis,* 2 as *A. lovaniensis*, 17 as *G. oxydans*, 12 as *G. cerinus*, 9 as *G. frateurii*, 7 as *G. thailandicus*, 21 as *Asaia*, 14 as *Gluconacetobacter*, 2 as *Swaminatania* and 2 as *Kozakia*. The tested strains of *Acetobacter* contained ubiquinone-9 as the major quinone while the rests contained ubiquinone-10. The DNA G+C contents of the tested isolates of *Acetobacter* (Group 1 to 3) ranged from 52 to 58.4 mol%.

Acetobacter strains were divided into three groups by 16S-23S rDNA ITS restriction analyses with *Hpa*II and *Hae*III. Group I contained fifty-three strains that were identified as *A. pasteurianus*, since they gave the same restriction patterns *f* and *h* as *A. pasteurianus* TISTR 1056^T, when digested respectively with *Hpa*II and *Hae*III. Group 2 contained forty-two strains that were identified as *A. orientalis*, since they gave the same restriction patterns *e* and *i* as *A. orientalis* NBRC 16606^T. Group 3 contained two strains that were identified as *A. lovaniensis*, since they gave the same restriction patterns *c* and *j* as *A. lovaniensis* NBRC 13753^T.

On the other hand, forty-five isolates did not oxidize acetate and lactate, were identified as *Gluconobacter*. They were divided into four groups by 16S-23S rDNA ITS restriction analyses with *Bsp*1286I, *Mbo*II and *Ava*II. Group 4 contained seventeen strains that were identified as *G. oxydans*, since they gave the same restriction patterns as *G. oxydans* NBRC 14819^T respectively with *Bsp*1286I and *Mbo*II. Group 5 contained twelve strains that were identified as *G. cerinus*, since they gave the same restriction patterns as *G. cerinus* NBRC 3267^T. Group 6 contained nine strains that were identified as *G. frateurii*, since they gave the same restriction patterns as *G. frateurii* NBRC 3264^T with *Bsp*1286I, *Mbo*II and *Ava*II. Group 7 contained seven strains that were identified as *G. thailandicus*, since they showed the same restriction patterns as

G. thailandicus NBRC 100600^T with *Bsp*1286I, *Mbo*II and *Ava*II. Except AN1-1 that gave a restriction pattern showing a band of 610 bp different from that of 714 bp found in the type strains of *G. frateurii* and *G. thailandicus*, when digested with *Ava*II.

In a phylogenetic analyses, PA 3-3 (Group 1) showed 99.8% similarity of 16S rDNA nucleotide with *A. pasteurianus* TISTR 1056^T. KLM13-1, MHM10-1, FBM4-3 and BBM91-1 (Group 2) showed 99.5, 99.4, 99.7 and 99.6% sequence similarities, respectively with *A. orientalis* NRIC 0481^T. Strains LBM3-1 (Group 3) showed 99.8% similarity with *A. lovaniensis* IFO 13753^T. Strains JR70-1 (Group 4) showed 99.8% similarity with *G. oxydans* NBRC 14819^T. Strains AK33-2 (Group 5) showed 99.7% similarity with *G. cerinus* NBRC 3267^T. Strains LD51-1(Group 6) showed 99.6% similarity with *G. frateurii* NBRC 3264^T. Strains MG71-2 (Group 7) showed 99.8% similarity with *G. thailandicus* F149-1^T while AN1-1 showed 98.7% similarity with *G. frateurii* NBRC 3264^T and 98.6% to *G. thailandicus* NBRC 100600^T. Strains MG71-1 (Group 8) showed 99.7% similarity with *As. bogorensis* NBRC 12264^T. Strains SIS32-2 (Group 9) showed 97.8% similarity with *Ga. liquefaciens* IFO 12388^T. Strains SI15-1 (Group 10) showed 97.7% similarity with *Sw. salitolerans* PA51^T. Group 11, CT8-1 showed 96.4% similarity with *K. baliensis* NRIC 0488^T.

In this study, a lot of known and novel species of acetic acid bacteria were found in many kinds of samples in Thailand. The 16S-23S restriction pattern analyses and the 16S rDNA sequencing were useful to indicate the strains as *Acetobacter* and *Gluconobacter* at the species level. However the DNA-DNA hybridization of the isolates with the closed type strains of each species are required for further studies in order to propose them as the new species. In addition, *A. pasteurianus* PA 3-3 was selected as a thermotolerant strain that could produce 28.87 g/l, 25.63 g/l and 18.75 g/l of acetic acid after 3 days incubation at 30°C, 37°C and 40°C, respectively. This strain will be useful for acetic acid fermentation.