

## Systematic Study of Acetic Acid Bacteria Isolated in Thailand

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Recently, acetic acid bacteria are intensively studied from the viewpoint of microbial diversity. This study is comprised of two parts. The first is concerned with the identification of strains of the genus *Gluconobacter* based on restriction analysis of 16S-23S rDNA internal transcribed spacer (ITS) and the proposal of the new species and the revived name, *Gluconobacter albidus* (ex Kondo and Ameyama 1958) Yukphan et al. 2005. The second is concerned with the classification and identification of acetic acid bacteria isolated from tropical flowers collected in Thailand and the proposals of the new species, *Asaia krungthepensis* Yukphan et al. 2004 and the new genus *Neoasaia* Yukphan et al. 2006 with *Neoasaia chiangmaiensis* Yukphan et al. 2006.

1) A phylogenetic tree based on 16S-23S rDNA ITS sequences represented that thirteen representative strains of the genus *Gluconobacter* constituted three clusters corresponding to *G. oxydans*, *G. cerinus* and *G. frateurii*. In restriction analysis of 16S-23S rDNA ITS with *Bsp*1286I and *Mbo*II, all the restriction patterns coincided with those of the type strains of the three *Gluconobacter* species except for one strain. However, the exceptional strain was included in the same cluster as the type strain of *G. frateurii*. All the representative strains examined were identified at the species level by sequence and restriction analyses of 16S-23S rDNA ITS.



2) Thirty *Gluconobacter* strains were divided into seven groups by digestion with *Mbo*II and *Bsp*1286I. Twenty-seven strains grouped into Group I, Group III, Group IV, Group V and Group VI were re-identified respectively as *G. oxydans*, *G. frateurii*, *G. cerinus*, *G. frateurii* and *G. oxydans*. There was no strain to be grouped into Group II and re-identified as *G. cerinus*. The remaining two strains of Group VII and one strain of Group VIII were suggested to constitute new taxa by sequencing of 16S-23S rDNA ITS.

3) The two strains, NBRC 3250<sup>T</sup> and NBRC



3273, of Group VII were phenotypically discriminated from the type strains of *G. oxydans*, *G. cerinus* and *G. frateurii*. The two strains showed low levels of DNA-DNA similarity of 8-43%, an independent cluster in phylogenetic trees based on 16S rDNA and 16S-23S rDNA ITS sequences and unique restriction patterns with *MboII*, *BsoBI* and *Tsp509I*. On the basis of these results, *Gluconobacter albidus* (ex Kondo and Ameyama 1958) sp. nov., nom. rev. was proposed for the two strains. Strain NBRC 3250<sup>T</sup> was designated as the type strain.

4) Three bacterial strains were isolated from heliconia flowers (paksasawan in Thai) collected in Bangkok, Thailand. Phylogenetic analysis based on 16S rDNA sequences showed that the isolates were located in the lineage of the genus *Asaia* but constituted a separate cluster. DNA base composition was 60.2-60.5 mol% G+C. The isolates constituted a separate taxon by DNA-DNA hybridization. The isolates had phenotypic characteristics similar to those of the type strains of *A. bogorensis* and *A. siamensis* but grew on maltose. On the basis of these results, *Asaia krungthepensis* sp. nov. was proposed. The type strain was isolate AA08<sup>T</sup> (= BCC 12978<sup>T</sup>).

5) A phylogenetic tree based on 16S-23S rDNA ITS sequences represented that the type strains of the three *Asaia* species constituted three clusters separately. The clustering of thirteen representative strains isolated from Thai sources and phenotypically assigned to the genus *Asaia* was complicated, which was composed of eight clusters. In restriction analysis of 16S-23S rDNA ITS with *TaqI* and *MvaI*, all the representative strains examined were divided into five groups. Nine strains of Group I, Group IV and Group VI were identified as *A. bogorensis*, two strains of Group V were identified as *A. siamensis*, and the remaining two strains of Group III were identified as *A. krungthepensis*. There was no strain belonging to Group II or *A. siamensis*.

6) In restriction analysis of 16S rDNA with *StyI*, *BsaJI* and *SnaBI*, the thirteen representative strains of the genus *Asaia* showed four restriction groups by combination of the resulting restriction patterns. Eight strains of Group A were identified as *A. bogorensis*. Two strains each of Group B and Group C were respectively identified as *A. siamensis* and *A. krungthepensis*. The remaining one strain of Group D was identified as *A. bogorensis*. The 16S rDNA restriction analysis was applicable to the species-level identification as well.

7) An acetic acid bacterium, isolate AC28<sup>T</sup> was isolated from a flower of red

ginger (khing daeng in Thai) collected in Chiang Mai, Thailand. A phylogenetic tree based on 16S rDNA sequences showed that isolate AC28<sup>T</sup> constituted a cluster along with the type strain of *Kozakia baliensis*. However, the isolate was located in an independent cluster in a phylogenetic tree based on 16S-23S rDNA ITS sequences. Restriction analysis of 16S-23S rDNA ITS discriminated the isolate from the type strains of *Asaia* and *Kozakia* species. Phenotypically, the isolate was differentiated especially from *Asaia* and *Kozakia* strains. On the basis of these results, *Neoasaia* gen. nov. was proposed with *Neoasaia chiangmaiensis* sp. nov. The type strain was isolate AC28<sup>T</sup> (= BCC 15763<sup>T</sup>).

8) In six phylogenetic trees based on 16S rDNA, 16S-23S rDNA, 23S rDNA, 16S rDNA/16S-23S rDNA ITS/23S rDNA, 23S rDNA D1/D2 and 23S rDNA down-stream sequences, *A. bogorensis*, *A. siamensis* and *A. krungthepensis* usually constituted a cluster, i.e., the *Asaia* cluster. The single species, *Swaminathania salitolerans* of the genus *Swaminathania* was located outside but inside the *Asaia*



cluster in the 23S rDNA and the 23S rDNA down-stream phylogenetic trees. *Kozakia baliensis* and *N. chiangmaiensis* were usually distant phylogenetically from the three species of the genus *Asaia*. These data demonstrated that the genera *Swaminathania* and *Asaia* are “phylogenetically congeneric” in the 23S rDNA sequence analysis.