Tanticharoenia sakaeratensis gen. nov., sp. nov., a New Osmotolerant Acetic Acid Bacterium in the α-Proteobacteria

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Tanticharoenia sakaeratensis gen. nov., sp. nov. is proposed for three strains isolated from soil collected in Thailand. The three strains, AC37T, AC38, and AC39, were included within a lineage comprising the genera Asaia, Kozakia, Swaminathania, Neosaaia, Acetobacter, Gluconobacter, and Saccharibacter in a phylogenetic tree based on 16S rRNA gene sequences, but formed a quite different, independent cluster. Pair-wise sequence similarities of strain AC37 were 96.5–92.1% to the type strains of Acetobacter aceti, Gluconobacter oxydans, Acidomonas methanolicus, Gluconacetobacter liquefaciens, Asaia bogorensis, Kozakia baliensis, Swaminathania salitolerans, Saccharibacter floridicus, Neosaaia chiangmaiensis, and Granulibacter bethesdensis. The three strains had DNA base compositions comprising respectively 65.6, 64.5, and 65.6 mol % G+C with a range of 1.1 mol %, and formed a single species. Phenotypically, the three strains did not oxidize acetate or lactate, but grew on 30% D-glucose (w/v). Chemotaxonomically, they had Q-10. The type strain is AC37T (= BCC 15772T = NBRC 103193T).

Key words: Tanticharoenia sakaeratensis gen. nov., sp. nov.; acetic acid bacteria; 16S rRNA gene sequences; Acetobacteraceae; taxonomy


During the course of studies on microbial diversity in the natural environment of Thailand, we isolated phylogenetically and phenotypically unique acetic acid bacteria on August 17, 2002. This paper proposes Tanticharoenia sakaeratensis gen. nov., sp. nov. for the isolates in the family Acetobacteraceae.

Materials and Methods

Three strains, AC37T, AC38, and AC39, were used in this study. They were isolated from soil collected at Sakaerat, Nakhon Ratchasima, Thailand by an enrichment culture approach with glucose/ethanol/acetic acid medium, which contained 1.5% D-glucose, 0.5% ethanol (v/v), 0.8% peptone, 0.5% yeast extract, and...
0.3% acetic acid (v/v), and was adjusted to pH 3.5, among the four kinds of media used.7,8,11,14–16 Acetobacter aceti NBRC 14818T, Gluconobacter oxydans NBRC 14819T, Gluconacetobacter liquefaciens NBRC 12388T, Acidomonas methanolica NRIC 0498T, Asaia bogorensis NBRC 16594T, Kozakia baliensis NBRC 16664T, Swaminathania salitolerans LMG 2129T, and Neoasaia chiangmaiensis BCC 15763T were used as reference strains.

PCR amplification for 16S rRNA genes was made, and amplified 16S rRNA genes were sequenced and analyzed, as described previously.16–18 Multiple sequence alignment was made with the program CLUSTAL x (version 1.8).19 Distinct matrices for the aligned sequences were calculated using the two-parameter method of Kimura.20 A phylogenetic tree based on 16S rRNA gene sequences for 1,374 bases was constructed by the neighbor-joining method of Saitou and Nei.21 The robustness of individual branches was estimated by bootstrapping with 1,000 replications.22 In addition, pair-wise sequence similarities were determined for 1,390 bases.

Chromosomal DNA was prepared as described previously.11,16,17 DNA base composition was determined by the method of Tamaoka and Komagata.23 Levels of DNA-DNA similarity (%) were determined by the fluorometric method, as described by Ezaki et al.24 Isolated, single-stranded, labeled DNAs were hybridized with DNAs from test strains at 50.0°C for 3 h in 2 × SSC containing 50% formamide. Fluorescence intensity was measured with Fluoroscan Ascent (Thermo Labsystems, Helsinki, Finland) at wavelengths of 335 nm for excitation and 460 nm for emission. The highest and lowest values obtained in each test sample were excluded, and the mean of the remaining three values was taken as the DNA-DNA similarity value.

Phenotypic characteristics were determined by the methods of Asai et al.,25 Yamada et al.,7,14 Katsura et al.,26 Lisdiyanti et al.,8 and Yukphan et al.11,16,17 Ubiquinone isoprenologue was determined by the method of Yamada et al.27

Results and Discussion

In a phylogenetic tree based on 16S rRNA gene sequences, the three strains, AC37T, AC38, and AC39, were included within a lineage comprising the genera Asaia, Kozakia, Swaminathania, Neoasaia, Acetobacter, Gluconobacter, and Saccharibacter, but formed a quite different, independent cluster (Fig. 1). Pair-wise sequence similarities of strain AC37T were calculated to be 95.3, 95.8, 96.3, 96.5, 95.8, 95.6, 92.5, 92.1, and 94.6% respectively to the type strains of Acetobacter aceti, Gluconobacter oxydans, Acidomonas methanolic acid, Gluconacetobacter liquefaciens, Asaia bogorensis, Kozakia baliensis, Swaminathania salitolerans, Saccharibacter floriboca, Neoasaia chiangmaiensis, and Granulibacter bethesdensis. The sequence similarity was 100% among the three strains. The phylogenetic data obtained above indicate that the three strains are distinguished at the generic level from the above-mentioned taxa.

The three strains, AC37T, AC38, and AC39, had DNA base compositions respectively of 65.6, 64.5, and 65.6 mol % G+C with a range of 1.1 mol %. The calculated DNA G+C contents were higher in the acetic acid bacteria tested (Table 1).

Labeled DNA of strain AC37T represented DNA-DNA similarities of 100, 100, 100, and 2% respectively to DNAs from strains AC37T, AC38, and AC39, and Gluconacetobacter liquefaciens NBRC 12388T, which was used as a reference strain. When isolate AC38 was labeled, the calculated DNA-DNA similarities were 97 and 100% respectively to strains AC37T and AC38. Similarly, the DNA-DNA similarity to strain AC37T was 100% when strain AC39 was labeled. The genetic data obtained above indicate that the three isolates are to be classified in an identical species.

The phenotypic characteristics of strains AC37T, AC38, and AC39 are described in the genus and the species descriptions.

The three strains were especially unique physiologically in that they showed no oxidation of acetate or lactate, vigorous growth on 30% d-glucose (w/v), and no assimilation of ammoniac nitrogen on d-glucose, d-mannitol, or ethanol. They had Q-10 as a major isoprenoid quinone. They were also unique ecologically, in that they were isolated from soil, differing in this respect from most acetic acid bacteria.

In the acetic acid bacteria, 10 genera are presently described in the family Acetobacteraceae: Acetobacter, Gluconobacter, Acidomonas, Gluconacetobacter, Asaia, Kozakia, Swaminathania, Saccharibacter, Neoasaia, and Granulibacter. The three strains form the 11th genus.

Morphologically, the three strains are discriminated by an absence of motility and of flagellation from peritrichously and polarly flagellated, motile strains of the genera Acetobacter, Gluconobacter, Acidomonas, Gluconacetobacter, Asaia, Kozakia, Swaminathania, Saccharibacter, Neoasaia, and Granulibacter species. In addition, the three strains are distinguished from strains of the remaining two genera, Gluconobacter and Neoasaia. Growth on 30% d-glucose (w/v) was found in the three isolates but not in Gluconobacter strains, and a water-soluble brown pigment was intensely produced in the three isolates but not in the Neoasaia strain. Characteristics differentiating the three strains from the Neoasaia strain were found additionally in weak growth on glutamate agar, dihydroxyacetone production from glycerol, and an absence of assimilation of ammoniac nitrogen in the presence of d-mannitol (Table 1).
As described above, the three strains, AC37T, AC38, and AC39, are phylogenetically, genetically, chemotaxonomically, and phenotypically discriminated at the generic level from strains of species of the above-mentioned 10 genera, and should be classified in a separate genus with a single species (Table 1). The name *Tanticharoenia sakaeratensis* gen. nov., sp. nov. is therefore proposed for the three strains.

**Description of Tanticharoenia gen. nov.**

*Tanticharoenia* [Tan.ti.cha.ro.e’ni.a. N. L. fem. n. *Tanticharoenia* after Dr. Morakot Tanticharoen, Director, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani, Thailand, who contributed to studies of acetic acid bacteria, and especially to their systematic study].

Gram-negative rods, non-motile, measuring 0.6–0.8 × 1.0–1.6 μm. Colonies were creamy and smooth with entire margin when grown on glucose/ethanol/peptone/yeast extract/calcium carbonate agar. Produces acetic acid from ethanol. Does not oxidize acetate or lactate. Grows on glutamate agar (weakly positive) and mannitol agar. Growth on 30% D-glucose (w/v) but not of 1% KNO₃. Does not grow on methanol. Ammoniac nitrogen is not assimilated in the presence of D-glucose, D-mannitol, or ethanol as carbon source. Production of dihydroxyacetone from glycerol is positive. Produces 2-keto-D-gluconate, 5-keto-D-gluconate, and 2,5-diketo-D-gluconate from D-glucose. Produces a water-soluble brown pigment intensely on glucose/peptone/yeast extract/calcium carbonate agar. Acid is produced from D-glucose, D-galactose, D-xylene, L-arabinose, D-fructose (weakly positive), meso-erythritol, glycerol, melibiose, sucrose (weakly positive), raffinose (weakly positive), or ethanol, but not from D-arabinose, L-rhamnose, L-sorbose, D-mannitol, D-sorbitol, dulcitol, maltose, or lactose. Grows on D-glucose, D-galactose, D-xylene, D-arabinose, D-fructose, D-mannitol, D-sorbitol, meso-erythritol, glycerol, or sucrose, but not on D-mannose, D-arabinose, L-rhamnose, L-sorbose, dulcitol, maltose, lactose, melibiose, raffinose, or ethanol. The major isoprenoid quinone is Q-10. DNA base composition is 64.5–65.6 mol % G+C with a range of 1.1 mol %. The type species is *Tanticharoenia sakaeratensis* sp. nov.
Description of Tanticharoenia sakaeratensis sp. nov.

Tanticharoenia sakaeratensis (sa.k.a.e.rat.en’sis. N. L. fem. adj. sakaeratensis of or pertaining to Sakaerat, Nakhon Ratchasima, Thailand, where the type strain was isolated).

Characteristics are the same as those described for the genus. The type strain is AC37T (= BCC 15772T = NBRC 103193T). The DNA base composition of the type strain is 65.6 mol% G+C. Strains were isolated from soil.

Acknowledgments

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References

4) Yamashita, S., Uchimura, T., and Komagata, K.,

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+, positive; –, negative; w, weakly positive; vw, very weakly positive; d, delayed; v, variable; nd, not determined.

Cited from "Loganathan and Nair" except for the data on Neosaia chiangmaiensis strain AC28 and Tanticharoenia sakaeratensis strain AC37, Jojima et al.,

Yukplan et al., Greenberg et al., Yamada et al., and Lidyantzi et al.

Abbreviations: T, Tanticharoenia; A, Acetobacter; G, Gluconobacter; Ac, Acidomonas; K, Kozakia; S, Saccharibacter; N, Neosaia; Gr, Granulibacter; 1, Tanticharoenia sakaeratensis strain AC37; 2, Acetobacter aceti NBRC 14818T; 3, Gluconobacter oxydans NBRC 14819T; 4, Acidomonas methanolicus NRRL 04985; 5, Gluconacetobacter liquefaciens NBRC 125888T; 6, Acidia bogorensis NBRC 16594T; 7, Kozakia baliensis NBRC 166647T; 8, Swaminathania saltolerans strain PAS1T; 9, Saccharibacter florculus strain S-877T; 10, Neosaia chiangmaiensis strain AC28; 11, Granulibacter bethesdensis CGDNIH15T.

Per, peritrichous; Pol, polar.


