NOTE

Transfer of *Acetobacter oboediens* Sokollek et al. 1998 and *Acetobacter intermedius* Boesch et al. 1998 to the genus *Gluconacetobacter* as *Gluconacetobacter oboediens* comb. nov. and *Gluconacetobacter intermedius* comb. nov.

Yuzo Yamada

Tel: +81 54 635 2316. Fax: +81 54 635 2316. e-mail: yamada-yuzo@mub.biglobe.ne.jp

Laboratory of General and Applied Microbiology,
Department of Applied Biology and Chemistry,
Faculty of Applied Bioscience, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

***Acetobacter oboediens* Sokollek et al. 1998 and *Acetobacter intermedius* Boesch et al. 1998 are transferred to the genus *Gluconacetobacter* as *Gluconacetobacter oboediens* comb. nov. and *Gluconacetobacter intermedius* comb. nov. because, on the basis of their 16S rRNA gene sequences, the type strains of both species are located in the cluster of the genus *Gluconacetobacter* along with those of *Gluconacetobacter xylinus*, *Gluconacetobacter europaeus*, *Gluconacetobacter hansenii*, *Gluconacetobacter liquefaciens* (the type species) and *Gluconacetobacter diazotrophicus*. The significance of growth on mannitol agar and the presence of a ubiquinone isoprenologue composed of Q-10 is discussed for characterization of the genus *Gluconacetobacter*.**

Keywords: *Gluconacetobacter oboediens* comb. nov., *Gluconacetobacter intermedius* comb. nov., acetic acid bacteria, new combination

Recently, Sokollek *et al.* (1998) described *Acetobacter oboediens* and Boesch *et al.* (1998) described *Acetobacter intermedius*. *Acetobacter oboediens* LTH 2460^T and *Acetobacter intermedius* TF2^T were actually included within the cluster of the genus *Gluconacetobacter* in phylogenetic trees constructed on the basis of complete or near-complete 16S rRNA gene sequences by Sokollek *et al.* (1998), Boesch *et al.* (1998) and Yamada *et al.* (2000). The type strains of both species constituted a subcluster along with those of *Gluconacetobacter xylinus*, *Gluconacetobacter hansenii* and *Gluconacetobacter europaeus*. This subcluster 1 was different from subcluster 2, in which *Gluconacetobacter liquefaciens* and *Gluconacetobacter diazotrophicus* were located.

The genus *Gluconoacetobacter* [sic] was introduced for acetate- and lactate-oxidizing acetic acid bacteria equipped with Q-10 as the major ubiquinone (Yamada *et al.*, 1997a). The generic name was validated and corrected as *Gluconacetobacter* nom. corrig. in accordance with Rule 61 of the Bacteriological Code (Yamada *et al.*, 1998).

Boesch *et al.* (1998) stated that the name *Gluconacetobacter* was not valid on the basis of the note of Stackebrandt & Goebel (1994), because Yamada *et al.*

(1997a) proposed the new genus without a complete or near-complete 16S rRNA gene sequence analysis. However, Stackebrandt & Goebel (1994) did not mention that such a proposal cannot be 'valid', though they noted that careful evaluation of the partial 16S rRNA sequence is required. In addition, Yamada *et al.* (1997a, b) discussed the phylogenetic and taxonomic positions of the species to be accommodated in the genus *Gluconacetobacter* by the use of their partial 16S rRNA sequences and the complete 16S rRNA gene sequences reported by Sievers *et al.* (1994a, b). The phylogenetic trees constructed by Sievers *et al.* (1994a, b) and by Yamada *et al.* (1997a, b, 2000) gave comparable results.

Franke *et al.* (1999) recognized the genus *Gluconacetobacter* and proposed a new species, *Gluconacetobacter sacchari*. In their paper, they mentioned "some newly sequenced and misidentified species, [*Acetobacter*] *oboediens* and '[*Acetobacter*] *intermedius*', which should be reassigned to the genus *Gluconacetobacter*."

Yamada *et al.* (2000) calculated the 16S rRNA gene sequence similarities between the type strains of *Gluconacetobacter* subcluster 1 to be 98.3–99.9% and those between the type strains of species belonging to

Gluconacetobacter subcluster 1 and 2 to be 96.5–97.3%. In contrast, 16S rRNA gene sequence similarities of the type strains within the genera *Acetobacter* and *Gluconobacter* were 96.7–99.4% and 98.1–98.9%, respectively. The 16S rRNA gene sequence similarities of *Acetobacter oboediens* LTH 2460^T and *Acetobacter intermedius* TF2^T to the type strains of *Acetobacter aceti*, *Gluconobacter oxydans*, *Acidomonas methanolica* and *Asaia bogorensis* were 95.6–95.7, 95.2–95.3, 95.7–95.9 and 96.6–96.7%, respectively.

Sokollek *et al.* (1998) and Boesch *et al.* (1998) reported similarities that were similar to those proposed by Yamada *et al.* (2000). These data all support the classification of *Acetobacter oboediens* and *Acetobacter intermedius* in the genus *Gluconacetobacter*.

Boesch *et al.* (1998) mentioned neither the quinone system of *Acetobacter intermedius* nor the usefulness of the ubiquinone isoprenologue for differentiation of the genus *Gluconacetobacter* from the genus *Acetobacter*, which had already been described by Yamada *et al.* (1969, 1997a, b). The genus *Gluconacetobacter* is characterized by Q-10 and the genus *Acetobacter* is characterized by Q-9. The type strain (LTH 2458^T) of *Acetobacter pomorum* is actually equipped with Q-9 as the major ubiquinone (Sokollek *et al.*, 1998) and is located phylogenetically within the cluster of the genus *Acetobacter* along with *Acetobacter aceti* and *Acetobacter pasteurianus* in the trees constructed by Sokollek *et al.* (1998) and by Yamada *et al.* (2000).

Boesch *et al.* (1998) mentioned in their paper that “one of the characteristics that differentiates the four proposed genera, growth on mannitol agar only for the genera *Gluconobacter* and *Gluconacetobacter*, is definitely wrong.” In addition, they cited ‘YPM agar’ from the BCCM LMG List of Cultures Bacteria 1989, which corresponds to medium 13 (mannitol/yeast extract/peptone agar). Medium 13 or medium 17 (glucose/yeast extract/CaCO₃ agar) is suggested by Janssens *et al.* (1998) for the cultivation of *Acetobacter*, *Gluconobacter* and *Frateriia* strains. But this does not mean that all *Acetobacter* strains can indeed grow on mannitol agar. In fact, Asai *et al.* (1964) reported that *Gluconobacter* strains and the peritrichously flagellated intermediate strains (i.e. strains of *Gluconacetobacter liquefaciens*) grew on mannitol agar, but *Acetobacter* strains showed scanty growth on this medium with the exception of two strains. In addition, Gosselé *et al.* (1983) described that 90% of strains of Phenon 2 (*Acetobacter liquefaciens*, 10 strains used), 50% of strains of Subphenon C (*Acetobacter hansenii*, 12 strains used), 100% of strains of Subphenon D (*Acetobacter aceti*, 7 strains used) and 15% of strains of Subphenon E (*Acetobacter pasteurianus*, 66 strains used) grew on mannitol medium. Of 98 strains that they examined, only 36% of strains (about 35 strains) grew on mannitol medium. Since *Acetobacter liquefaciens*, *Acetobacter hansenii* and *Acetobacter xylinus* were transferred to the genus *Gluconacetobacter* (Yamada *et al.*, 1997a), only Subphenon D and Subphenon E can be regarded as *Acetobacter* species.

Therefore, of 73 strains tested, only 17 (23%) grew on mannitol medium. Thus, the statement of Boesch *et al.* (1998) that all *Acetobacter* strains can grow on mannitol agar has lost standing.

Members of the genus *Gluconacetobacter* can be characterized by the combination of the ubiquinone isoprenologue composed of Q-10 as the major quinone, growth on mannitol agar for the majority of members and their 16S rRNA gene sequence. As a consequence, *Acetobacter oboediens* and *Acetobacter intermedius* should be transferred to the genus *Gluconacetobacter* as new combinations.

Description of *Gluconacetobacter oboediens* (Sokollek, Hertel & Hammes 1998) comb. nov.

Basonym *Acetobacter oboediens* Sokollek, Hertel & Hammes. The description of the species was provided by Sokollek *et al.* (1998). The type strain is LTH 2460^T (= DSM 11826^T).

Description of *Gluconacetobacter intermedius* (Boesch, Trčák, Sievers & Teuber 1998) comb. nov.

Basonym *Acetobacter intermedius* Boesch, Trčák, Sievers & Teuber. The description of the species was provided by Boesch *et al.* (1998). The type strain is TF2^T (= DSM 11804^T).

References

- Asai, T., Iizuka, H. & Komagata, K. (1964). The flagellation and taxonomy of genera *Gluconobacter* and *Acetobacter* with reference to the existence of intermediate strains. *J Gen Appl Microbiol* **10**, 95–126.
- Boesch, C., Trčák, J., Sievers, M. & Teuber, M. (1998). *Acetobacter intermedius* sp. nov. *Syst Appl Microbiol* **21**, 220–229.
- Franke, I. H., Fegan, M., Hayward, C., Leonard, G., Stackebrandt, E. & Sly, L. I. (1999). Description of *Gluconacetobacter sacchari* sp. nov., a new species of acetic acid bacterium isolated from the leaf sheath of sugar cane and from the pink sugar-cane mealy bug. *Int J Syst Bacteriol* **49**, 1681–1693.
- Gosselé, F., Swings, J., Kersters, K., Pauwels, P. & De Ley, J. (1983). Numerical analysis of phenotypic features and protein gel electrophoregrams of a wide variety of *Acetobacter* strains. Proposal for the improvement of the taxonomy of the genus *Acetobacter* Beijerinck 1898, 215. *Syst Appl Microbiol* **4**, 338–368.
- Janssens, D., Vereecke, C., Vanhonacker, K. & 7 other editors (1998). *BCCM LMG Catalogue Bacteria 1998*. Universiteit Gent (RUG) Laboratorium voor Microbiologie, Belgian Co-ordinated Collections of Micro-organisms, Belgian Office for Scientific, Technical and Cultural Affairs.
- Sievers, M., Ludwig, W. & Teuber, M. (1994a). Phylogenetic positioning of *Acetobacter*, *Gluconobacter*, *Rhodopila* and *Acidiphilium* species as a branch of acidophilic bacteria in the α -subclass of *Proteobacteria* based on 16S ribosomal DNA sequences. *Syst Appl Microbiol* **17**, 189–196.
- Sievers, M., Ludwig, W. & Teuber, M. (1994b). Revival of the species *Acetobacter methanolicus* (ex. Uhlig *et al.*, 1986) nom. rev. *Syst Appl Microbiol* **17**, 352–354.

- Sokollek, S. J., Hertel, C. & Hammes, W. P. (1998).** Description of *Acetobacter oboediens* sp. nov. and *Acetobacter pomorum* sp. nov., two new species isolated from industrial vinegar fermentations. *Int J Syst Bacteriol* **48**, 935–940.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Yamada, Y., Aida, K. & Uemura, T. (1969).** Enzymatic studies on the oxidation of sugar and sugar alcohol. V. Ubiquinone of acetic acid bacteria and its relation to classification of *Gluconobacter* and *Acetobacter*, especially of the so-called intermediate strains. *J Gen Appl Microbiol* **15**, 186–196.
- Yamada, Y., Hoshino, K. & Ishikawa, T. (1997a).** The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA: the elevation of the subgenus *Gluconoacetobacter* to the generic level. *Biosci Biotechnol Biochem* **61**, 1244–1251.
- Yamada, Y., Hoshino, K. & Ishikawa, T. (1997b).** The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA. *Bull Fac Agric Shizuoka Univ* **47**, 37–44.
- Yamada, Y., Hoshino, K. & Ishikawa, T. (1998).** *Gluconacetobacter* corrig. (*Gluconoacetobacter* [sic]). In *Validation of Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 64. *Int J Syst Bacteriol* **48**, 327–328.
- Yamada, Y., Katsura, K., Kawasaki, H., Widyastuti, Y., Saono, S., Seki, T., Uchimura, T. & Komagata, K. (2000).** *Asaia bogorensis* gen. nov., sp. nov., an unusual acetic acid bacterium in the α -*Proteobacteria*. *Int J Syst Evol Microbiol* **50**, 823–829.