Short Communication

Gluconobacter roseus (ex Asai 1935) sp. nov., nom. rev., a pink-colored acetic acid bacterium in the Alphaproteobacteria

Taweesak Malimas, Pattaraporn Yunkhan, Mai Takahashi, Yuki Muramatsu, Mika Kaneyasu, Wanchern Potacharoen, Somboon Tanasupawat, Yasuyoshi Nakagawa, Morakot Tanticharoen, and Yuzo Yamada

1 BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani 12120, Thailand
2 Biological Resource Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation (NITE), Kisarazu 292-0818, Japan
3 Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

(Received October 9, 2007; Accepted December 26, 2007)

Key Words——acetic acid bacteria; Alphaproteobacteria; Gluconobacter roseus sp. nov., nom. rev.; restriction analysis; 16S–23S rDNA ITS sequences

The genus Gluconobacter Asai 1935 is characterized physiologically by no ability to oxidize acetate and chemotaxonomically by ubiquinone-10 (Q-10) as a major isoprenoid quinone (Asai, 1935; Yamada et al., 1969). These two characteristics clearly differentiates the genus Gluconobacter from the genus Acetobacter Beijerinck 1898, which is characterized by the ability to oxidize acetate to carbon dioxide and water and by Q-9. The phenotypic features including no oxidation of acetate and lactate and Q-10 are actually utilized for classifying isolated acetic acid bacteria into the genus Gluconobacter (Tanaka et al., 1999; Yamada et al., 1999).

Asai (1935) first proposed “Gluconobacter roseus,” a new species for the strain that was morphologically pink-colored and physiologically did not oxidize acetate. However, this species was not accepted in The Approved List of Bacterial Names (Skerman et al., 1980).

Yamada and Akita (1984a) represented that “G. roseus” IFO 3990, strain IFO 3266 (=G. kondonii NBRC 3266T), strains IFO 3250 and IFO 3273 (=G. albidus NBRC 3250T and NBRC 3273), and G. oxydans subsp. sphaericus IFO 12467T (Ameyama, 1975) formed clusters different from G. oxydans NCIB 9013T in the electrophoretic pattern analysis of six enzymes. The calculated similarity values of the strains to the type strain of G. oxydans were respectively 50, 33, 33, and 18%.
Yukphan et al. (2004a, b) examined a number of *Gluconobacter* strains, which were once isolated in Japan, deposited, and maintained in NBRC, for a 16S–23S rDNA ITS restriction analysis. Of the forty strains examined, strain NBRC 3990 of Group VI showed a different kind of restriction patterns, when digested with restriction endonuclease *Bsp*1286I, among the *Gluconobacter* strains examined. A phylogenetic analysis based on 16S–23S rDNA ITS sequences represented that strain NBRC 3990 formed a cluster along with *G. oxydans* NBRC 14819T, but the phylogenetic distance between the strain and the type strain was not very close. Considering the peculiar restriction pattern and the phylogenetic relationship obtained, strain NBRC 3990 was supposed to form a separate taxon. However, the strain was re-identified as *G. oxydans*.

Takahashi et al. (2006) showed a similar phylogenetic relationship of strain NBRC 3990 in a 16S–23S rDNA ITS sequence analysis, although the strain was completely included in the cluster of *G. oxydans* NBRC 14819T in a phylogenetic tree based on 16S rDNA sequences. The molecular-biological data obtained above suggested that strain NBRC 3990 constitutes a new taxon.

This paper describes *Gluconobacter roseus* (ex Asai 1935) sp. nov., nom rev., for the pink-colored strain, NBRC 3990T.


Phylogenetic analyses based on 16S rDNA sequences and 16S–23S rDNA ITS sequences were made for strain NBRC 3990T, as described previously (Malimas et al., 2006, 2007; Yukphan et al., 2004b, c). Multiple sequence alignments were made with the program CLUSTAL X (version 1.81) (Thompson et al., 1997). Sequence gaps and ambiguous bases were excluded. Distance matrices were calculated by the two-parameter method of Kimura (1980). The neighbor-joining method was used for constructing phylogenetic trees (Saitou and Nei, 1987). Robustness of individual branches was estimated by bootstrapping with 1,000 replications (Felsenstein, 1985).

In a phylogenetic tree based on 16S rDNA sequences of 1,428 bases, strain NBRC 3990T formed an identical cluster with *G. oxydans* NBRC 14819T (Takahashi et al., 2006), and the two strains were closely related phylogenetically (Fig. 1A). In a phylogenetic tree based on 16S–23S rDNA ITS sequences of 690 bases, strain NBRC 3990T formed a cluster along with *G. oxydans* NBRC 14819T likewise (Fig. 1B). However, the phylogenetic distance between strain NBRC 3990T and *G. oxydans* NBRC 14819T was almost identical with that between *G. albidus* NBRC 3250T and *G. kondonii* NBRC 3266T (Takahashi et al., 2006; Yukphan et al., 2004a).

The calculated pair-wise 16S rDNA sequence similarities of strain NBRC 3990T for 1,430 bases were 99.9, 99.3, 99.2, 97.9, 98.1, and 97.9% respectively to the type strains of *G. oxydans*, *G. albidus*, *G. kondonii*, *G. cerinus*, *G. frateurii*, and *G. thailandicus*. In the 16S–23S rDNA ITS sequences, the calculated pair-wise sequence similarities of the strain for 714 bases were respectively 96.3, 95.4, 93.4, 85.4, 83.1, and 83.6%.

The 16S–23S rDNA ITS sequence of strain NBRC 3990T was analyzed by computer analysis using the program NEBcutter (version 2.0, New England Bio-Labs, Beverley, MA, USA) for the recognition sequence, 5′-G(G/A/T)GC(C/A/T)C-3′ of restriction endonuclease *Bsp*1286I (Malimas et al., 2006; Yukphan et al., 2004b). The 16S–23S rDNA ITS PCR products of strain NBRC 3990T and the type strains of the six *Gluconobacter* species were prepared and examined for restriction analysis using restriction endonucleases *Mbo*I (Fermentas, Hanover, MD, USA) and *Bsp*1286I (New England BioLabs) (Malimas et al., 2006; Trček and Teuber, 2002; Yukphan et al., 2004a, b). As shown in Fig. 2, strain NBRC 3990T was clearly discriminated from the type strains of the six *Gluconobacter* species by producing restriction fragments comprising 189, 177, 173, 92 and 82 bp, which differed from those of *G. oxydans* NBRC 14819T comprising 362, 177, 92, and 82 bp, when digested with restriction endonuclease *Bsp*1286I. The phylogenetic and molecular-biological data obtained above suggested that strain NBRC 3990T constitutes a new species.

Extraction and isolation of bacterial chromosomal DNAs were performed, as described previously (Ezaki et al., 1983; Marmur, 1961; Saito and Miura, 1963; Yukphan et al., 2004c, d). DNA base composition was determined by the method of Tamaoka and Komagata (1984). The DNA G+C content of strain NBRC 3990T was 60.5 mol%. The data obtained showed that strain
A. G. albidus NBRC 3250T (AB162710)
G. kondonii NBRC 3266T (AB162712)
G. oxydans NBRC 14819T (AB111899)
G. roseus NBRC 3990T (AB163865)
G. cerinus NBRC 3267T (AB111898)
G. frateurii NBRC 3264T (AB127941)
G. thailandicus strain F149-1T (AB128050)
A. aceti NCIB 8621T (X74066)

B. G. albidus NBRC 3250T (AB162710)
G. kondonii NBRC 3266T (AB162712)
G. oxydans NBRC 14819T (AB111899)
G. roseus NBRC 3990T (AB163865)
G. cerinus NBRC 3267T (AB111898)
G. frateurii NBRC 3264T (AB127941)
G. thailandicus strain F149-1T (AB128050)
A. aceti NCIB 8621T (X74066)

Fig. 1. Phylogenetic relationships of *Gluconobacter roseus* NBRC 3990T.
The phylogenetic trees based on 16S rDNA sequences (A) and 16S–23S rDNA ITS sequences (B) were constructed by the neighbor-joining method. The type strain of *Acetobacter aceti* was used for an outgroup. Numerals at the nodes indicate bootstrap percentages derived from 1,000 replications.

Fig. 2. Restriction analysis of 16S–23S rDNA ITS PCR products from *Gluconobacter roseus* NBRC 3990T by digestion with restriction endonucleases *MboII* and *Bsp1286I*.
The restriction patterns were obtained by digestion with restriction endonucleases *MboII* (A) and *Bsp1286I* (B). For estimation of the resulting restriction fragments, 50-bp DNA markers were used in agarose gel electrophoresis. Abbreviations: 1, *G. oxydans* NBRC 14819T; 2, *G. cerinus* NBRC 3267T; 3, *G. frateurii* NBRC 3264T; 4, *G. albidus* NBRC 3250T; 5, *G. thailandicus* BCC 14116T; 6, *G. kondonii* NBRC 3266T; 7, *G. roseus* NBRC 3990T; M, 50-bp DNA marker.

NBRC 3990T is included in the higher DNA G+C content-having group or the sublineage of *G. oxydans* (Yamada and Akita, 1984a; Yamada et al., 1984, 2000).

DNA-DNA hybridization was carried out by the photobiotin-labeling method using microdilution wells, as described previously (Ezaki et al., 1989; Malimas et al., 2007; Yukphahn et al., 2004c, d). Levels of DNA-DNA similarity were determined colorimetrically (Verlander, 1992). The color intensity was measured at A450 on a model VersaMax microplate reader ( Molecular Devices, Sunnyvale, CA, USA). When an isolated, single-stranded, and labeled DNA from strain NBRC 3990T was hybridized with DNAs from test strains in 2 × SSC and 50% formamide at 49.0°C for 15 h, strain NBRC 3990T represented DNA-DNA similarities of 49, 47, 37, 100, 8, 9, 10, and 3% respectively to *G. oxydans* NBRC 14819T, *G. albidus* NBRC 3250T, *G. kondonii* NBRC
3266T, strain 3990T, G. cerinus NBRC 3267T, G. frateurii NBRC 3264T, G. thailandicus BCC 14116T, and A. aceti NBRC 14818T. A labeled DNA of G. oxydans NBRC 14819T gave DNA-DNA similarities respectively of 100, 37, 41, 34, 8, 8, 8, and 3%, and those of G. albidus NBRC 3250T and G. kondonii NBRC 3266T gave DNA-DNA similarities respectively of 37, 100, 51, 32, 11, 9, 10, and 3% and 37, 52, 100, 38, 16, 7, 9, and 4%. The data obtained above indicated that strain 3990T is genetically separated at the species level from the type strains of the six Gluconobacter species.

Strain NBRC 3990T was examined for morphological, physiological, biochemical, and chemotaxonomic characteristics, as described previously (Asai et al., 1964; Gosselé et al., 1983; Katsura et al., 2002; Malimas et al., 2007; Mason and Claus, 1989; Yamada et al., 1969, 1976, 1999; Yukphan et al., 2004c, d). The phenotypic characteristics determined are described in the species description of Gluconobacter roseus, the new species and the revived name.

In the genus Gluconobacter, six species are currently described: Gluconobacter oxydans (Henneberg 1897) De Ley 1961 (the type species), Gluconobacter cerinus Yamada and Akita 1984, Gluconobacter frateurii Mason and Claus 1989, Gluconobacter albidus (ex Kondo and Ameyama 1958) Yukphan et al. 2005, Gluconobacter thailandicus Tanasupawat et al. 2005, and Gluconobacter kondonii Malimas et al. 2008 (Katsura et al., 2002; Mason and Claus, 1989; Malimas et al., 2007, 2008; Skerman et al., 1980; Tanaka et al., 1999; Tanasupawat et al., 2004, 2005; Yamada and Akita, 1984a, b; Yamada et al., 1999; Yukphan et al., 2004d, 2005).

Strain NBRC 3990T produced acid very weakly from meso-erythritol, differing from the type strains of G. oxydans, G. kondonii, G. albidus (weakly positive), G. cerinus, G. frateurii (weakly positive), and G. thailandicus, and from maltose, differing from the type strains of G. oxydans and G. frateurii (weakly positive) (Table 1). Concerning growth on pentitol, the strain showed growth on D-arabitol and meso-ribitol (very weakly positive) but not on L-arabitol, differing from the type strains of G. frateurii and G. thailandicus (weakly positive). The strain was distinguished from the type strains of G. cerinus, G. frateurii, and G. thailandicus, the members of the lower DNA G+C content-having group; the former required nicotinic acid for growth, however, the latter grew without nicotinic acid. Phylogenetically, strain NBRC 3990T had a similar 16S rDNA sequence showing 99.9% similarity to the type strain of G. oxydans; however, the former was distinguished from the latter by a 16S–23S rDNA ITS sequence showing 96.3% similarity. Additionally, the former is clearly discriminated from the latter by the G. roseus type of restriction patterns, when digested with restriction endonuclease Bsp1286I, in the 16S–23S rDNA ITS restriction analysis.

As described above, strain NBRC 3990T is genetically, molecular-biologically, morphologically, physiologically, and biochemically distinguished from the type strains of the six Gluconobacter species, i.e., G. oxydans, G. albidus, G. kondonii, G. cerinus, G. frateurii, and G. thailandicus (Table 1). A new species is therefore proposed for the pink-colored strain, NBRC 3990T in the higher DNA G+C content-having group, or the sublineage of G. oxydans, and the name of the new species is Gluconobacter roseus.

Description of Gluconobacter roseus (ex Asai 1935) sp. nov., nom. rev.

Gluconobacter roseus (ro.se’us. L. masc. adj. roseus rose, referring to the colony color of the type strain)

Gram-negative rods, measuring 0.6–0.8 × 1.0–1.4 μm, with polar flagella. Grows at pH 3.5 and at 35°C. However, the maximum growth is shown at pH 5.5 and at 25°C. Does not oxidize acetate or lactate. Grows on mannitol agar but not on glutamate agar. Produces dihydroxyacetone from glycerol, and 2-keto-d-gluconate and 5-keto-d-gluconate from d-gluconate, but not 2,5-diketo-d-gluconate and a water-soluble brown pigment. Acid is produced from d-glucose, d-mannose, D-galactose, D-xylene, D-arabinose (weakly positive), L-arabinose, L-rhamnose (very weakly positive), d-fructose, d-mannitol, d-sorbitol (weakly positive), meso-erythritol (very weakly positive), glycerol, maltose (very weakly positive), melibiose, sucrose, raffinose, and ethanol. From L-sorbitose, dulcitol, and lactose, acid is not produced. Grows on d-glucose (very weakly positive), d-fructose, d-mannitol, d-sorbitol (very weakly positive), glycerol (very weakly positive), sucrose, and raffinose. No growth is shown in D-mannose, D-galactose, D-xylene, D-arabinose, L-arabinose, L-rhamnose, L-sorbitose, dulcitol, meso-erythritol, maltose, melibiose, lactose, and ethanol. Growth on pentitol is shown in D-arabitol and meso-ribitol (very weakly positive) but not in L-arabitol. Requires nicotinic acid for growth. Restriction analysis of 16S–23S rDNA ITS represents the G. oxydans type and the G. roseus type of restriction
Table 1. Differential characteristics for *Glucobacter roseus* NBRC 3990T.

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<td>D-Fructose</td>
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<td>w or −</td>
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<td>D-Mannitol</td>
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<td>D-Sorbitol</td>
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<td>Growth without nicotinic acid</td>
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+ , positive; w, weakly positive; vw, very weakly positive; −, negative; Gr, *G. roseus*; Gk, *G. kondoii*; Go, *G. oxydans*; Gc, *G. cerinus*; Gf, *G. frateurii*; nd, not determined.

a Cited from Malimas et al. (2007).
b Cited from Yukhan et al. (2004d).
c Cited from Tanasupawat et al. (2004).

patterns by digestion respectively with Mbol and Bsp1286I. Major ubiquinone is Q-10. DNA G+C content is 60.5 mol%.

The type strain is NBRC 3990T (strain G-2, T. Asai; =IAM 1839T, =IFO 3990T), which has a DNA G+C content of 60.5 mol%. The type strain, which was isolated from fruit of persimmon ("kaki" in Japanese, Diospiros sp.), transferred to IFO Culture Collection, Osaka, Japan via IAM Culture Collection, The University of Tokyo, Tokyo, Japan, and was maintained as a strain of "G. roseus" in NBRC, is also deposited in BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand as BCC 14456T.

Acknowledgments

This study was supported in part by The Biodiversity Research and Training Program, Bangkok, Thailand.

References


