Leuconostoc amelibiosum Schillinger, Holzapfel, and Kandler 1989 Is a Later Subjective Synonym of *Leuconostoc citreum* Farrow, Facklam, and Collins 1989

MASAAKI TAKAHASHI,¹ SANAE OKADA,^{2*} TAI UCHIMURA,¹ AND MICHIO KOZAKI¹

Laboratory of Applied Microbiology, Department of Agricultural Chemistry,¹ and Culture Collection Center,²
Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo 156, Japan

Leuconostoc amelibiosum Schillinger, Holzapfel, and Kandler 1989 is described as a later subjective synonym of *Leuconostoc citreum* Farrow, Facklam, and Collins 1989 on the basis of the results of phenotypic, chemotaxonomic, and DNA-DNA hybridization studies. The name *Leuconostoc citreum* should be retained; the type strain is strain NCDO 1837 (= NRIC 1776).

The taxon *Leuconostoc citreum* was separated from other *Leuconostoc* species and established for strains previously placed in *Leuconostoc mesenteroides* mainly on the basis of DNA relatedness data by Farrow et al. in 1989 (2). The taxon *Leuconostoc amelibiosum* was proposed for the tentatively

named taxon "*Leuconostoc mesenteroides* subsp. *amelibiosum*" on the basis of DNA relatedness data, peptidoglycan type, and other characteristics (12). These two species were described and validated independently at almost the same time (2, 5), and in each case the authors did not compare

TABLE 1. Characteristics of *Leuconostoc citreum* NCDO 1837^T, *Leuconostoc amelibiosum* DSM 20188^T, *Leuconostoc mesenteroides* subsp. *mesenteroides* NRIC 1541^T, *Leuconostoc lactis* NRIC 1540^T and "*Lactobacillus batatas*" strains

Characteristic	<i>Leuconostoc citreum</i> NCDO 1837 ^T	<i>Leuconostoc amelibiosum</i> DSM 20188 ^T	<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i> NRIC 1541 ^T	<i>Leuconostoc lactis</i> NRIC 1540 ^T	" <i>Lactobacillus batatas</i> " NY2-4 and NY2-8
Cell size (μm)	1.2–1.5 × 1.5–2.1	0.9–1.2 × 1.2–1.8	0.9–1.5 × 1.5–2.1	0.9–1.5 × 1.5–4.5	0.9–1.2 × 1.2–1.8
Isomer of lactic acid	D(–)	D(–)	D(–)	D(–)	D(–)
Hydrolysis of arginine	–	–	–	–	–
Dextran produced from sucrose	+	+	+	–	+
Growth at pH 4.8	+	+	+	–	+
Growth at 15°C	+	+	+	–	+
Growth at 45°C	–	–	–	–	±
Production of yellow pigment	+	–	–	–	+
Acid produced from:					
L-Arabinose	+	+	+	–	+
D-Ribose	–	–	–	–	–
D-Xylose	–	–	+	–	–
Gluconate	+	+	–	–	+
D-Glucose	+	+	+	+	+
D-Fructose	+	+	–	+	+
D-Galactose	–	–	+	+	–
D-Mannose	+	+	+	–	+
L-Rhamnose	–	–	–	–	–
D-Cellobiose	+	+	+	–	+
Lactose	–	–	+	+	–
D-Maltose	+	+	+	+	+
D-Melibiose	–	–	+	–	–
Sucrose	+	+	+	+	+
Raffinose	–	–	+	–	–
Salicin	+	+	+	–	+
D-Trehalose	+	+	+	–	+
D-Melezitose	–	–	–	–	–
D-Mannitol	–	–	+	–	–
D-Sorbitol	–	–	–	–	–
Starch	–	–	–	–	–
Vitamin requirements	Thiamine, biotin, niacin, calcium pantothenate	Thiamine, niacin, calcium pantothenate	NT	Thiamine, riboflavin, niacin, folic acid	Thiamine, niacin, calcium pantothenate

^a NT, not tested.

* Corresponding author.

TABLE 2. Chemotaxonomic characteristics of *Leuconostoc citreum* NCDO 1837^T, *Leuconostoc amelibiosum* DSM 20188^T, *Leuconostoc mesenteroides* subsp. *mesenteroides* NRIC 1541^T, *Leuconostoc lactis* NRIC 1540^T, and "*Lactobacillus batatas*" strains

Strain ^a	G+C content (mol%) ^b	Peptidoglycan type	Mobility of ^c :	
			D-LDH	L-LDH
<i>Leuconostoc citreum</i> NCDO 1837 ^T	40.0	Lys-Ala ₂	1.21	ND ^d
<i>Leuconostoc amelibiosum</i> DSM 20188 ^T	39.9	Lys-Ala ₂	1.21	ND
<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i> NRIC 1541 ^T	39.7	Lys-Ser-Ala ₂	1.36	ND
<i>Leuconostoc lactis</i> NRIC 1540 ^T	43.9	Lys-Ala ₂	1.40	ND
" <i>Lactobacillus batatas</i> " strains				
NY2-4 (= NRIC 1579)	40.6	Lys-Ala ₂	1.21	ND
NY2-8 (= NRIC 1581)	39.6	Lys-Ala ₂	1.21	ND

^a NCDO, National Collection of Dairy Organisms, Reading, United Kingdom; NRIC, Nodai Research Institute-Culture Collection, Tokyo University of Agriculture, Tokyo, Japan; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany.

^b Determined by using high-performance liquid chromatography.

^c Electrophoretic mobility on a polyacrylamide gel.

^d ND, not detected.

their new species with the type strain of the other species taxonomically.

During the course of identification of lactic acid bacteria obtained from a fermented starter of sakamoto-manju (fermented steamed cake), Okada et al. isolated some heterofermentative lactic acid bacteria and named them "*Lactobacillus batatas*" (10). Later, some of these isolates were found to be similar to *Leuconostoc citreum* and *Leuconostoc amelibiosum* phenotypically. In this study we reinvestigated these organisms and compared the characteristics of *Leuconostoc citreum*, *Leuconostoc amelibiosum*, and "*Lactobacillus batatas*" strains with one another.

The strains used in this study were *Leuconostoc citreum* NCDO 1837^T (= NRIC 1776^T) (T = type strain), *Leuconos-*

toc amelibiosum DSM 20188^T (= NRIC 1772^T), *Leuconostoc mesenteroides* subsp. *mesenteroides* NRIC 1541^T (= ATCC 8293^T), *Leuconostoc lactis* NRIC 1540^T (= ATCC 19256^T), and "*Lactobacillus batatas*" NY2-4 and NY2-8 (isolated by Okada et al.) (10). *Leuconostoc lactis* was selected referring to the evolutionary distance with *Leuconostoc citreum* NCDO 1837^T and *Leuconostoc amelibiosum* DSM 20188^T (9). The organisms were cultivated stationarily in GYP broth (10, 16a) at 30°C. The optical form of lactic acid which each strain produced was determined enzymatically as described by Okada et al. (11). Vitamin requirements were determined by using the defined medium of Tamura et al. (15). Cell wall peptidoglycan was prepared from whole cells by using the modified method of Schleifer and Kandler (13), and the peptidoglycan type was determined by qualitative and quantitative analyses of the hydrolysate by using the amino acid autoanalyzer. The cellular lactic acid dehydrogenases (LDH) profiles (i.e., optical specificity, electrophoretic pattern) were determined electrophoretically by using extracts from disrupted cells. Electrophoresis was performed in 4.5% (stacking gel) and 10% polyacrylamide gel according to Laemmli's system (6a) without SDS, and LDH activity was determined by using the method of Hensel et al. (4). D-(−)-LDH of *Lactobacillus leichmannii* (Boehringer Mannheim Yamanouchi, Tokyo, Japan) was used as a reference. Cellular DNA was prepared from exponential-phase cells by using Marmur's method (8), with some modifications, and DNA base composition was determined by high-performance liquid chromatography (14) with a DNA-GC kit (Yamasa Shoyu, Choshi, Chiba, Japan). Levels of DNA relatedness were determined by using an SDS-membrane filter method with a tritium-labeled system (6, 13a) and by using a microdilution well method with photobiotin labeling and a fluorometric system (1). In the former method, DNA was labeled with a nick translation kit (Amersham) and hybridization was conducted at 60°C for 40 h in 2× SSC containing 0.1% SDS. In the latter method, hybridization was conducted at 42°C for 16 h in 2× SSC containing 50% formamide.

Table 1 shows several biochemical and physiological characteristics of *Leuconostoc citreum* NCDO 1837^T, *Leuconostoc amelibiosum* DSM 20188^T, *Leuconostoc mesen-*

TABLE 3. Levels of DNA relatedness among *Leuconostoc citreum* NCDO 1837^T, *Leuconostoc amelibiosum* DSM 20188^T, *Leuconostoc mesenteroides* subsp. *mesenteroides* NRIC 1541^T, *Leuconostoc lactis* NRIC 1540^T, and "*Lactobacillus batatas*" strains

Strain	G+C content (mol%)	% Homology				
		³ H-labeled DNA from:		Photobiotin-labeled DNA from:		
		Strain NCDO 1837 ^T	Strain DSM 20188 ^T	Strain NCDO 1837 ^T	Strain DSM 20188 ^T	Strain NRIC 1581
<i>Leuconostoc citreum</i> NCDO 1837 ^T	40.0	100	83	100	82	85
<i>Leuconostoc amelibiosum</i> DSM 20188 ^T	39.9	81	100	94	100	82
<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i> NRIC 1541 ^T	39.7	14	17	14	16	16
<i>Leuconostoc lactis</i> NRIC 1540 ^T	43.9	15	13	22	24	19
" <i>Lactobacillus batatas</i> " strains						
NY2-4 (= NRIC 1579)	40.6	NT ^a	NT	92	85	93
NY2-8 (= NRIC 1581)	39.6	83	101	86	79	100

^a NT, not tested.

teroides subsp. *mesenteroides* NRIC 1541^T, *Leuconostoc lactis* NRIC 1540^T, and “*Lactobacillus batatas*” strains. Most of the phenotypic characteristics of *Leuconostoc citreum* NCDO 1837^T, *Leuconostoc amelibiosum* DSM 20188^T, and “*Lactobacillus batatas*” strains were identical. However, *Leuconostoc citreum* NCDO 1837^T required biotin for growth, and *Leuconostoc amelibiosum* DSM 20188^T did not produce a yellow pigment. Our data coincided with the data given in the original descriptions (2, 12). The characteristics of *Leuconostoc mesenteroides* subsp. *mesenteroides* NRIC 1541^T were different from those of above-mentioned strains in some kinds of fermentative sugars (especially D-melibiose), and *Leuconostoc lactis* NRIC 1540^T was also different from them in many other characteristics. Furthermore, cells of most strains appeared to range from spherical to short and rod shaped. Many distinctly rod-shaped cells were observed in *Leuconostoc lactis* NRIC 1540^T in particular.

Chemotaxonomic characteristics are shown in Table 2. The DNA base compositions of *Leuconostoc citreum* NCDO 1837^T, *Leuconostoc amelibiosum* DSM 20188^T, and “*Lactobacillus batatas*” NY2-4 and NY2-8 ranged from 39.6 to 40.6 mol% guanine plus cytosine. The cell wall peptidoglycan type was the same in all of the strains which we studied except *Leuconostoc mesenteroides* subsp. *mesenteroides* NRIC 1541^T, as reported previously (12). We report the peptidoglycan type of *Leuconostoc citreum* here for the first time. The similarity in the electrophoretic mobilities of D-(−)-LDH in *Leuconostoc* species was studied by Garvie (3). However, *Leuconostoc citreum* NCDO 1837^T and *Leuconostoc amelibiosum* DSM 20188^T have not been tested. *Leuconostoc citreum* NCDO 1837^T, *Leuconostoc amelibiosum* DSM 20188^T, and “*Lactobacillus batatas*” NY2-4 and NY2-8 contained D-(−)-LDH and produced identical electrophoretic mobility patterns. *Leuconostoc mesenteroides* subsp. *mesenteroides* NRIC 1541^T and *Leuconostoc lactis* NRIC 1540^T contained D-(−)-LDH and produced an electrophoretic mobility pattern different from the patterns of the strains discussed above. The results of DNA relatedness experiments are shown in Table 3. The data obtained by the two different methods were substantially the same. *Leuconostoc citreum* NCDO 1837^T and *Leuconostoc amelibiosum* DSM 20188^T were related to each other at a homology level of more than 81%, and the two type strains and isolates were regarded as belonging to a single species on the basis of the criteria of an ad hoc committee of the International Committee of Systematic Bacteriology (16). The level of similarity of the 16S rRNA sequences (99.1%) of *Leuconostoc citreum* and *Leuconostoc amelibiosum* (9) supports this conclusion.

Since the name *Leuconostoc citreum* has nomenclatural priority over *Leuconostoc amelibiosum* according to the *International Code of Nomenclature of Bacteria* (7), *Leuconostoc amelibiosum* is a later subjective synonym of *Leuconostoc citreum*. Therefore, we propose that the name *Leuconostoc citreum* should be retained; the type strain is strain NCDO 1837. Furthermore, we identified “*Lactobacillus batatas*” NY2-4 and NY2-8 as *Leuconostoc citreum* strains.

REFERENCES

1. Ezaki, T., Y. Hashimoto, and E. Yabuuchi. 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.* 39:224–229.
2. Farrow, J. A. E., R. R. Facklam, and M. D. Collins. 1989. Nucleic acid homologies of some vancomycin-resistant leuconostocs and description of *Leuconostoc citreum* sp. nov. and *Leuconostoc pseudomesenteroides* sp. nov. *Int. J. Syst. Bacteriol.* 39:279–283.
3. Garvie, E. I. 1969. Lactic dehydrogenases of strains of the genus *Leuconostoc*. *J. Gen. Microbiol.* 58:85–94.
4. Hensel, R., U. Mayr, K. O. Stetter, and O. Kandler. 1977. Comparative studies of lactic acid dehydrogenases in lactic acid bacteria. *Arch. Microbiol.* 112:81–93.
5. International Journal of Systematic Bacteriology. 1989. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. List no. 31. *Int. J. Syst. Bacteriol.* 39:495–497.
6. Kaneko, T. 1982. DNA-DNA hybridization, p. 243–271. In K. Komagata (ed.), *Biseibutsu no Kagakubunrui Jikkenhou* (Manual of microbial chemotaxonomy; in Japanese). Gakkai Shuppan Center, Tokyo.
- 6a. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680–685.
7. Lapage, S. P., P. H. A. Sneath, E. F. Lessel, V. B. D. Skerman, H. P. R. Seeliger, and W. A. Clark (ed.) 1975. International code of nomenclature of bacteria. 1975 Revision. American Society for Microbiology, Washington, D.C.
8. Marmur, J. 1960. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* 3:208–218.
9. Martinez-Murcia, A. J., and M. D. Collins. 1991. A phylogenetic analysis of an atypical leuconostoc: description of *Leuconostoc fallax* sp. nov. *FEMS Microbiol. Lett.* 82:55–60.
10. Okada, S., N. Ohara, and M. Kozaki. 1982. Lactic acid bacteria in the fermentation starter (manju-moto) of sakamoto-manju. *Hakkokogaku Kaishi* 60:403–408. (In Japanese.)
11. Okada, S., T. Toyoda, and M. Kozaki. 1978. An easy method for the determination of optical type of lactic acid produced by lactic acid bacteria. *Agric. Biol. Chem.* 42:1781–1783.
12. Schillinger, U., W. Holzapfel, and O. Kandler. 1989. Nucleic acid hybridization studies on *Leuconostoc* and heterofermentative lactobacilli and description of *Leuconostoc amelibiosum* sp. nov. *Syst. Appl. Microbiol.* 12:48–55.
13. Schleifer, K. H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* 36:407–477.
- 13a. Suzuki, K., T. Kaneko, and K. Komagata. 1981. Deoxyribonucleic acid homologies among coryneform bacteria. *Int. J. Syst. Bacteriol.* 31:131–138.
14. Tamaoka, J., and K. Komagata. 1984. Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* 25:125–128.
15. Tamura, G., T. Tsunoda, J. Kirimura, and S. Miyazawa. 1952. The microbiological determination of amino acids by lactic acid bacteria. I. *Nippon Nogeikagaku Kaishi* 26:464–470. (In Japanese.)
16. Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Trüper. 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int. J. Syst. Bacteriol.* 37:463–464.
- 16a. Yanagida, F., K. Suzuki, T. Kaneko, M. Kozaki, and K. Komagata. 1987. Morphological, biochemical, and physiological characteristics of spore-forming lactic acid bacteria. *J. Gen. Appl. Microbiol.* 33:33–45.