Proposal To Reclassify *Leuconostoc oenos* as *Oenococcus oeni* [corrig.] gen. nov., comb. nov.

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Wine strains belonging to the genus *Leuconostoc* were classified as *Leuconostoc oenos* by Garvie in 1967, and this name was confirmed on the Approved Lists of Bacterial Names in 1980. *L. oenos* is distinguished from other *Leuconostoc* spp. by its growth in acidic media, by its requirement for a growth factor in tomato juice, and by a number of carbohydrate fermentation characteristics. In addition, the results of a total soluble cell protein analysis, an electrophoretic analysis of NAD-dependent D-(–)-lactate dehydrogenase, 6-phosphogluconate dehydrogenase, and alcohol dehydrogenase, and an analysis of cross-reactivity with anti-glucose-6-phosphate dehydrogenase and anti-NAD-dependent D-(–)-lactate dehydrogenase performed with other *Leuconostoc* spp. clearly indicated that *L. oenos* should be distinguished from the other *Leuconostoc* species. Phylogenetic studies, in particular 16S and 23S rRNA sequencing studies, have revealed that *L. oenos* represents a distinct subline that is separate from other *Leuconostoc* spp. and lactic acid bacteria. In view of the phenotypic and phylogenetic distinctiveness of *L. oenos*, we propose that this species should be assigned to a new genus as *Oenococcus oeni* [corrig.] gen. nov., comb. nov. The type strain of *O. oeni* is NCDO 1674 (= ATCC 23179).

At the present time the genus *Leuconostoc* consists of the type species, *Leuconostoc mesenteroides*, and the following eight other species: *Leuconostoc oenos*, *Leuconostoc pseudomesenteroides*, *Leuconostoc lactis*, *Leuconostoc citreum*, *Leuconostoc gelidum*, *Leuconostoc carnosum*, *Leuconostoc fallax*, and *Leuconostoc argentinum* (2, 5, 11, 17, 19, 20). *L. amelabiosum* is now considered a subjective synonym of *L. citreum* (21), whereas organisms formerly designated *Leuconostoc cremoris* and *Leuconostoc dextranicum* are now classified as subspecies of *L. mesenteroides* (10). *Leuconostoc paramesenteroides* has recently been assigned to the new genus *Weissella*, as *Weissella paramesenteroides* (1).

L. oenos is the only acidophilic member of the genus *Leuconostoc* and occurs naturally in wine and related habitats (6, 11, 12). Strains of *L. oenos* are distinguished from the other *Leuconostoc* spp. by their growth at an initial pH of 4.8 and in media containing 10% (vol/vol) ethanol, by their requirement for a tomato juice growth factor (most strains), and by their lack of NAD-dependent glucose-6-phosphate dehydrogenase (11). The electrophoretic mobilities of NAD-dependent D-(–)-lactate dehydrogenase, 6-phosphogluconate dehydrogenase, and alcohol dehydrogenase also clearly distinguish *L. oenos* from the other *Leuconostoc* spp. (3, 7, 11, 12). Furthermore, antisera prepared from *L. oenos* exhibit no cross-reactivity with *L. lactis* anti-glucose-6-phosphate dehydrogenase and anti-NAD-dependent D-(–)-lactate dehydrogenase (14) and *L. mesenteroides* subsp. *mesenteroides* anti-glucose-6-phosphate dehydrogenase (13). *L. oenos* is also distinguished from other *Leuconostoc* spp. by its total soluble cell protein pattern (4).

Further evidence that *L. oenos* is a distinct taxon comes from genotypic investigations. Chromosomal DNA-DNA pairing has been used extensively to determine the genetic interrelationships of members of the genus *Leuconostoc* (4, 5, 8, 15, 19). Although *L. oenos* has been shown to be a distinct species by this approach, more significant are the very low levels of DNA

homology detected between this species and other members of the genus (Table 1). Such low levels of homology indicate that there is a very distinct genomic relationship between *L. oenos* and the nonacidophilic species of the genus. DNA-rRNA hybridization analyses (9, 19) and rRNA sequence analyses (16, 22) have also revealed the genotypic distinctness of *L. oenos*. In particular, 16S rRNA sequencing (16, 22) and, more recently, 23S rRNA (18) sequencing have revealed that the leuconostocs form three distinct lines of descent (viz., *Leuconostoc sensu stricto*, including *L. mesenteroides*; the *L. paramesenteroides* group of species, including some atypical lactobacilli; and *L. oenos*) (Fig. 1).

Yang and Woese (22) and Martinez-Murcia et al. (16–18) have shown that *L. oenos* is unusual in that it exhibits a relatively long rRNA branch. It has been suggested by Yang and Woese (22) that this characteristic, together with the phenotypic peculiarities of *L. oenos* and its negligible levels of DNA homology with other *Leuconostoc* spp., indicates that *L. oenos* is a fast-evolving organism. However, irrespective of whether *L. oenos* is a fast-evolving organism, it is clear from the results of comparative 16S and 23S rRNA sequencing analyses that this species forms a distinct line within the so-called “*Leuconostoc* branch” that is worthy of separate generic status (18). Recently, a taxonomic reorganization of the leuconostocs was initiated with the introduction of the genus *Weissella* for *L. paramesenteroides* and its close relatives (1). On the basis of the phenotypic and phylogenetic evidence mentioned above, we propose that *L. oenos* should be reclassified in a new genus, *Oenococcus*, thereby completing the necessary taxonomic revision of the *Leuconostoc* branch of the lactic acid bacteria. It should be noted that when this taxon was assigned to the genus *Leuconostoc* by Garvie (6), the specific epithet was incorrectly presented as *oenos*, which is the nominative form of the noun; however, the genitive is required. The specific epithet was also incorrectly Latinized as a nominative; the correct Latinized form is *oinus*. We therefore propose that the specific epithet *oenos* should be changed to the correct Latin genitive form, *oeni*.

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TABLE 1. Levels of DNA-DNA homology for leuconostocs^a

Species or subspecies	% Homology with [³ H]DNA from:				
	<i>L. mesenteroides</i> subsp. <i>mesenteroides</i>	<i>L. pseudomesenteroides</i>	<i>L. paramesenteroides</i>	<i>L. lactis</i>	<i>L. carnosum</i>
<i>L. mesenteroides</i> subsp. <i>mesenteroides</i>	73–108	24–63	7–18	7–38	1–17
<i>L. mesenteroides</i> subsp. <i>cremoris</i>	66–106	20–50	5–10	5–35	4–10
<i>L. mesenteroides</i> subsp. <i>dextranicum</i>	84–110	38–49	5–19	6–35	5–8
<i>L. pseudomesenteroides</i>	18–48	70–100	9–22	13–36	ND ^b
<i>L. paramesenteroides</i>	6–19	7–30	82–100	8–14	0–3
<i>L. lactis</i>	16–49	23–61	0–25	74–100	7–22
<i>L. amelibiosum</i>	39	34	22	32	ND
<i>L. carnosum</i>	19–32	ND	0–6	0–25	78–116
<i>L. gelidum</i>	9–31	ND	0–3	0–10	3–21
<i>L. citreum</i>	21–30	27–56	10–20	23	ND
<i>L. oenos</i>	8–15	5–10	5–7	3–11	ND
<i>L. fallax</i>	28–41	ND	25	ND	ND
<i>L. argentinum</i>	9	ND	ND	35–39	ND

^a Data from references 2, 4, 5, 11, 15, 17, 19, and 20.^b ND, no data.

Description of *Oenococcus* gen. nov. *Oenococcus* (Oe.no. coc'cus. Gr. n. oinos, wine; Gr. masc. n. kokkus, berry, little round thing; N.L. masc. n. *Oenococcus*, little round thing from wine). Gram-positive, nonmotile, asporogenous, ellipsoidal to spherical cells usually occur in pairs or chains. Cell morphology varies from strain to strain and is influenced by the growth medium. Chemoorganotrophic. Facultatively anaerobic. Catalase negative. No cytochromes are present. Nonproteolytic.

Nitrates are not reduced. Nonhemolytic. Indole is not formed. Acidophilic; prefers an initial growth pH of 4.8. Grows in media supplemented with 10% (vol/vol) ethanol. Growth in broth is slow and usually uniform. Surface growth is enhanced by incubation in a 10% CO₂ atmosphere. Colonies usually develop only after 5 days and are less than 1 mm in diameter. Grows at temperatures between 20 and 30°C. Requires a rich medium containing complex growth factors and amino acids.

FIG. 1. Unrooted phylogenetic tree showing the relationship of *O. oeni* to *Leuconostoc* spp. and "lactic acid bacteria" based on 16S rRNA sequences. Abbreviations: W., *Weissella*; Lb., *Lactobacillus*; L., *Leuconostoc*; P., *Pediococcus*; A., *Aerococcus*; C., *Carnobacterium*; E., *Enterococcus*; S., *Streptococcus*; V., *Vibrio*; Lc., *Lactococcus*.

Grows only in the presence of tomato juice, grape juice, pantothenic acid, or 4'-*O*-(β -glucopyranosyl)-D-pantothenic acid. Glucose is fermented to equal amounts of D-(–)-lactic acid, CO₂, and ethanol or acetate via a pathway that is not yet fully elucidated. L-Malate is decarboxylated to L-(+)-lactate in the presence of a fermentable carbohydrate. NAD-dependent glucose-6-phosphate dehydrogenase is not present. The amino acid sequence of the interpeptide bridge of the cell wall peptidoglycan is of the L-Lys-L-Ala-L-Ser or L-Lys-L-Ser-L-Ser type. The DNA base composition is 37 to 42 mol% G+C (as determined by the thermal denaturation method). The type species is *Oenococcus oeni* (6) [corrig.] comb. nov. (*Leuconostoc oenos*).

Description of *Oenococcus oeni* [corrig.] comb. nov. *Oenococcus oeni* (oe'ni. Gr. gen. n. *oinou*, of wine; N.L. gen. n. *oeni*, of wine). Acidophilic; grows in grape must and wine at pH 3.5 to 3.8. Prefers an initial growth pH of 4.8. Growth is not inhibited in the presence of 10% (vol/vol) ethanol. The optimum growth temperature is 22°C. Growth at 15°C is slow. Requires a rich growth medium supplemented with grape juice, tomato juice, pantothenic acid, or a glucose derivative of pantothenate. Converts L-malate to L-(+)-lactate and CO₂ in the presence of a fermentable carbohydrate. Glucose is fermented to D-(–)-lactic acid, CO₂, and ethanol or acetate. Metabolically inactive; ferments only a few carbohydrates. Prefers fructose and usually ferments trehalose. In grape must or wine, pentose sugars (arabinose or xylose) are fermented before glucose, resulting in diauxic growth. Variable reactions for arabinose, cellobiose, galactose, mannose, melibiose, salicin, and xylose fermentation. Sucrose, lactose, maltose, mannitol, and raffinose are not fermented. Esculin is hydrolyzed. Arginine is hydrolyzed by certain strains, but only in wine or related habitats. Certain strains ferment citrate in the presence of a fermentable carbohydrate, but this characteristic is often lost when strains are subcultured. Glucose-6-phosphate dehydrogenase activity is observed only in the presence of NADP. NAD-dependent D-(–)-lactate dehydrogenase migrates slowly, and at least nine electrophoretically different NAD-dependent D-(–)-lactate dehydrogenase bands have been observed. Growth in yeast glucose milk is unusual, but if it occurs, acid and gas are not produced. Certain strains produce extracellular polysaccharides in medium supplemented with grape juice. The amino acid sequence of the interpeptide bridge of the cell wall peptidoglycan is L-Lys-L-Ala-L-Ser or L-Lys-L-Ser-L-Ser. The G+C content of the DNA is 38 to 42 mol% (as determined by the thermal denaturation method). The type strain is NCDO 1674 (= ATCC 23179). Isolated from wine.

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REFERENCES

- Collins, M. D., J. Samelis, J. Metaxopoulos, and S. Wallbanks. 1993. Taxonomic studies on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. *J. Appl. Bacteriol.* **75**:595–603.
- Dicks, L. M. T., L. Fantuzzi, F. C. Gonzalez, M. Du Toit, and F. Dellaglio. 1993. *Leuconostoc argentinum* sp. nov., isolated from Argentine raw milk. *Int. J. Syst. Bacteriol.* **43**:347–351.
- Dicks, L. M. T., and H. J. J. van Vuuren. 1990. Differentiation of *Leuconostoc* species by nicotinamide adenine dinucleotide-dependent D-(–)-lactic dehydrogenase profiles. *FEMS Microbiol. Lett.* **67**:9–14.
- Dicks, L. M. T., H. J. J. van Vuuren, and F. Dellaglio. 1990. Taxonomy of *Leuconostoc* species, particularly *Leuconostoc oenos*, as revealed by numerical analysis of total soluble cell protein patterns, DNA base compositions, and DNA-DNA hybridizations. *Int. J. Syst. Bacteriol.* **40**:83–91.
- 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.
- Garvie, E. I. 1967. *Leuconostoc oenos* sp. nov. *J. Gen. Microbiol.* **48**:431–438.
- Garvie, E. I. 1969. Lactic dehydrogenases of strains of the genus *Leuconostoc*. *J. Gen. Microbiol.* **58**:85–94.
- Garvie, E. I. 1976. Hybridization between the deoxyribonucleic acids of some strains of heterofermentative lactic acid bacteria. *Int. J. Syst. Bacteriol.* **26**:116–122.
- Garvie, E. I. 1981. Sub-divisions within the genus *Leuconostoc* as shown by RNA/DNA hybridization. *J. Gen. Microbiol.* **127**:209–212.
- Garvie, E. I. 1983. *Leuconostoc mesenteroides* subsp. *cremoris* (Knudsen and Sorensen) comb. nov. and *Leuconostoc mesenteroides* subsp. *dextranicum* (Beijerinck) comb. nov. *Int. J. Syst. Bacteriol.* **33**:118–119.
- Garvie, E. I. 1986. Genus *Leuconostoc*, p. 1071–1075. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams & Wilkins Co., Baltimore.
- Garvie, E. I., and J. A. E. Farrow. 1980. The differentiation of *Leuconostoc oenos* from non-acidophilic species of *Leuconostoc*, and the identification of five strains from the American Type Culture Collection. *Am. J. Enol. Vitic.* **31**:154–157.
- Gasser, F., and M. Hontebeyrie. 1977. Immunological relationships of glucose-6-phosphate dehydrogenase of *Leuconostoc mesenteroides* NCDO 768 (= ATCC 12291). *Int. J. Syst. Bacteriol.* **27**:6–8.
- Hontebeyrie, M., and F. Gasser. 1975. Comparative immunological relationships of two distinct sets of isofunctional dehydrogenases in the genus *Leuconostoc*. *Int. J. Syst. Bacteriol.* **25**:1–6.
- Hontebeyrie, M., and F. Gasser. 1977. Deoxyribonucleic acid homologies in the genus *Leuconostoc*. *Int. J. Syst. Bacteriol.* **27**:9–14.
- Martinez-Murcia, A. J., and M. D. Collins. 1990. A phylogenetic analysis of the genus *Leuconostoc* based on reverse transcriptase sequencing of 16 S rRNA. *FEMS Microbiol. Lett.* **70**:73–84.
- 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.
- Martinez-Murcia, A. J., N. M. Harland, and M. D. Collins. 1993. Phylogenetic analysis of some leuconostocs and related organisms as determined from large-subunit rRNA gene sequences: assessment of congruence of small- and large-subunit rRNA derived trees. *J. Appl. Bacteriol.* **74**:532–541.
- 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.
- Shaw, B. G., and C. D. Harding. 1989. *Leuconostoc gelidum* sp. nov. and *Leuconostoc carnosum* sp. nov. from chill-stored meats. *Int. J. Syst. Bacteriol.* **39**:217–223.
- Takahashi, M., S. Okada, T. Uchimura, and M. Kozaki. 1992. *Leuconostoc amelibiosum* Schillinger, Holzapfel, and Kandler 1989 is a later subjective synonym of *Leuconostoc citreum* Farrow, Facklam, and Collins 1989. *Int. J. Syst. Bacteriol.* **42**:649–651.
- Yang, D., and C. R. Woese. 1989. Phylogenetic structure of the "Leuconostocs": an interesting case of a rapidly evolving organism. *Syst. Appl. Microbiol.* **12**:145–149.