Proposal of Neotype Strains for *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem, *Leuconostoc dextranicum* (Beijerinck) Hucker and Pederson, and *Leuconostoc cremoris* (Knudsen and Sørensen) Garvie

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National Collection of Dairy Organisms strains 523, 529, and 543 are proposed as the neotype strains of *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem, *L. dextranicum* (Beijerinck) Hucker and Pederson, and *L. cremoris* (Knudsen and Sørensen) Garvie, respectively. Descriptions of the three strains are given.

Sneath and Skerman (15) listed National Collection of Dairy Organisms (NCDO) 523, NCDO 529, and NCDO 543, respectively, as "working types" of *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem (16, 17), *L. dextranicum* (Beijerinck) Hucker and Pederson (1, 12), and *L. cremoris* (Knudsen and Sørensen) Garvie (4, 13), respectively. No type strains exist for these three species. Since 1966 some or all of these strains have been used in several different laboratories studying various aspects of bacterial classification (5, 8-11; W. H. Holzapfel, Inaugural dissertation, Tech. Hochschule München, Munich, 1969). The properties of these strains have, therefore, been checked, and, from the results which have been published, there appears to be no reason why NCDO 523, NCDO 529, and NCDO 543 should not be proposed as neotype strains for the above-mentioned species. They were selected by Garvie for publication in the list of type and reference strains (6) because they were typical of their species as described earlier (4).

A second widely used strain of *L. mesenteroides*, strain 39, was considered as a possibility for the neotype of this species. This strain has been used in biochemical work and has normally been included in taxonomic studies of these organisms. However, strain 39 has been found to be different from the majority of other strains of *L. mesenteroides* in several ways, namely, the end products of glucose fermentation (2), the structure of its lactate dehydrogenase and glucose-6-phosphate dehydrogenase as judged by immunological techniques (10), the guanine-plus-cytosine (G+C) content of its deoxyribonucleic acid (DNA) (6), and its DNA-DNA relationships (8, 11).

L. mesenteroides contains at least two subtypes, and strain 39 belongs to the subtype containing the minority of known strains. Strain 39

would therefore be unsuitable as the neotype strain of the species. Tsenkovskii (16) gives some excellent drawings of the cocci which he observed to form dextran from sucrose, and there can be no doubt that the bacteria he observed are the same as those that are known today as *L. mesenteroides*.

Beijerinck (1) described a coccus which formed dextran from sucrose and which did not ferment xylose (arabinose was not tested), and the proposed neotype strain of *L. dextranicum*, NCDO 529, has both these properties. The characters of NCDO 529 differ from those given in the original description of this species in that it ferments neither lactose nor maltose, but both of these properties have been found to be variable in species of *Leuconostoc*, and consequently they have neither taxonomic nor diagnostic significance. Old records indicate that *L. dextranicum* NCDO 529 was sent many years ago to the National Institute for Research in Dairying by Orla-Jensen as a strain of *Betacoccus bovis*. *B. bovis* included strains which fermented xylose as well as those which did not ferment arabinose or xylose (14). Hucker and Pederson (12) adhered to Beijerinck's original description and put only strains which did not ferment arabinose or xylose in *L. dextranicum*. More recently (5) it has been suggested that xylose-positive, arabinose-negative strains should be included in *L. dextranicum* rather than *L. mesenteroides*, thus reverting to the division of species proposed by Orla-Jensen. The xylose-fermenting strains are in the majority of those whose properties are known (4), but the neotype strain must be selected from the smaller group of non-xylose fermenters.

In the past, *L. citrovorum* (Hammer) Hucker and Pederson and *L. cremoris* have been regarded as synonyms. In 1971 the Judicial Commission (3) issued Opinion 45, which rejected

TABLE 1. *Physiological and other properties of Leuconostoc mesenteroides* NCDO 523, *L. dextranicum* NCDO 529, and *L. cremoris* NCDO 543

Property	<i>L. mesenteroides</i> NCDO 523	<i>L. dextranicum</i> NCDO 529	<i>L. cremoris</i> NCDO 543
Morphology	Gram-positive coccus or coccobacillus, usually in pairs	Gram-positive coccus in pairs and chains	Gram-positive coccus or coccobacillus in long chains of pairs
Reaction in litmus milk	Slightly acid	—	Slightly acid
Reaction in yeast, glucose, litmus, milk	Acid, clot, gas, and reduction	Acid, clot, and reduction	Acid, clot, and slight reduction
Growth at:			
10°C	+	+	+
37°C	+	+	—
39°C	+	—	—
45°C	—	—	—
Growth in:			
3% NaCl	+	—	—
6.5% NaCl	+	—	—
Dextran from sucrose	+	+	—
Citrate used	—	+	+
Acetoin formed in citrate medium	—	+	+
Survival at 55°C for:			
15 min	+	—	—
30 min	—	—	—
Growth at pH 4.8	—	—	—
Acid formed from:			
Arabinose	+	—	—
Xylose	+	—	—
Fructose	+	+	—
Glucose	+	+	+
Galactose	+	—	+
Mannose	+	+	—
Cellobiose	+	—	—
Lactose	+	—	+
Maltose	+	—	—
Sucrose	+	+	—
Trehalose	+	+	—
Melibiose	+	—	—
Raffinose	+	—	—
Esculin	+	—	—
Salicin	+	—	—
Mannitol	+	—	—
Requirement for:			
Uracil	—	—	+
Riboflavin	—	+	+
Pyridoxal	+	+	+
Nicotinic acid	+	+	+
Thiamine	+	+	+
B12	—	—	—
Folic acid	—	+	+
Pantothenate	+	+	+
Guanine + adenine + uracil + xanthine	—	—	+
Cell wall peptide	Lys-ser-(ala) ₇	Lys-ser-(ala) ₇	
LDH group	D	D	D
G-6-PDH group	d	d	d
G+C content (mol%) of			
DNA	38.5	39.4	38.6
T _m ^a			
Buoyant density	38.7		38.5

^a Data from Gavie et al. (9) corrected so that *Escherichia coli* K-12 under the same conditions has a G+C value of 51 to 52 mol%.

the name *L. citrovorum*. The Judicial Commission stated that with this action "...there was no need to adjudicate on the request (7) for the conservation of the name *Leuconostoc cremoris*." Knudsen and Sørensen (13) originally described two forms (A and X) of *Betacoccus cremoris* (syn. *L. cremoris*). NCDO 543 has the properties of the X type as given by Knudsen and Sørensen (13), and, because it was isolated from a Danish commercial starter, it was selected as the working type. It is here proposed as the neotype strain of *L. cremoris*.

The properties of the three strains proposed as neotypes are given in Table 1. The techniques used in determining these properties have been previously published (4, 5, 9, 11; Holzapfel, Inaugural dissertation, Tech. Hochschule München, Munich, 1969).

Recent work using DNA-DNA hybridization techniques (8, 11) has shown that *L. dextranicum* and *L. cremoris* may only be subspecies of *L. mesenteroides* (8, 11). Further work on the relationship of these bacteria is needed.

The proposed neotype strains have been sent to or were received from other culture collections, and they have the histories given below:

L. mesenteroides

Vaughn 37Y →
 ATCC 8293 → NRRL B-1118
 ↘
 NCIB 8023 → NCDO 523

L. dextranicum

? Orla-Jensen →
 NIRD → NCDO 529 → NRRL B-3469
 ↘
 ATCC 19255

L. cremoris

Starter powder → NCDO 543 → ATCC 19254

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