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REQUEST FOR AN OPINION THAT THE NAME
LEUCONOSTOC CITROVORUM BE REJECTED AND
THE NAME LEUCONOSTOC CREMORIS BE CONSERVED

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ABSTRACT. The names Streptococcus citrovorus, Leuconostoc citrovorus and Betacoccus cremoris have been regarded as synonyms. Evidence is given which suggests that Hammer used the name Streptococcus citrovorus for strains which in fact belonged to several species of bacteria. One of these strains which is a sucrose fermenting leuconostoc is indicated in the 1968 ATCC catalogue as the representative strain of Leuconostoc citrovorum. The strain indicated as representative under Leuconostoc cremoris is a sucrose nonfermenting leuconostoc. An Opinion is requested whether the specific epithet citrovorum should have been perpetuated in the species name as Leuconostoc citrovorum and also whether the name Leuconostoc cremoris should be conserved for the sucrose nonfermenting species of Leuconostoc. An Opinion on whether Leuconostoc citrovorum and Leuconostoc cremoris are synonyms is also requested.

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Rule 24 of the International Code of Nomenclature of Bacteria (1966) states "A name must be rejected if it is illegitimate..." The reasons why a name falls into this category are several: section (e) states "If it (i. e. the name) is used with different meanings and so has become a long persistent source of error" (nomen ambiguum) and section (f) states "If its application is uncertain" (nomen dubium). For the reasons to be stated it is argued that the name Leuconostoc citrovorum is illegitimate under both these sections. It is requested that the Judicial Commission of the International

Committee on Nomenclature of Bacteria give a ruling as to the status of the name and also an Opinion whether or not Leuconostoc cremoris is a suitable alternative. It is further suggested that in view of the ambiguity which surrounds the work using Leuconostoc citrovorum that it should be made clear that this name is not a synonym of Betacoccus cremoris but that Leuconostoc citrovorum is a dubious species.

It is now generally accepted that the catalase negative cocci which form chains of cells and which produce gas as a result of the fermentation of glucose are placed together in the genus Leuconostoc. In the past microorganisms with these characteristics found in milk and dairy products were given other generic names. Hammer (1920) included them with the streptococci and Orla-Jensen (1920) called them betacocci. In 1931 Hucker and Pederson studied strains from milk and dairy products as well as strains from sugar and fermenting vegetables. From their studies they concluded that all the strains belonged to the same genus and that it should be called Leuconostoc. When considering the species in this genus Hucker and Pederson recognized the specific epithet citrovorus used by Hammer (1920) in the new combination Leuconostoc citrovorus (sic) and cite as a synonym Betacoccus cremoris (Knudsen and Sørensen 1929).

Streptococcus citrovorus was described by Hammer (1920) as a slightly elongated coccus which formed chains in liquid media. It was Gram-positive, did not grow at 37°C, and in litmus milk there was no pronounced change. Slight acidity was formed in beef extract bouillon containing fructose, galactose, glucose, lactose or maltose, but with glycerol, sucrose, mannitol, salicin, raffinose, inulin or starch there was no increase in acidity. Gas production was not evident in milk or bouillon. From this description it might reasonably be concluded that Hammer was dealing with the species studied in greater detail later by Hucker and Pederson (1931). Several strains of Streptococcus citrovorus originating from Hammer's collection are either still available or sufficient is known about them to be certain that they are not Leuconostoc citrovorum of Hucker and Pederson and furthermore no strain originating from Hammer's collection has been found which can be placed in this species. Unfortunately none of the strains used by Hucker and Pederson appear to have survived.

Table 1 lists all the strains of Streptococcus citrovorus which have been traced and Table 2 gives the names under which they would now be classified and the evidence for this classification.

Hucker and Pederson (1931) included some strains received from Hammer in their work. On p.81 they remark "Strains of both S. citrovorus and S. paracitrovorus as secured from Hammer upon inoculation in sucrose gelatin produced the characteristic slimy growth similar to the growth produced by Leuconostoc strains from sugar solutions and from fermenting vegetables" and on p. 100 when discussing Leuconostoc citrovorum "in connection with the study of the systematic relationship of this species it is interesting to note that, although the description given to this species included the qualifications that it does not ferment sucrose, all of the strains of Leuconostoc citrovorus received from Hammer have produced an appreciable amount of acid from sucrose. For this reason the strains received from him as Streptococcus citrovorus must be classified with the sucrose-fermenting types. This apparent discrepancy between the actual organisms and the description is easily explained as these fresh isolations had not been studied previously in relation to their ability to attack sucrose but only from the standpoint of their ability to produce a high volatile acidity with a correspondingly low production of residual acid. The latter characters do not designate species but are common to the entire genus. For this reason, inasmuch as the sucrose-fermenting types are the more common, such types are more easily secured."

These remarks suggest that the three strains 51, 53 and 55 (Table 1) had not been included in the work on which Hammer based his description of Streptococcus citrovorus. Similarly ATCC 797 isolated in 1926 would not be studied in work published in 1920. The other three strains listed in Table 1 were deposited in the ATCC in 1938 or 1941. It appears probable that they were also isolated more recently than 1920.

The position then appears to be that Hammer described and named a species Streptococcus citrovorus which later workers have believed to be the nonsucrose fermenting species of leuconostoc. It is possible that after 1920 Hammer isolated strains which he considered to be Streptococcus citrovorus but which are now known to belong to Leuconostoc mesenteroides, L. dextranicum and Pediococcus cerevisiae (nomina ambigua). The question is, were Hucker and Pederson correct in perpetuating the specific epithet citrovorus as their nonsucrose fermenting Leuconostoc when the strains of Streptococcus citrovorus which they received from Hammer did not belong to the nonsucrose fermenting species?

Table 1. Strains of Streptococcus citrovorus originating from Hammer's collection.

Strain identification	Published source	Additional published information
ATCC 797	ATCC catalogue 1927	Catalogue entry: B.W. Hammer, Iowa State College. Isolated from natural starter 1926, deposited 1926
ATCC 7013	ATCC catalogue 1938	Catalogue entry: B.W. Hammer, Iowa State College, Ames, Strain No.122 through W.B. Sarles, University of Wisconsin, Madison, "Slow acid-production, delicate flavour" deposited 1938.
ATCC 8081	ATCC catalogue 1949	Catalogue entry: B.W. Hammer, Iowa State College. Strain 32, deposited 1941.
ATCC 8082	ATCC catalogue 1949	Catalogue entry: B.W. Hammer, Iowa State College. Strain 146, deposited 1941.
Strain 51	Hucker and Pederson (1931)	<u>Streptococcus citrovorus</u> Hammer 33B or 338)
Strain 53	" "	<u>Streptococcus citrovorus</u> from Hammer)
Strain H55	" "	<u>Streptococcus citrovorus</u> from Hammer)

Table 1
p. 68

ATCC = American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA

Table 2. Published information on strains of Streptococcus citrovorus.

Strain identification	Published information
ATCC 797	Fermentation pattern of <u>Leuconostoc mesenteroides</u> (Camien et al. 1947)
=	NCDO 824. Received by NCDO from NCTC as NCTC 3739 - forms slime from sucrose but does not ferment arabinose or xylose - placed as <u>Leuconostoc dextranicum</u> (Garvie 1960)
ATCC 7013	Fermentation pattern of <u>Leuconostoc mesenteroides</u> (Camien et al. 1947)
ATCC 8081	<u>Pediococcus cerevisiae</u> (Felton and Niven 1953)
ATCC 8082 =	NCDO 516. Received by NCDO from NCIB as NCIB 8189) forms slime from sucrose -
=	NCDO 812, " " " " ATCC as ATCC 8082) ferments xylose but not arabinose
	<u>Leuconostoc dextranicum</u>
Strain 51)	
Strain 53)	Form acid from sucrose - and not <u>Leuconostoc citrovorus</u> Hucker and Pederson (1931, 100)
Strain H55)	

NCDO = National Collection of Dairy Organisms, National Institute for Research in Dairying,
Shinfield, Reading, England

NCTC = National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue,
London NW 9, England

NCIB = National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland

Hammer had described two species of bacteria which produced a high volatile acidity and recognized that Streptococcus paracitrovorus was the more usual organism isolated from dairy products (Hammer 1923). Both Hammer and Hucker and Pederson agree that the nonsucrose fermenting species of Leuconostoc is the most difficult to grow. Despite this, strains called Streptococcus citrovorus have survived.

Orla-Jensen did not describe any betacocci which did not ferment sucrose but Knudsen and Sørensen (1929) describe Betacoccus cremoris (X form) which formed acid from glucose, galactose and lactose but not from glycerin, xylose, arabinose, rhamnose, sorbitol, mannitol, laevulose, mannose, maltose, raffinose, inulin, dextrin, starch or salicin. Acid was sometimes formed by some strains from sucrose and slight acid from fructose. They also describe Betacoccus cremoris (A form), which usually formed acid from sucrose and which is considered by Knudsen and Sørensen to resemble Streptococcus paracitrovorus. Usage has established Betacoccus cremoris to mean the X form, and this name has not become confused by being applied to strains of species other than the nonsucrose fermenting leuconostoc.

Several of Hammer's strains bearing the label Streptococcus citrovorus were deposited in the American Type Culture Collection and they have been used by other workers. No strains of Leuconostoc citrovorum or Betacoccus cremoris from sources other than Hammer's collection have been found listed in culture collection catalogues prior to 1954. The result is that only Hammer's strains were generally available for study and therefore much published work on Leuconostoc citrovorum is not concerned with the sucrose nonfermenting Leuconostoc species. For example, Dunn et al. (1947) examined 3 ATCC strains of Leuconostoc citrovorum. In this case the workers concerned were aware of the discrepancy between the name and the sugar fermenting ability of the strains and point it out in a later paper (Camien et al. 1947). Recognition of such discrepancies was not, however, always so quick. For example, a large volume of work was published on the folinic acid requirement of Leuconostoc citrovorum ATCC 8081, but only when the bulk of this work was completed was it realized that ATCC 8081 was a pediococcus (Felton and Niven, 1953).

To complicate matters still further the 1968 catalogue of the American Type Culture Collection contains the following entries:

Leuconostoc citrovorum (Hammer) Hucker and Pederson.
8082* B. W. Hammer 146 (Streptococcus citrovorus)
Comment Leuconostoc mesenteroides
(J. Dairy Res. 27:289 (1960)).

Leuconostoc cremoris (Knudsen and Sørensen) Garvie.
19254* NCDO 543 Ellen I. Garvie (LF2) Hansen's
dried starter powder. Reference Strain
(Intl. J. Syst. Bacteriol. 16(1):70-71 (1966)).

"The asterisk suffixed to the ATCC accession number denoted the strain is a type strain, a neotype strain, a proposed or suggested neotype, an original strain not yet designated in the literature as a type strain or an ATCC representative strain i.e. a strain whose identification has been varified." These two strains belong to different species. ATCC 8082 forms dextran and ferments sucrose, NCDO 543 does neither. If Leuconostoc citrovorum and Leuconostoc cremoris are synonyms the ATCC is indicating "type" strains of the same species while in fact these cultures belong to different species.

To prevent further misunderstanding and muddle a clarification of the position of the names and designation of type species at international level is required urgently.

Sources of Betacoccus cremoris

The description of Streptococcus citrovorus (Hammer 1920) could apply to a nonsucrose fermenting Leuconostoc. Hammer was working with starter and dairy products. As already pointed out none of the seven of his strains about which much is known were Betacoccus cremoris. Knudsen and Sørensen who described Betacoccus cremoris were also examining starters. Hucker and Pederson studied 35 strains of the nonsucrose fermenting Leuconostoc and of these 9 were examined in detail. In their paper they do not make clear where they obtained their strains but by comparing information in the text with the strains listed in their Table 1, it is almost certain that the 9 strains were from the H group, all of which came from starter or dairy products.

The National Collection of Dairy Organisms has 13 strains of sucrose nonfermenting leuconostocs. These have either come from starter from cheese or from Dairy Institutes, and 11 are from European centres.

While it is easy to isolate the sucrose-fermenting species from fermenting vegetables and also as contaminants in dairy products no evidence has been found showing that Betacoccus

cremoris has been isolated except from starter or sources which could be contaminated by starter.

Other species of Leuconostoc are extremely varied in their properties (Garvie 1960, 1967; Whittenbury 1966) but Betacoccus cremoris is much more homogeneous (Garvie 1967). In view of the artificial nature of the source of the strains examined the question arises whether this is a true species or a variant which has been propagated because it is a useful ingredient of starter.

REFERENCES

- Camien, M.N., M.S. Dunn and A.J. Salle. 1947. The effect of carbohydrates on acid production by twenty-four lactic acid bacteria. J. Biol. Chem. 168:33-42.
- Dunn, M.S., S. Shankman, M.N. Camien and H. Block. 1947. The amino acid requirements of twenty-three lactic acid bacteria. J. Biol. Chem. 168:1-22.
- Felton, E.A. and C.F. Niven, Jr. 1953. The identity of 'Leuconostoc citrovorum strain 8081.' J. Bact. 65:482-3
- Garvie, E.I. 1960. The genus Leuconostoc and its nomenclature. J. Dairy Res. 27:283.
- _____. 1967. The growth factor and amino acid requirements of species of the genus Leuconostoc including Leuconostoc paramesenteroides (sp. nov.) and Leuconostoc oenos. J. gen. Microbiol. 48:439-447.
- Hammer, B.W. 1920. Volatile acid production of S. lacticus and the organisms associated with it in starters. Agr. Exp. Sta. Iowa Res. Bull. 63.
- _____. and M.P. Baker. 1923. Studies on Streptococcus paracitrovorus group. Agr. Exp. Sta. Iowa Res. Bull. 81.
- Hucker, G.J. and C.S. Pederson. 1931. A study of the physiology and classification of the genus Leuconostoc. Zbl. Bakt. II Abt. 85:65-114.
- International Code of Nomenclature of Bacteria. 1966. Intl. J. Syst. Bact. 16(4):459-490.
- Knudsen, S. and A. Sørensen. 1929. Bidrag til Syrevaekernes Bakteriologi. Kgl. Veterinaer-og-Landbohøjskole Aarsskrift.
- Orla-Jensen, S. 1919. The lactic-acid bacteria. Andr Fred Høst and Søn, Copenhagen.
- Whittenbury, R. 1966. A study of the genus Leuconostoc. Arch. Mikrobiol. 53:317-327.