THE TAXONOMIC STATUS OF SPOROSARCINA URAEAE
(BEIJERINCK) ORLA-JENSEN

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SUMMARY. A study of the taxonomic status of 9 strains designated as Sarcina ureae. In view of the fact that the present classification of this species is not appropriate, we recommend that it should be classified as belonging to a separate genus Sporosarcina. We suggest that the genus Sporosarcina should be placed in the family Bacillaceae. As the type culture we recommend the strain BS 860 (ATCC 13881).

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Sarcina ureae (Beijerinck) Löhnis is one of the interesting species of bacteria commonly placed in the family Micrococcaceae. It is the only representative of cocci that produces endospores. From the taxonomic point of view, this species has been studied quite inadequately. Gibson (1935) and Wood (1946) have contributed to a more profound knowledge of this species. Orla-Jensen (1909) and later Kluyver and van Niel (1936) proposed placing this species in the genus Sporosarcina Orla-Jensen. The lack of adequate taxonomic studies of this species is apparent in the present manuals (Krasilnikov 1949; Bergey's Manual 1957) in which it has various classifications. Thus the description of Sarcina ureae is insufficient and does not satisfy the present demands. We have, therefore, made it our task to give a more precise description of Sarcina ureae in this study and to revise its nomenclature and classification.

MATERIALS AND METHODS

In this work we studied 9 strains of Sarcina ureae received

from various laboratories. The list of them is given in Table 1. Ascertaining their morphological, cultural and biochemical properties we proceeded as in our preceding works (Kocur and Martinec 1959; Martinec and Kocur 1960). The formation of spores was followed on the medium recommended by Gibson (1935) by means of phase contrast microscopy.

Table 1.

<u>Sarcina ureae</u>	
<u>Strain No.</u>	<u>Source of strains</u>
204	A. Pijper, Institute of Pathology Pretoria, S. Africa
380	Dept. of Microbiology, Technical Highschool, Delft
634	J. Evans, American Meat Institute Foundation, Chicago
752	W. Haynes, NRRL, Peoria, Illinois
858, 860	C.B. van Niel, Stanford University, Pacific Grove, California
684	ATCC 6474
871	S. Aaronson, Haskins Labs., New York
981	NCIB 869

RESULTS

Morphology. All the strains formed Gram-positive cocci which occurred in characteristic packets of 8 cells. On the sporulation medium recommended by Gibson (1935) all the strains formed spherical spores centrally located. The cells measured 1.5-2 μ , the spores 0.8-1.2 μ . All the strains were motile only when grown on nutrient broth with 1% urea. They were nonmotile when grown on a nutrient agar or broth.

Cultural characteristics. On nutrient agar all the strains formed small (2-3 mm) circular colonies with a straight margin, slightly convex, grey or brown. In nutrient broth all the strains formed a slight turbidity and a thick sediment on the bottom and partly also on the walls of the tube. The strains did not grow on potato. All the strains were able to resist heating up to 80°C for 10 minutes.

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Biochemical characteristics. The results of the studies of biochemical characteristics are quoted in Table 2.

DISCUSSION

The studies of morphological, cultural, and biochemical characteristics of 9 strains of Sarcina ureae have shown that there are no essential differences between the strains. Having compared them with other aerobic micrococci also studied by us it became evident that the biochemical properties of Sarcina ureae are much more constant than those of most other micrococci.

The results of the studies of sporulation, resistance to high temperature, and motility are in accordance with the data of other authors (Gibson 1935; Pijper et al. 1955; Halle and Bisset 1958; Thompson and Leadbetter 1962). The other results also agree with both the original description, and other published data (Gibson 1935; Krasilnikov 1949; Breed 1957).

One of the unsolved but very important problems remains the question of nomenclature and classification of Sarcina ureae. Since 1901 when it was described, it has several times been renamed and placed in different genera. Indistinct opinions of the precise classification of this species in the system have become evident even in contemporary guides and keys (Krasilnikov 1949; Bergey's Manual 1957) in which this species has been classified quite differently.

In our opinion none of the authors classified this organism in a satisfactory manner. Krasilnikov places it, in accordance with Beijerinck, in the genus Planosarcina.

Breed places the species in the genus Sarcina, but this is not an appropriate solution either, because the genus Sarcina is heterogeneous and contains both aerobic and anaerobic species substantially differing from one another in their biochemical properties. There is only one common character that connects them in the genus Sarcina, namely, the characteristic grouping of cells in packets.

Orla-Jensen proposed in 1909 that Beijerinck's Planosarcina ureae should be placed in a new genus—Sporosarcina. Although the author does not concern himself with this problem in detail, one can conclude even from his brief data that the new name Sporosarcina was published validly and

Table 2. Results of biochemical tests with 9 strains of Sarcina ureae.

Tests	Number positive	Number negative
Glucose	0	9
Lactose	0	9
Sucrose	0	9
Maltose	0	9
Galactose	0	9
Fructose	0	9
Rhamnose	0	9
Mannose	0	9
Inulin	0	9
Xylose	0	9
Arabinose	0	9
Glycerol	0	9
Adonitol	0	9
Sorbitol	0	9
Mannitol	0	9
Dulcitol	0	9
Starch	0	9
Esculin	0	9
Salicin	0	9
Sodium gluconate	0	9
Sodium formicate	0	9
Sodium acetate	0	9
Sodium lactate	0	9
Sodium succinate	0	9
Sodium benzoate	0	9
Sodium tartarate	0	9
Sodium citrate	0	9
Acetylmethylcarbinol	0	9
Methyl red	0	9
Gelatin hydrolysis	0	9
Casein hydrolysis	0	9
Arginine dihydrolase	9	0

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Table 2. (cont.)

Tests	Number positive	Number negative
Lysine decarboxylase	6	3
Nitrate reduction	9	0
Nitrite reduction	0	9
Hydrogen sulfide	3	6
Indole	0	9
Phenylalanine	0	9
Catalase	9	0
Oxidase	0	9
Lipase	0	9
Urease	9	0
Tyrosinase	0	9
Hemolysis	0	9
Coagulase	0	9
Phosphatase	0	9
5% NaCl	9	0
7.5% NaCl	9	0
10% NaCl	0	9
Pigment	0	9
Survival at 60°/30 min.	9	0
Survival at 80°/10 min.	9	0
Penicillin 10 units	9	0
Streptomycin 20 µg	9	0
Chloramphenicol 20 µg	9	0
Chlortetracycline 100 µg	9	0
Terramycin 50 µg	9	0
Tetracycline 50 µg	9	0
Erythromycin 20 µg	9	0
Neomycin 40 µg	9	0
Tyrothricin 40 µg	8	1
Nystatin 20 units	9	0
Bacitracin 5 units	9	0
Lysozyme	2	7

that he had designated one species—Sporosarcina ureae (Beijerinck) Orla-Jensen. The generic name Sporosarcina has also been recommended by Kluver and van Niel (1936). It is of interest that though this is a much more suitable designation for Beijerinck's organism, it was not accepted in compiling determinative keys and manuals.

It should be pointed out that both these names, Planosarcina ureae Beijerinck and Sporosarcina ureae (Beijerinck) Orla-Jensen are valid. Which of them is to be used depends on whether one thinks that the motile sarcinae should be classified only in the genus Planosarcina or that the motile spore-forming sarcinae should be placed in a separate genus.

Therefore we think it right to classify Beijerinck's urea-decomposing organism as belonging to a separate genus—Sporosarcina—which would for the present have one species only—Sporosarcina ureae (Beijerinck) Orla-Jensen. As for the placing of this genus in the bacterial system we think it right to place it in the family Bacillaceae, as follows:

Family:	<u>Bacillaceae</u>
genus I:	<u>Sporosarcina</u>
genus II:	<u>Bacillus</u>
genus III:	<u>Clostridium</u>

Such a classification seems more correct phylogenetically; because Sporosarcina ureae forms spores it is much more closely related to the members of the family Bacillaceae than to the species of the family Micrococcaceae.

At the same time we propose a new characterization of the genus Sporosarcina.

Genus Sporosarcina Orla-Jensen 1909

Spherical cells dividing in three planes perpendicular to one another; this causes the formation of packets. Spores are formed. Motile. Gram-positive. Growth occurs in aerobic conditions. No pigment produced. No acid or gas from carbohydrates. Urea is converted into ammonium carbonate. Nitrites produced from nitrates. Nitrites not reduced. Acetylmethylcarbinol not produced. Gelatin usually not liquefied, hydrogen sulfide and indole not produced. Saprophytes. There is only one species Sporosarcina ureae in the genus.

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Sporosarcina ureae (Beijerinck 1901) Orla-Jensen 1909

Synonyms: Planosarcina ureae Beijerinck 1901

Sarcina ureae (Beijerinck 1901) Löhnis 1911

Cocci measuring 1-1.5 μ occur singly, in pairs and in packets. They form endospores measuring 0.8-1.0 μ which are located centrally. Motile, each cell possessing a single long flagellum. Gram-positive. Colonies on nutrient agar: circular, with a straight margin, flat, smooth, greyish brown.

Colonies on gelatin: Similar to those on the nutrient agar.
Broth: Slight turbidity, sediment on the bottom and walls of the tube.

Gelatin stab: Growth of grey colour, does not liquefy gelatin.

Agar slant: Average grey growth, not translucent, glistening; old cultures light yellow.

Potato: No growth.

Peptone water with carbohydrates: No acid or gas from glucose, lactose, sucrose, maltose, galactose, fructose, mannitol, sorbitol, dulcitol, arabinose, xylose, rhamnose, glycerol, adonitol, inositol, mannose and salicin.

Milk: No change, but it can be alkaline if a heavy inoculum is used. No indole produced.

Hydrogen sulfide usually not produced.

Acetylmethylcarbinol not produced.

The methyl red test is negative.

Nitrates: Reduced to nitrites, only occasional strains fail to reduce them.

Nitrites not reduced.

Aerobic. Catalase produced.

Oxidase, coagulase and phosphatase not produced.

Sheep or human blood not hemolyzed.

Gelatin, casein, starch and esculin not hydrolyzed.

Phenylalanine not decomposed. Decarboxylation of lysin variable.

Temperature relations: Optimum, 25°C. Maximum, 37°C.
Minimum, 10°C.

Spore-containing cells resist heating up to 80°C for 10 min.

Optimum pH: 8.8.

Growth in higher concentrations NaCl: 7.5% +; 10% -.

Sensitivity to antibiotics: Usually sensitive to penicillin, streptomycin, choramphenicol, chlortetracycline, terramycin, tetracycline, erythromycin, nystatin, bacitracin.

Source: Isolated from garden soil.

Ecology: Distributed in swamp soil. Also found in sea water.

The culture BS 860 (ATCC 13881) is suggested as neotype.

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