CONTRIBUTION TO THE TAXONOMIC STUDIES
OF SARCINA LUTEA SCHROETER AND SARCINA
FLAVA DE BARY

M. Kocur and T. Martinec

(With the technical assistance of J. Benešová)

Department of Microbiology
Faculty of Natural Sciences in Brno

Taxonomic studies of the genus Sarcina, particularly of the two closely cognate species Sarcina lutea Schroeter and Sarcina flava De Bary, are inadequate in many details. The original concise description of Sarcina lutea (1886) and Sarcina flava (1887) were given under the strong influence of the monomorphic concept of species and were accepted by later authors without any essential emendation that would be of great importance for further taxonomic studies.

The descriptions have been more adequately formulated in Bergey's Manual (1948, 1957) and Krassilnikov's Guide (1949). Recently studies not directly connected with the taxonomy of the aforesaid microorganisms have appeared. These later investigations, especially relating to Sarcina lutea, for example, identify nucleic acid (Dutta and Jones, 1956) and certain carotenoid pigments (Arnaki and Stary, 1952; Miller, 1954; Besborodov, 1956). Mutants of these species were investigated by Witkus (1948) and Bellini (1951). The contributions of these authors, although not primarily aimed at taxonomy [as the recent Aaronson's studies (1956)], nevertheless include results that can be used in the taxonomic studies of the genus Sarcina.

Some authors, e.g. Lehmann and Neumann (1927), have suggested that Sarcina lutea and S. flava might be identical. As a result of this expression of opinion we have undertaken to ascertain the characteristics of the two species, to determine their identity or differences and on the basis of the results obtained to place them correctly in the genus Sarcina.

MATERIAL AND METHODS

In our work we have studied 16 strains of Sarcina lutea

and 28 of S. flava, of these 26 strains were acquired from various collections in Czechoslovakia and abroad, 18 strains were isolated by us from water and air. The list of strains is given in table 1. We have studied morphological, cultural and biochemical characteristics of these cultures. We have

Table 1.

Species	Strain No.	Source of Strain
<u>Sarcina lutea</u>	352, 353, 354, 355	Dr. Stárka, Dept. of
	356	Microbiol., Prague
	357	Biological Institute,
		Prague
	362	Institute for Fermentation,
		Osaka, Japan
	336	Dept. of Applied Biology,
		NRC., Ottawa
	249	Institute of Hygiene,
<u>Sarcina flava</u>		Perugia, Italy
	407, 408, 409	Dept. of Microbiol.,
		Bratislava
	443	Institute of Hygiene,
		Warsaw
	149, 310, 373	Own isolates
	268	State Inst. of Public
		Health, Prague
	358	Dr. Starka, Dept. of
<u>Sarcina flava</u>		Microbiol., Prague
	296	Dept. of Biological Sci.,
		Purdue Univ., USA
	335	Dept. of Applied Biology,
		NRC., Ottawa
	163, 166, 372, 308	Own isolates
	309, 365, 366, 367	
	368, 369, 370, 371	
	374, 437, 438, 439	
<u>Sarcina flava</u>	511, 512, 513, 514	
	515, 516, 517, 518	

used the following methods: Gram's stain as modified by Hucker, carbohydrate fermentation was determined in peptone water with 1% sugar, gelatin liquefaction was tested by Frazier's method and by stab inoculation on 15% gelatin, production of hydrogen sulfide by means of lead acetate test strips, indole by Kovács' reagent, nitrate reduction by Gries-Ilosway's reagent, starch hydrolysis by Lugol solution. These methods are quoted in the "Manual of Methods for Pure Culture Study of Bacteria" (1946).

RESULTS

Morphology. The strains of the two species of Sarcina used by us are morphologically identical. They were differentiated from each other only by the size of individual cells that varied from 1.0-2.0 μ . All the strains were Gram positive and formed characteristic packets, strains No. 362, 335, 358, 268 only in broth, all the other strains also on nutrient agar.

Cultural characteristics. Colonies both of Sarcina lutea and S. flava were usually round, 0.4-0.6 cm in diameter, raised, granular, profile slightly convex. They were yellow or non-pigmented. Most cultures growing in nutrient broth produced yellow or yellowish sediment. Some cultures deposited a granular sediment on the test tube walls, while broth remained clear. Slight turbidity was formed by strains No. 362, 386, 268. Nearly all the investigated strains produced pigments on nutrient agar, starch agar, blood agar and on potato. An exception occurred with the strains No. 163, 166, and 266, which produced no pigment in the media used.

Biochemical characteristics. The ascertained biochemical characteristics are shown in the table 2.

DISCUSSION

In this study we have endeavored to determine the correctness of present recognition of S. lutea and S. flava as distinct species.

Sarcina lutea Schroeter. Cells spherical, from 1-2 μ in diameter. Our findings agree with the original description and with those in Krassilnikov's Guide and in Bergey's Manual (ed. 7). The colony characteristics are essentially identical.

Table 2.

Species	Gelatin		Nitrate		Indole		Hydrogen Sulfide		Milk		Hemolysis		Glucose		Fructose		Galactose	
	No.		No.		No.		No.		No.		No.		No.		No.		No.	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
<u>Sarcina lutea</u>	12	4	1	15	0	16	0	16	3	13	0	16	0	16	0	16	0	16
<u>Sarcina flava</u>	26	2	2	26	0	28	0	28	3	25	0	28	0	28	0	28	0	28
	Lactose		Sucrose		Maltose		Glycerol		Mannitol		Sorbitol		Dulcitol		Sodium Citrate		Acetylmethyl-carbinol	
	No.		No.		No.		No.		No.		No.		No.		No.		No.	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
<u>Sarcina lutea</u>	0	16	0	16	0	16	0	16	0	16	0	16	0	16	0	16	0	16
<u>Sarcina flava</u>	0	28	0	28	0	28	0	28	0	28	0	28	0	28	0	28	0	28
	Methyl Red Test		Starch Hydrolysis		5% NaCl		7.5% NaCl		Urease		Catalase		Lipase					
	No.		No.		No.		No.		No.		No.		No.					
	+	-	+	-	+	-	+	-	+	-	+	-	+	-				
<u>Sarcina lutea</u>	0	16	0	16	0	0	16	0	16	0	16	0	16	0				
<u>Sarcina flava</u>	0	28	0	28	0	28	0	28	0	28	0	28	0	28				

BACTERIOLOGICAL NOMENCLATURE
AND TAXONOMY

Comparison on findings relative to cultural characteristics with those of other workers has been somewhat limited by lack of availability of literature. Most strains studied grow and produce some pigment in synthetic media; we have found no accounts of such studies by others. Our data with reference to optimum growth temperature agree with those of other workers.

Data relative to biochemical activity as recorded in the literature show marked differences among strains. Schroeter states that *S. lutea* usually does not liquefy gelatin, Breed and Smit in Bergey's Manual (1957) record slow liquefaction, Krassilnikov's Guide states no liquefaction. We recorded liquefaction in 12 of 16 strains.

Breed and Smit (Bergey's Manual) state "nitrites generally produced from nitrates"; we found nitrate reduction in one strain of 16. Breed and Smit record production of H_2S , we found all 16 strains negative. These authors also state that litmus milk is coagulated and becomes alkaline. We agree with Krassilnikov that in general milk is not changed (three milk +, 13 -). Krassilnikov states that glucose is fermented, we agree with Breed and Smit that there is no fermentation. It is evident that the Bergey's Manual and Krassilnikov's Guide are not in agreement.

Sarcina flava De Bary. The original description by de Bary states that the cells are $1-2\mu$ in diameter, as do also Breed and Smit, and we agree. Our results with reference to cultural characters agree with statements of Krassilnikov and of Breed and Smit. De Bary states that *S. flava* liquefied gelatine. With this conclusion both manuals agree. We found 2 only of 28 strains which were non-gelatin liquefying. Breed and Smit state that this organism does not reduce nitrates, Krassilnikov found that not all strains reduce nitrates, we recorded nitrate reduction in 2 only of 28 strains. All agree that neither indole nor hydrogen sulfide are produced. No coagulation of milk is recorded in the manuals; we found slight peptonization of milk in 3 strains only. We confirm the fermentation of sugars as given by the manuals.

We conclude that the published taxonomic differences, on the basis of which the two species, *Sarcina lutea* and *S. flava* were separated were insufficient. In more detailed study of a greater number of strains from both species it was shown that the differences between the two species were

not so outstanding that they can be distinguished by their use. As can be seen in the table 2, investigated biochemical characteristics of both species did not show any differences in the biochemical activity, except in starch hydrolysis. Therefore we assume that this single distinguishing feature, in spite of its importance, is inadequate as a reason for separation of the species. These two species differ neither in gelatin liquefaction as de Bary (1886) and Krassilnikov (1949) indicate, nor in milk coagulation according to Bergey's Manual (1957). Therefore these characteristics cannot be used for species differentiation as these authors suggest. Instead of this we propose differentiation on the basis of starch hydrolysis.

Therefore we suggest the following key for the differentiation of the two species:

I. Aerobic

A. Not halophilic

1. Non-motile.

a. Yellow pigment produced

(1) Starch is hydrolyzed

Sarcina lutea

(2) Starch not hydrolyzed

Sarcina lutea subsp. flava subsp. nov.

Sarcina lutea Schroeter

Spheres 1-2 μ in diameter, in packets of 8. Non-motile.
Gram positive. Some strains produce packets only in liquid media.

Agar colonies: (After 2 days) Yellow, circular, usually 3-6 mm in diameter, granular, moist, glistening.

Broth: (After 2 days) Clear, yellow sediment.

Potato: (After 5 days) Slight growth, yellow pigment.

Gelatin liquefaction: Most strains liquefied.

Nitrite not produced from nitrates. Only single strains +.
Indole not produced.

Hydrogen sulfide not produced.

Milk: (After 10 days) Not coagulated, milk slight alkaline or unchanged, only single strains +.

Hemolysis: Negative.

No acid from glucose, fructose, galactose, lactose, sucrose, maltose, glycerol, mannitol, sorbitol, dulcitol.

Sodium citrate: No growth.

BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

Acetylmethylcarbinol: Negative.

Methyl red test: Negative.

Urease: Negative.

Starch hydrolysis: Positive.

Sarcina lutea subsp. flava subsp. nov.

Differs from S. lutea in that it does not hydrolyze starch.

SUMMARY

We have studied 16 strains of Sarcina lutea and 28 strains of Sarcina flava.

We have found that the two species are distinguished only by starch hydrolysis.

Because of minor differences in morphology and biochemical characteristics, we reduce the species Sarcina flava to rank of a subspecies, with the name Sarcina lutea subsp. flava.

REFERENCES

- Aaronson, S. 1956. A biochemical-taxonomic study of a marine micrococcus Gaffkya homari and a terrestrial counterpart. J. Gen. Microbiol. 15:478-484.
- Arnaki, M. and Z. Stary. 1952. Étude sur la biosynthese des carotenoides chez la Sarcina lutea. Folia Pharmaceutica II(7):3-6.
- Bellini, C. C. 1951. A preliminary investigation of mutation production in Sarcina lutea Schroeter. Jour. of Heredity 42:278-284.
- De Bary, A. 1887. Vorlesungen über Bakterien.
- Besborodov, A. M. 1956. Osobēnosti sintēza karotinoidov u želtych sarcin. Mikrobiologiya 24:25-29.
- Breed, R. S., E. G. Murray and A. P. Hitchens. Bergey's Manual of Determinative Bacteriology. Baltimore. 1948.
- _____, _____ and N. R. Smith. Bergey's Manual of Determinative Bacteriology. Baltimore. 1957. Ed. 6 and 7.
- Krassilnikov, N. A. 1949. Oprēdelitel' baktērij i aktinomycetov. Moskva.
- Lehmann, K. B. and R. O. Neumann. 1927. Bakteriologische Diagnostik. München.
- Manual of Methods for Pure Culture Study of Bacteria. 1946. Geneva, New York.

- Miller, M.A. 1954. Observations of morphological and pigment variation in Sarcina lutea. Turtox News 32:11.
- Pijper, A. 1955. The Sarcinae. Kind of flagella and specific agglutination. Jour. Bact. 69:151-158.
- Shaw, C., J.M. Stitt and S.T. Cowan. 1951. Staphylococci and their classification. Jour. Gen. Microbiol. 5:1010-1023.
- Schroeter, J.R. 1886. Kryptogamenflora von Schlesien, III (1):154.
- Winslow, C.E.A. and A.R. Winslow. 1908. The systematic relationship of the Coccaceae. New York.
- Witkus, R. 1948. A predictable mutation in bacteria. Proc. Nat. Acad. Sci., Washington. 34:442.