THE TAXONOMIC STATUS OF MICROCOCOCCUS ROSEUS FLÜGGE, 1886

M. Kocur and Zdena Páčová

Czechoslovak Collection of Microorganisms
J.E. Purkyně University, Brno, Czechoslovakia

ABSTRACT. A revised characterization of Micrococcus roseus Flüge 1886, 183 is proposed on the basis of taxonomic analysis of 29 strains.

- - - - -

In the course of the past 10 years several authors have contributed to our knowledge of Micrococcus roseus Flüge 1886, 183. Hill (1959) was the first who showed that M. roseus forms one of the natural groups among aerobic catalase-positive and Gram-negative cocci (Kocur and Martinec 1962, Eisenberg and Evans 1963, Baird-Parker 1965).

Recently a few papers have appeared which contribute to the knowledge of M. roseus. Energy and nitrogen requirements of M. roseus were studied by Eisenberg and Evans (1963). These authors proposed a synthetic basal medium. A defined medium for growth and pigmentation of M. roseus was also proposed by Cooney and Thierry (1966) and these authors (Thierry and Cooney 1966) studied physiochemical factors influencing growth and pigment synthesis. The chemical composition of pigment of M. roseus was studied by Cooney, Marks and Smith (1966) and Ungers and Cooney (1968).

Oeding (1966) surveyed the serological properties of several strains of M. roseus and found that this organism and some other micrococci are related to staphylococci as they have more shared agglutinogens. The GC content in the DNA of some strains of M. roseus has been studied by Silvestri and Hill (1965) and by Boháček, Kocur and Martinec (1969).

The characterization of M. roseus given by Hucker and Breed (1957) is no longer adequate. The purpose of the present paper is to give a revised characterization of M. roseus.

MATERIAL AND METHODS

Twenty-nine strains of M. roseus were investigated (Table 1).

All strains studied are from Czechoslovak Collection of Microorganisms, Brno. The phages of M. lysodeikticus (N1 and N6) were obtained from Dr. A.N. Naylor, Cornell University, Ithaca, New York. They were isolated by Naylor and Burgi (1956). Stock cultures were maintained on nutrient agar at 4°C. For morphological, cultural and biochemical examinations, cultures 24 hrs old were used. All tests were carried out at 30°C with the exception of strains CCM 2131 and 2390 which were cultivated at 22°C. Morphological properties, Gram staining and starch hydrolysis were studied according to the methods recommended in the Manual of Microbiological Methods (1957). Most of cultural and biochemical methods were those described by Mortensen and Kocur (1967). The production of phenylalanin deaminase, growth on

Table 1. List of strains used.

<u>Micrococcus roseus</u> CCM No.	Other names and numbers
189, 293, 347, 570, 574, 590 626, 677, 908, 1145, 1679	
239	NCIB 9147
385	ATCC 185, NCIB 9149, NCTC 7515
557	ATCC 177, NCTC 7512
560, 618, 839	ATCC 179, NCTC 7514; ATCC 534, NCTC 7522; ATCC 516, NCTC 7521
562	<u>Micrococcus flavoroseus</u> ATCC 397, NCTC 7528, G. J. Hucker No. 349
633	ATCC 412, NCIB 8175, NCTC 7517, G. J. Hucker No. 213
679	<u>Micrococcus rubens</u> ATCC 186, NCTC 7523, IAM 1315, IFO 3768
691	ATCC 9815, NCTC 7738, IAM 1295, IFO 3764
706	<u>Sarcina erythromyxa</u> ATCC 187
758	NRRL B-245
837	ATCC 416, NCTC 7518, G. J. Hucker No. 565
845, 861	<u>Sarcina</u> sp. ATCC 188, C. B. van Niel L. D. 2. 1. ATCC 385, C. B. van Niel L. D. 2. 2.
2131	<u>Micrococcus agilis</u> ATCC 998, NCTC 2676
2257	<u>Staphylococcus roseus</u> ATCC 144, NCTC 7511
2390	<u>Micrococcus corallinus</u> ATCC 966, NCTC 7509, NCDO 983, BP-97, W.O. 219.

Explanation:

ATCC = American Type Culture Collection

CCM = Czechoslovak Collection of Microorganisms, Brno

IAM = Institute of Applied Microbiology, Tokyo

IFO = Institute for Fermentation, Osaka

NCTC = National Collection of Type Cultures, London

NCDO = National Collection of Dairy Organisms, Reading

Simmons' citrate agar were studied by means of methods recommended by Ewing (1960). Aesculin hydrolysis and nitrate reduction were studied by means of methods recommended by Sneath (1956). Sensitivity to phages N1 and N6 was tested by the method recommended by Blair and Williams (1961). Sensitivity to antibiotics was tested by using multodiscs "Oxoid."

RESULTS

Morphology. All strains studied were Gram-positive cocci. The data on their size and cell arrangements are given in Table 2.

Cultural characteristics. As it follows from the Table 2, most of the strains formed smooth colonies on nutrient agar. Only two strains (CCM 560 and 679) formed rough colonies. As regards the pigment production the strains of *M. roseus* could be divided into three groups. The first group including 24 strains produced a relative stable pink pigment on all solid media studied. No differences in intensity of pigment production of these strains was observed. The second group formed strains CCM 626, 590 and 574 which produced on nutrient agar pink pigment; however, on nutrient agar with 5% NaCl on yeast-glucose agar, phenylalanine agar, and egg-yolk medium they produced an orange pigment. The third group encompasses two psychrophilic strains (CCM 2131 and 2390) which produced on nutrient agar, nutrient agar with 5% NaCl, and starch agar, pink pigment while on egg-yolk agar, tyrosine and phenylalanine agar developed a deep red pigment. In nutrient broth all strains produced slight turbidity, and the sediment. All strains were aerobic and most of them grew well within the range of 20-37°C. Two strains, CCM 2131 and 2390, were psychrophilic. They grew well at 22°C and did not grow at 37°C.

Biochemical characteristics. The data on biochemical characteristics of the strains studied are given in Table 2. The more detailed biochemical characteristics of 20 strains of *M. roseus* with known GC content in the DNA, are given in Table 3.

DISCUSSION

The purpose of this paper is to provide a more extensive description of *M. roseus* on the basis of the examination of 29 strains which came from various sources. Our study confirmed the opinion of Hill (1959) that *M. roseus* forms one of the natural groups among aerobic Gram-positive, catalase positive cocci and can be recognized as separate species.

Our results agree (with minor exceptions) with the data of other authors (e.g., Eisenberg and Evans 1963, Baird-Parker 1965) who also studied *M. roseus*. As it can be seen from the Tables 2 and 3, certain differences occurred among strains—particularly in biochemical tests. Most of them were observed also by other authors (Shaw et al. 1951, Hill 1959) and others.

A remarkable exception deserving attention were two strains (CCM 2131 and 2390) which differed from other strains studied by several features (see Table 3). They did not grow at 37°C and did not reduce nitrate but hydrolyzed gelatin and produced red pigment. In our opinion, these psychrophilic strains are aberrant forms of *M. roseus* and therefore we do not recognize them as separate species.

Table 2. Complete characters of 29 strains of Micrococcus roseus. *

Test	No. strains responding	
	+	-
Cell grouping		
(1) tetrads	5	
(2) tetrads and clumps	11	
(3) clumps	13	
Size		
< 1 μ	24	
1 to 1.5 μ	5	
Colony		
(1) Smooth, glistening, convex, regular edge	20	
(2) Smooth, opaque, convex, regular edge	7	
(3) Rough, irregular edge	2	
Pink pigment	27	
Red pigment	2	
Aerobic acid from glucose	0 (8 w)	21
Hydrolysis of		
Gelatin	2	27
Aesculin	1	28
Starch	13	16
Nitrate reduction	26 (1 w)	2
Growth in 10% NaCl	0 (1 w)	28
Oxidase	0 (2 w)	27
Growth at 37°C	27	2

* All strains produced catalase, were sensitive to lysozyme, penicillin, streptomycin, chloramphenicol, tetracycline, erythromycin, oleandomycin, novobiocin, kanamycin, colistin, vancomycin, and neomycin.

No strains produced acid from anaerobic glucose, acetoin, phosphatase, urease, haemolysis, reduced nitrite, split arginine, grew on Simmons' citrate agar, were sensitive to phage N-1 or grew in 15% NaCl.

w = weak reaction.

As shown in Table 3 there is no such agreement among GC content in the DNA and some of the other studied tests that would enable the grouping of M. roseus strains into biotypes.

Good correlation, however, was observed among three features: formation of pink pigment and negative tests for nitrate and gelatin hydrolysis. The mentioned tests are of great importance for differentiation of M. roseus from the other species of the genus Micrococcus, at present.

At the same time we propose a new characterization of Micrococcus roseus which is as follows:

Micrococcus roseus Flüge 1886, 183.

Neisseria rosea (Flüge 1886) Trevisan 1889, 32.

Merismopedia rosea (Flüge 1886) Dyar 1895, 354.

Micrococcus rubens Migula 1900, 177.

Rhodococcus roseus (Flüge 1886) Winslow and Rogers 1906, 545.

Staphylococcus roseus (Flüge 1886) Tavel acc. to Lehmann and Neumann 1896, 177.

Micrococcus roseofulvus Hucker 1928, 27.

Micrococcus roseofulvus Pribram 1933, 44.

Ro'se.us. L. adj. roseus - rose colored.

Spheres, 1-15 μ in diameter occurring singly in pairs or tetrads and in clumps. Nonmotile, nonsporeforming. Gram-positive.

Agar colonies: Circular, with straight margin 2-3 mm in diameter, slightly convex, smooth, occasionally rough, pink or red colour.

Agar slant: Good growth with pink or orange pigment.

Nutrient broth: Moderate turbidity with sediment. Some strains form slight pellicle with ring.

Catalase activity is present.

Porphyrin respiratory enzymes are present (method of Deibel and Evans 1960).

Produce no acid or gas from glucose and other carbohydrates in Standard medium (Subcommittee 1965).

Final pH in glucose broth 5.9 - 6.8.

Acetylmethylcarbinol not produced.

Methyl red test is negative.

Indole and hydrogen sulfide is not produced.

Ammonia is not produced from arginine.

Nitrate is reduced (exceptionally not reduced).

Nitrite is not reduced.

Gelatin is not hydrolyzed (variation-hydrolyzed).

Starch hydrolysis: different reactions.

Aesculin is hydrolyzed exceptionally.

Oxidase is produced only exceptionally.

Simmons' citrate agar: No growth.

Phenylalanine deaminase, phosphatase, or urease are not produced.

Nucleoside transphosphorylase is not produced (Komagata and Kocur 1967).

Egg-yolk reaction is negative.

Tween 80 is split.

Human and rabbit plasma is not coagulated.

Haemolysis is not produced.

Pigment: produce pink or red water insoluble pigment canthaxanthin, chemically it is 4.4 diketo β -carotene (Cooney et al. 1966, Unger and Cooney 1968).

Guanine plus cytosine content in the DNA is 66-75% (Boháček, Kocur and Martinec 1969).

Optimum temperature: 20-30°C (some strains are psychrophilic).

Salt tolerance: Good growth in 0-7.5% NaCl.

Saprophytic.

Habitat: Soil, water.

Neotype culture is strain ATCC 186 (NCTC 7523; CCM 679; IFO 3768; IAM 1315).

Table 3. The biochemical characteristics of Micrococcus roseus strains with known GC content in the DNA.

<u>M. roseus</u> CCM No.	% GC	pH in fluid medium after 6 days incubation		Glucose (aerobic)	Aesculin hydrolysis	Starch hydrolysis	Gelatin hydrolysis	Nitrate reduction	Tyrosinase	Oxidase	Growth at 37°C	5% NaCl	7.5% NaCl
		aerobic	anaerobic										
2131	68.4	6.4	6.7	-	-	+	+	-	-	-	-	-	-
2390	69.0	6.3	6.6	-	-	+	+	-	-	-	-	-	-
679	69.0	6.3	6.5	-	-	-	-	+	-	-	+	+	-
908	69.2	6.0	6.7	-	-	-	-	+	-	-	+	+	-
837	69.7	6.1	6.3	-	-	-	-	+	-	-	+	+	-
239	71.1	6.3	6.4	-	-	+	-	+	-	-	+	+	+
385	71.0	5.9	6.4	-	-	+	-	+	-	-	+	+	-
560	71.1	6.3	6.8	-	-	-	-	+	-	-	+	+	-
618	71.1	5.9	6.6	-	-	-	-	+	-	-	+	+	-
1679	71.1	6.0	6.5	-	-	-	-	+	+	-	+	+	-
347	71.5	6.0	6.6	-	-	-	-	+	-	-	+	+	-
839	72.0	6.0	6.6	-	-	-	-	+	-	-	+	+	-
706	72.0*	6.1	6.5	-	-	-	-	+	-	-	+	+	-
189	72.2	6.0	6.5	-	-	+	-	+	-	-	+	+	-
557	72.8**	6.2	6.6	-	-	-	-	+	+	-	+	+	-
663	72.8	6.2	6.4	-	-	-	-	+	-	-	+	+	-
562	72.8	6.0	6.4	-	-	-	-	+	-	-	+	+	-
691	73.3	6.0	6.7	-	-	+	-	-	-	-	+	+	-
1145	73.6	5.9	6.6	-	+	+	-	+	-	-	+	+	+
570	73.8	6.0	6.7	-	-	+	-	+	-	-	+	+	-
2257	75.0	6.0	6.4	-	-	-	-	+	-	-	+	+	-

(See legends on facing page)

Legends for Table 3.

All strains produced catalase, were sensitive to lysozyme, penicillin, streptomycin, chloramphenicol, tetracycline, erythromycin, oleandomycin, novobiocin, kanamycin, colistin, vancomycin.

No strains produced acid from anaerobic glucose, acetoin, phosphatase, urease, haemolysis, split arginine, hydrolyzed gelatine, grew on Simmons' citrate agar, were sensitive to phage N1 or N6 or grew in 15% NaCl.

* Data by Rosypalová et al. (1966)

** Data by Silvestri and Hill (1966)

The remaining data on GC content in the DNA of strains are by Boháček et al. (1969)

+ = positive reaction; - = negative reaction; . = weak reaction.

- - - - -

REFERENCES

- Baird-Parker, A. C. 1965. The classification of staphylococci and micrococci from world-wide sources. *J. gen. Microbiol.* 38:363-387.
- Blair, J. E. and R. E. O. Williams. 1961. Phage typing of staphylococci. *Bull. World Hlth. Org.* 24:771-784.
- Boháček, J., M. Kocur and T. Martinec. 1969. Deoxyribonucleic acid base composition of Micrococcus roseus. *Antonie van Leeuwenhoek* 39:
- Cooney, J. J. and O. C. Thierry. 1966. A defined medium for growth and pigment synthesis of Micrococcus roseus. *Canad. J. Microbiol.* 12:83-89.
- _____, H. W. Marks and A. M. Smith. 1966. Isolation and identification of canthaxanthin from Micrococcus roseus. *J. Bact.* 92:342-345.
- Deibel, R. H. and J. B. Evans. 1960. Modified benzidine test for the detection of cytochrome-containing respiratory systems in microorganisms. *J. Bact.* 79:356-360.
- Dyar, H. G. 1895. On certain bacteria from the air of New York City. *Ann. N. Y. Acad. Sci.* 8:322-380.
- Eisenberg, R. C. and J. B. Evans. 1963. Energy and nitrogen requirements of Micrococcus roseus. *Canad. J. Microbiol.* 9:633-642.
- Ewing, W. H. 1960. Enterobacteriaceae. Biochemical methods for group differentiation. *Hlth. Serv. Publ.* 734:1-30.
- Flügge, C. 1886. *Die Mikroorganismen*. Part 2. 3rd ed. Leipzig.
- Hill, L. R. 1959. The Adansonian classification of the staphylococci. *J. gen. Microbiol.* 20:277-283.
- Hucker, G. J. 1928. Studies on the Coccaceae. IV. The classification of the genus Micrococcus Cohn. *New York State Agr. Expt. Sta. Tech. Bull.* No. 135.
- _____, and R. S. Breed. 1957. In: *Bergey's Manual of Determinative Bacteriology*, 7th ed. p. 455. Ed. R. S. Breed, E. G. D. Murray and N. R. Smith. London: Baillière, Tindall and Cox.
- Kocur, M. and T. Martinec. 1962. A taxonomic study of the genus Micrococcus. *Folia Fac. Sci. Univ. Purkynianae Brunensis* 3:3-121.
- Komagata, K. and M. Kocur. 1967. The nucleoside phosphotransferase test and differentiation of staphylococci and micrococci. *Publ. Fac. Sci. Univ. J. E. Purkyně* K40:236-237.

- Lehmann, K. B. and R. Neumann. 1896. Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik. Teil II. 1-448.
- Manual of microbiological methods. 1957. Ed. H. J. Conn, New York, McGraw-Hill.
- Migula, W. 1900. System der Bakterien. Gustav Fischer, Jena.
- Mortensen, N. and M. Kocur. 1967. Correlation of DNA base composition and acid formation from glucose of staphylococci and micrococci. Acta path. microbiol. scand. 69:445-457.
- Naylor, H. B. and E. Burgi. 1956. Observation of abortive infections of Micrococcus lysodeikticus with bacteriophage. Virology 2:577-593.
- Oeding, P. 1967. Antigenic studies on Micrococcus strains. Acta path. microbiol. scand. 70:120-128.
- Pribram, E. 1933. Klassifikation der Schizomyceten. Leipzig. 1-143.
- Rosypalová, A., J. Boháček and S. Rosypal. 1966. Deoxyribonucleic acid base composition of some micrococci and sarcinae. Antonie van Leeuwenhoek 32:192-196.
- Shaw, C., J. M. Stitt and S. T. Cowan. 1951. Staphylococci and their classification. J. gen. Microbiol. 5:1010-1023.
- Silvestri, L. G. and L. R. Hill. 1966. Agreement between DNA base composition and taxonomic classification of Gram-positive cocci. J. Bacteriol. 90:136-140.
- Sneath, P. H. A. 1956. Cultural and biochemical characteristics of the genus Chromobacterium. J. gen. Microbiol. 15:70-98.
- Subcommittee on taxonomy of staphylococci and micrococci. 1965. Recommendation. Intl. Bull. Bact. Nomencl. Taxon. 15:109-110.
- Thierry, O. C. and J. J. Cooney. 1966. Physiochemical factors influencing growth and pigment synthesis by Micrococcus roseus. Canad. J. Microbiol. 12:691-698.
- Trevisan, V. 1889. I generi e le specie delle Batteriacee. Milan. 1-35.
- Ungers, E. G. and J. J. Cooney. 1968. Isolation and characterization of carotenoid pigments of Micrococcus roseus. J. Bact. 96:234-241.
- Winslow, C. A. E. and A. F. Rogers. 1906. A statistical study of generic characters in the Coccaceae. J. Inf. Dis. 3:385-546.