Taxonomic Status of *Micrococcus varians* Migula 1900 and Designation of the Neotype Strain

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An amended description of *Micrococcus varians* Migula 1900 is given on the basis of a study of 20 strains. CCM 884 (= ATCC 15306 = NCTC 7564) is designated as the neotype strain of *M. varians*. *Staphylococcus Zactis* Shaw et al. 1951 is a later objective synonym and *Microccus pulcher* Müller 1961 is a later subjective synonym of *M. varians* Migula 1900.

Recent advances in molecular biology, chemical analyses of the cell walls, and other factors contributed considerably to the discovery of so-called natural taxonomic groups (taxa) among the micrococci. Some of these taxa, e.g., *Micrococcus roseus*, were reexamined (12) and were found to form stable points around which a new classification of the genus *Micrococcus* could be established.

A rather confusing situation remains, however, in the classification of yellow-pigmented species of the genus *Micrococcus*. At present, two groups can be recognized among them: one contains the cell wall peptidoglycan of the lysine-tetrapeptide type (10, 17) and does not attack glucose; the other has the cell wall peptidoglycan of the lysine-alanine$_3$-4 type (10, 17) and attacks glucose oxidatively. The first group of organisms is considered to be the species *Micrococcus luteus* (13). The second group is without any designation, and the present paper deals with its taxonomy and nomenclature.

MATERIALS AND METHODS

**Organisms.** In the present work, 20 strains of aerobic, yellow-pigmented micrococci were investigated (Table 1). All the strains came from the Czechoslovak Collection of Microorganisms (CCM), Brno. The strains were maintained on nutrient agar at 4°C. All morphological, nutritional, and biochemical studies and tests were made on cultures grown at 37°C.

**Methods.** Morphological properties, Gram staining, starch hydrolysis, and nitrate reduction were determined according to methods described in the *Manual of Microbiological Methods* (4). Most of the cultural and biochemical methods were those described by Mortensen and Kocur (15). Two different media were used for glucose utilization: (i) the medium of Evans, Bradford, and Niven (6), and (ii) the medium recommended by the ICSB Subcommittee on the Taxonomy of Staphylococci and Micrococci (21). The latter was also employed for the study of other carbohydrates. The utilization of ammonium tartrate was observed on a medium recommended by Gordon and Mihm (9). Esculin hydrolysis and nitrite reduction were studied by methods recommended by Sneath (20). Gelatin hydrolysis was determined by the method of Clarke (3). Production of indole, hydrogen sulfide, acetoin, phenylalanine deaminase, and arginine dihydrolase and the methyl red test and growth on Simmons citrate agar were studied by methods recommended by Ewing (7). Oxidase was detected by the method of Gaby and Hadley (8). Salt tolerance was observed on nutrient agar with 5, 7.5, 10, and 15% NaCl. Sensitivity to antibiotics was determined by the use of Oxoid Multidiscs.

**RESULTS**

**Morphology.** All 20 strains examined were gram-positive cocci, 0.9 to 1.5 μm in diameter, arranged in tetrads and in irregular clusters of tetrads. Two strains, CCM 1414 and 2133, formed packets.

**Cultural characteristics.** Colonies of 17 strains were circular, slightly convex, smooth, and glistening. Colonies of strains CCM 552, 2139, and 2510 were rough, wrinkled, matted, and dry. All of the strains produced a light to dark yellow, water-insoluble pigment on most solid media tested. After 3 days of cultivation, seven strains formed a brown, water-soluble pigment on nutrient agar with gelatin. In nutrient broth, all of the strains produced a slight turbidity and sediment.

**Biochemical characteristics.** The mole % of the guanine plus cytosine (GC) content of the deoxyribonucleic acid (DNA) of the strains...
studied ranged from 66.2 to 72% (average 69.8%). All strains produced acid aerobically from glucose; they differed, however, in the intensity of the terminal pH values, which averaged from pH 4.3 to 5.9. The aerobic production of acid from glucose in the standard medium of the ICSB Subcommittee (21) developed slowly and occurred in 2 to 3 days. Utilization of other carbohydrates as well as the results of the other tests varied from strain to strain. Of the strains which grew on Simmons citrate agar, five utilized ammonium tartrate and two hydrolyzed arginine.

Susceptibility to antibiotics. All of the strains studied were susceptible to penicillin, streptomycin, chloramphenicol, tetracycline, erythromycin, and novobiocin. Only 10 strains were susceptible to lysozyme.

**DISCUSSION**

The strains used in this work form natural groups and have the following characters. (i) The moles % GC of their DNA ranges from 66 to 72% (1,2; Boháček, Kocur, and Martinec, in press); (ii) the peptidoglycan of their cell walls is of the Lys-Ala$_{3-4}$ type (10, 17; Schleifer, personal communication); (iii) 70% of them failed to transform M. luteus (11; Kloos, personal communication); (iv) they produce a yellow, water-insoluble pigment; (v) they produce acid from glucose and some other carbohydrates.

These strains were received at the CCM under various names (Table 1), but they belong to one species only.

A comparison of the characteristics of the 20 strains studied with the original descriptions of micrococci whose names have been validly published revealed that the earliest named species whose description agrees with these strains is *Micrococcus varians* Migula 1900 (14). Therefore, the name of the species which includes the 20 strains studied here is *M. varians*.

CCM 884 (= ATCC 15306 = NCTC 7564) is here designated as the neotype strain of *M. varians* Migula 1900. The characters of this strain agree with those recorded in the original description of *M. varians* and are presented below.

Because CCM 884 is also the type strain of *Staphylococcus lactis* Shaw et al. 1951 (17), *S. lactis* is a later objective synonym of *M. varians* Migula 1900, for both names are based on the
same type. Furthermore, inasmuch as the type strain (CCM 2127 [= ATCC 15936]) of Micrococcus pulcher Müller 1961 (16) has characters which are in general agreement with those of the neotype of M. varians, M. pulcher is here considered to be a later subjective synonym (a name based on a different, but similar, type) of M. varians.

The original description of M. varians is insufficient in terms of present-day knowledge; therefore we suggest that it be amended as follows:

_Micrococcus varians_ Migula 1900, 135.


(Percentages in parentheses indicate number of strains studied which are positive for the character cited.)

**TABLE 2. Biochemical characters of 20 strains of _Micrococcus varians_**

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<tr>
<th>Strain (CCM no.)</th>
<th>% GC</th>
<th>pH in glucose medium after 6 days</th>
<th>Galactose</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Maltose</th>
<th>Starch hydrolysis</th>
<th>Simmons citrate</th>
<th>Tartrate</th>
<th>Methyl red</th>
<th>Gelatin hydrolysis</th>
<th>Nitrates reduction</th>
<th>Nitrite reduction</th>
<th>Alkaline hydrolys</th>
<th>Urease</th>
<th>Growth in 10% NaCl</th>
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</tbody>
</table>

*a* All strains studied had positive catalase and benzidine tests, produced a yellow, water-insoluble pigment, produced acid aerobically from glucose and fructose, and grew at 37°C and on a solid medium with 7.5% NaCl. None of the strains studied produced oxidase, free or bound coagulase, acid from mannitol, arabinose, raffinose, rhamnose, mannose, salicin, adonitol, sorbitol, glycerol, or inositol; none produced deoxyribonuclease, acetoin, or phosphatase; none split Tween 80, hydrolyzed esculin, produced hydrogen sulfide, indole, phenylalanine deaminase, or hemolysis; none gave a positive egg-yolk reaction or grew on a solid medium with 15% NaCl.

*b* Reaction: +, positive; --, negative; •, weak

*c* Data by Silvestri and Hill (19); the other data on the GC contents are by Boháček et al. (1, 2; in press).
TABLE 3. Differentiation of species of the genus Micrococcus

<table>
<thead>
<tr>
<th>Character</th>
<th>M. luteus</th>
<th>M. varians</th>
<th>M. roseus</th>
<th>M. freudenreichii</th>
<th>M. mucilaginosus</th>
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</thead>
<tbody>
<tr>
<td>Pigment</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Pink</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Growth in 5% NaCl</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aerobic acid from glucose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Acetoin production</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of esculin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

of the ICSB Subcommittee (21). Acid and gas not produced from arabinose, raffinose, rhamnose, salicin, adonitol, mannitol, sorbitol, glycerol, or inositol.

Final pH in aerobic glucose broth varies with the strain from 4.3 to 5.9.

Catalase is produced (100%).

Porphyrin respiratory enzymes are produced (100%) (method of Deibel and Evans [5]).

Acetymethylcarbinol not produced.

Simmons citrate agar: Growth occurs with some strains (60%).

Ammonium tartrate is utilized by some strains (20%).

Methyl red test is positive in some strains (30%).

Indole and hydrogen sulfide are not produced.

Ammonia usually not produced from arginine.

Nitrates and nitrites are reduced (80%).

Gelatin is hydrolyzed (60%) (method of Clarke [3]).

Starch is hydrolyzed (50%).

Esculin is not hydrolyzed.

Oxidase is not produced (method of Gaby and Hadley [8]).

Phenylalanine deaminase and phosphatase not produced.

Egg-yolk reaction is negative.

Tween 80 is not split.

Urease is produced (90%).

Human and rabbit plasmas are not coagulated.

Hemolysis is not produced.

Pigment: Produce yellow, water-insoluble pigment (probably a carotenoid).

Good growth between 22 and 37 C.

Good growth on solid media with 0 to 7.5% NaCl.

Susceptibility to antibiotics: Susceptible to penicillin, streptomycin, chloramphenicol, tetracycline, erythromycin, oleandomycin, and novobiocin. Resistant to lysozyme.

Micrococcus varians can be distinguished from other species of the genus Micrococcus by means of the characters listed in Table 3.

**LITERATURE CITED**


884 = NCDO 777 = T. Gibson strain G33), the neotype strain of Micrococcus varians.

Spheres, 0.9 to 1.1 μm in diameter, occurring in pairs and tetrads. Nonmotile. Nonsporeforming. Gram positive.

The cell wall contains peptidoglycan of the lysine-alanine3-4 type (17).

Agar colonies: Circular entire, 2 to 3 mm in diameter, slightly convex, smooth with glistening surface, yellow.

Agar slant: Good growth with yellow, water-insoluble pigment.

Nutrient broth: Moderate turbidity and sediment are formed.

Chemoorganotroph: Metabolism respiratory. Strictly aerobic.

The biochemical characteristics and the GC composition of the DNA of this strain are given in Table 2.

Susceptibility to antibiotics: Susceptible to penicillin, streptomycin, chloramphenicol, tetracycline, erythromycin, oleandomycin, and novobiocin. Resistant to lysozyme.

Micrococcus varians can be distinguished from other species of the genus Micrococcus by means of the characters listed in Table 3.