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PRIORITY OF MICROCOCOCCUS MUCILAGINOSUS MIGULA
1900 OVER STAPHYLOCOCCUS SALIVARIUS ANDREWES
AND GORDON 1907 WITH PROPOSAL OF A
NEOTYPE STRAIN

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ABSTRACT. Andrewes and Gordon (1907) isolated a species to which they applied the name Staphylococcus salivarius. This has been re-isolated (Gordon 1967; Bergan et al. 1969) and by virtue of its biochemical properties and DNA base composition must belong in the genus Micrococcus. Inherent in the change of classification is the question of the specific epithet for the species. The combination M. salivarius would become illegitimate, being a later homonym to another species described by Migula (1900). Also, Andrewes and Gordon's use of the epithet salivarius is found to be illegitimate per se, because the species was named Micrococcus mucilaginosus by Migula (1900). This is substantiated both by our own studies (Bergan et al. 1969) and by additional biochemical criteria listed for M. mucilaginosus by Krasil'nikov (1949). A neotype strain, 5762/67, is proposed for M. mucilaginosus and an emended description of this species is given.

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Gordon in 1967 isolated a species to which he applied the name Staphylococcus salivarius Andrewes and Gordon 1907. This is considered in Index Bergeyana (1966) to constitute a legitimate name. There is no reason to doubt that Gordon's strains are, indeed, true re-isolates of S. salivarius. However, extensive biochemical studies and DNA base composition analysis in a material of analogous isolates necessitate transfer of this entity to the genus Micrococcus (Bergan et al. 1969).

In accordance with Rule 24b of the International Code of Nomenclature of Bacteria (1966) the corresponding reclassification would yield the combination M. salivarius (cfr. Rule 18b). This binomial, however, had been employed previously for another species when Migula (1900) substituted it for the trinomial Coccus salivarius septicus, which appeared in a description by Biondi (1887). Even Migula's use of the name was illegitimate, because the species had already been named M. sialosepticus by Trevisan (1889). Nevertheless, if the combination M. salivarius were applied to our strains (Bergan et al. 1969), it would become a later homonym and therefore illegitimate (Rule 24d).

However, the epithet salivarius was illegitimate even as used by Andrewes and Gordon (1907); it was nomenclaturally superfluous because an earlier, validly published epithet was available for this taxon, the use of which is mandatory by Rule 25d (cfr. 24a and 24b). The name with priority for the taxon involved is Micrococcus mucilaginosus Migula 1900, and it is the purpose of the following to substantiate this.

Migula's description of M. mucilaginosus is fairly detailed for its time and includes several independent characters found in the strains studied by us (vide infra). The information of Migula that M. mucilaginosus did not grow in broth may have been due to its composition. For instance, relatively small amounts of salt will inhibit the growth of this organism (Bergan et al. 1969).

M. mucilaginosus was first described by von Rätz in 1890. He used only a vernacular designation, however. Migula had personally studied a strain of this species. Index Bergeyana (1966) lists the name of this species as Micrococcus mucilaginosus Migula 1900, but the status of the species may be summarized as follows:

It is not listed among the recognized species in any of the editions of Bergey's Manual, nor in several larger systematic works (Chester 1901; Hucker 1924; Pribram 1933; Winslow and Winslow 1908). Winslow and Winslow (1908) considered it a synonym for M. albus, the 6th edition of Bergey's Manual listed it in an appendix of insufficiently characterized species, whereas Krasil'nikov (1949) listed it as a bona fide species. He added to the characteristics noted by Migula (1900) that M. mucilaginosus reduces nitrate to nitrite and that inorganic nitrogen compounds are not utilized, both consistent with the behaviour of the strains studied by us (Bergan et al. 1969).

The original description of M. freudenreichii (Guillebeau 1891) differs from that of M. mucilaginosus Migula 1900 in presumably important aspects, such as cell shape and behaviour in milk and gelatin. In addition, Krasil'nikov (1949) and Bergey's Manual (1957) state that M. freudenreichii is able to grow with ammonium salts as a nitrogen source and that it does not reduce nitrate, which also differ from the characteristics of M. mucilaginosus as studied by Krasil'nikov (1949) and us (Bergan et al. 1969). We compared available strains named M. freudenreichii with M. mucilaginosus and concluded that they were not identical.

Krasil'nikov (1949) listed three names as applied to the same species (sic!) as M. mucilaginosus: M. gelatinosus Isachenko 1914, M. marinus Isachenko 1914, and M. gelatinicus (Isachenko 1914) Krasil'nikov 1949. These are, however, not adequately described, making an accurate taxonomic evaluation impossible. A fourth entity listed by Krasil'nikov (1949) as similar to M. mucilaginosus, M. cellaris (Schröter) Migula 1900, is easily distinguishable from our strains and does not, in this context, require further attention. Diplococcus crassus von Lingelsheim 1906 has morphological characteristics similar to M. mucilaginosus, but its description is so inadequate that no reisolate can with certainty be identified as D. crassus. Also, it appears that the name has been used with different meanings and has become a persistent source of error. This point has been clarified earlier (Henriksen 1937). There is no type strain available for the species.

The original description of M. tetragenus by Gaffky (1883) reports a distinct guinea-pig pathogenicity. For this reason and because it is virtually impossible to identify this species due to the inadequacy of the early descriptions (see also Biondi 1887) and the lack of a type strain, M. tetragenus Gaffky 1883 cannot be considered identical with M. mucilaginosus as identified by us (Bergan et al. 1969). Gaffkya tetragena (Gaffky 1883) Trevisan 1885 as recorded in Bergey's Manual (1923, 1957) is distinctly different from the latter organism.

There is no type strain available for M. mucilaginosus Migula 1900. After a detailed study of a collection of strains considered to represent this entity (Bergan et al. 1969), we regard the strain 5762/67 as typical of the species, and propose that this strain be designated as the neotype for M. mucilaginosus Migula 1900. This strain has been deposited in the Czechoslovak Collection of Microorganisms, in the National Type Culture Collection, London, and in the American Type Culture Collection.

Original description of
Micrococcus mucilaginosus Migula 1900

"Unbewegliche, etwas ovale Kokken von 1.2 μ Breite und 2.1 μ Länge, meist in Diplokokkenform. In Milch gezüchtet, besitzt er eine schwach färbbare Kapsel, die in anderen Nährböden nicht auftritt. Die ovale Form ist wahrscheinlich als Ausdruck der Teilung anzusehen, sonst müsste die Art zu den Bakterien gestellt werden.

Auf Gelatineplattenkulturen erscheinen die Kolonien nach 24 Stunden als kleine weisse Pünktchen, die nach 3 Tagen grauweise, stecknadelkopfgrosse Kügelchen darstellen.

In Gelatinestichkulturen entwickelt er sich im Stich rascher als an der Oberfläche; es bilden sich, auch an der Oberfläche, kleine, lange Zeit isoliert bleibende, glasig durchscheinende Kügelchen.

Auf schrägem Agar bilden sich kleine, glasige Tröpfchen, welche nach einigen Tagen zu einem feinen, durchsichtigen schwach glänzenden Belage zusammenfliessen.

In Milch ruft er bei 22°C nach 30-48 Stunden schwache Gerinnung hervor; unter dem Rahm findet sich eine dünne Molkenschicht. Die Rahmschicht wie das geronnene Casein ist klebrig-schleimig, aber nicht fadenziehend. Bei 35° tritt kaum noch Gerinnung ein. Altes Material ruft sehr viel langsamer Gerinnung und Schleimigwerden hervor. Der Organismus bildet gleichzeitig Säure.

In Bouillon und flüssigem Blutserum wächst er nicht, dagegen in neutraler oder schwach alkalischer Molkenflüssigkeit."

...

"Eine Kultur, die ich aus einer schleimigen Milch isolierte und die mit der eben beschriebenen Art übereinstimmte, begann nach 3 Wochen Gelatine langsam zu verflüssigen."

The author groups the species in the category "Gelatine verflüssigend."

Emended description of
Micrococcus mucilaginosus Migula 1900

Micromorphology. Coffeebean-shaped cocci, mostly arranged as diplococci with their adjacent sides flattened. Cells often irregularly sized, 1 to 1.5 μ in diameter. Gram-positive. Capsules are formed. Nonmotile.

Colonies. Overnight blood agar colonies are 1-1.5 mm, mucoid, dome-shaped with entire edges and glistening surface. They are either transparent or whitish. Tendency towards turning white after several days at room temperature. Coherent consistency. Adherent to the agar surface, especially after some days. No haemolysis.

Relation to oxygen. Aerobe. Will grow slowly in anaerobic jar with hydrogen atmosphere.

Temperature of growth and viability. Optimal growth at 30-37°C, but growth also observed at room temperature (20°C) and at 40°C. Killed by exposure to 56°C for 30 minutes. Remains alive on blood agar at room temperature for at least 1-2 weeks.

Growth requirements. Grows well in usual broth media, better if aerated. The growth is first turbid, then viscid precipitate forms, and supernatant clears. Less growth in 1% peptone water. No growth in Koser's citrate or in Baird-Parker's (1963) ammonium sulfate medium. No growth on bromothymolblue lactose agar.

Biochemical reactions. Catalase reaction strongly or weakly positive, but may also be negative. Oxidase reaction negative. Acetoin produced, pH 4.5-4.8 reached in acetoin broth. Aesculine hydrolysis positive. Egg albumen not liquefied. Gelatin liquefied slowly. Indole or H₂S not formed. Nitrate reduced with accumulation of nitrite and without gas formation. Tyrosine not decarboxylated. Coagulase, lipase, phosphatase, phenylalanine-deaminase and urease reactions all negative. Tween-80 not split. No production of ammonia from arginine. Changes in skim milk after 3-4 days proceeding within 14 days to formation of slightly viscid curd with clear or nearly clear whey above. Litmus milk not coagulated, but may be slightly reduced with pH of 6.1-6.6 after 14 days. Mostly no growth with 5 per cent NaCl. Acid produced from the following sugars or alcohols: fructose, galactose, glucose, glycerol, mannose, maltose, saccharose, salicin and trehalose. No acid from: adonitol, arabinose, cellobiose, dulcitol, inositol, inulin, lactose, mannitol, melibiose, raffinose, rhamnose, sorbitol, or xylose.

Antibiotic sensitivity. Sensitive to ampicillin, bacitracin, chloramphenicol, erythromycin, fusidic acid, lincomycin, neomycin, novobiocin, oleandomycin, oxytetracycline, and penicillin G. Usually sensitive to sulfonamide and streptomycin.

Lysostaphin resistance. Uniformly not lysed.

Glycose-bromocresol purple agar (Subcommittee on Taxonomy of Staphylococci and Micrococci, 1965). Typical micrococcal reaction: acid production apparent by indicator change only in the upper 0.5-2 cm of the agar when aerobically grown. Distinctly less change when anaerobically grown.

Habitat. Frequently isolated from the human pharynx and cavum oris. Also found in bronchial secretions and blood cultures. Reportedly also found in milk and as epiphyte on plants (Migula 1900; Krasil'nikov 1949).

Pathogenicity. Low. Subcutaneous injection of undiluted living culture in mice produces local abscesses. Large doses may produce fatal disease in mice. No pathogenicity for guinea-pigs.

DNA base composition. By buoyant density gradient centrifugation the DNA density of two strains was 1.710 g/cm³ corresponding to a % (G+C) content of 59. One strain showed a DNA density of 1.720 g/cm³ equal to 60% (G+C) (Bergan et al. 1969).

Serology. All strains examined had identical capsular antigen.

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