Proposal of *Mycoplasma buccale* nom. nov. and *Mycoplasma faucium* nom. nov. for *Mycoplasma orale* "Types" 2 and 3, Respectively

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Following recommendations made by the Subcommittee on the Taxonomy of *Mycoplasmatales* of the International Committee on Systematic Bacteriology, it is proposed that *Mycoplasma orale* 2 and *Mycoplasma orale* 3 be recognized as two separate species, *Mycoplasma buccale* nom. nov. (type strain: CH20247; ATCC 23636) and *Mycoplasma faucium* nom. nov. (type strain: DC-333; ATCC 25293), respectively. The general properties and distinctive characteristics of the newly named species are summarized.

At present, three "types" of *Mycoplasma orale* are recognized: *M. orale* 1 Taylor-Robinson et al. 1964 (21), *M. orale* 2 Taylor-Robinson et al. 1965 (22), and *M. orale* 3 Fox et al. 1969 (7). However, the authors who described the latter two "types" or "serotypes" did, in fact, regard them as distinct new species rather than subspecies of *M. orale* 1. In consequence, the Subcommittee on the Taxonomy of *Mycoplasmatales* of the International Committee on Systematic Bacteriology (18), in considering the taxonomic status of the three proposed "types" of *M. orale*, recommended that *M. orale* 1 and 2 be recognized as two separate species and that a binary name be proposed for *M. orale* 2. As to the taxonomic status of *M. orale* 3, it was agreed by the Subcommittee that a recommendation should await further comparative studies based on nucleic-acid homology and gel-electrophoresis techniques. In reconsidering presently available data, including those from polyacrylamide gel-electrophoresis studies recently published (24), the Subcommittee later felt that there was sufficient evidence to justify recognizing *M. orale* 3 as a separate species as well (Subcommittee on the Taxonomy of *Mycoplasmatales*: Minutes of Meeting, 8 January 1973, New York, unpublished data).

Following the recommendations of the Subcommittee on the Taxonomy of *Mycoplasmatales*, we herewith propose that: (i) *Mycoplasma orale* 2 Taylor-Robinson et al. 1965

(22) be recognized as a species under the new name *Mycoplasma buccale* (L. adj. *buccalis* buccal), and (ii) *Mycoplasma orale* 3 Fox et al. 1969 (7) be recognized as a species under the new name *Mycoplasma faucium* (L. noun *fauces* the throat; L. gen. pl. noun *faucium* of throats).

Consequently, *Mycoplasma orale* 1 should henceforward be referred to simply as *Mycoplasma orale*.

We designate strain CH20247 (22) as the type strain of *M. buccale*; this strain has been deposited in the American Type Culture Collection (ATCC) under the number 23636. The type strain of *M. faucium* is DC-333 (7); it has been deposited in the ATCC under the number 25293. Both strains were isolated from the human oropharynx. Their characters agree throughout with those described for the species they represent.

The following descriptions of the two newly named species are based mostly on previously published work, supplemented with a few new observations in order to comply with the proposed minimal standards for descriptions of species of the order *Mycoplasmatales* (19).

Mycoplasma buccale nom. nov.

Morphology and staining characteristics.
Dark-field microscopy of broth cultures of

strain CH20247 in the logarithmic growth phase showed minute coccoid, coccobacillary, and very short filamentous forms. Thin-section electron micrographs of colonies on solid medium fixed and embedded in situ revealed pleomorphic cells bounded by a triple-layered membrane and devoid of a cell wall. Gram-negative. (F. T. Black and E. A. Freundt 1973, unpublished data).

Colonial appearance. Colonies on agar medium containing 20% horse serum attain a maximum diameter of approximately 200 μ m in 3 to 4 days. Fully developed colonies show the typical "fried egg" appearance of *Mycoplasma* colonies, with a small central nipple (22).

Absence of reversion. No reversion to a bacterium occurred when an early-passage culture of strain CH20247 was subcultured five consecutive times on media devoid of penicillin and thallium acetate (E. A. Freundt, 1973, unpublished data).

Filterability characteristics. A suspension of strain CH20247 used for filtration contained 4×10^8 colony-forming units (CFU)/ml, and after passage through 450-, 200-, and 100-nm membrane filters (Acropor), the filtrates yielded 4×10^6 , 4×10^6 , and 0 CFU/ml, respectively (F. T. Black, 1973, unpublished data).

Gaseous requirements. The organism is a facultative anaerobe, although the growth of fresh isolates is better under anaerobic conditions, i.e., in an atmosphere of 5% CO₂ and 95% nitrogen (2, 21).

Growth requirements. Growth occurs best at 36 to 37 C in a medium of pH 7.0 to 8.0 containing PPLO broth (Difco), seven parts; uninactivated horse serum, two parts; and 25% yeast extract (Fleischmann type 20-40 or Distillers Company Ltd. dry yeast), one part (23). Deoxyribonucleic acid (DNA) (0.02%) appears to stimulate growth (6), but the evidence for this is not conclusive. Fresh yeast extract is not required for growth (7). Cholesterol is required (14).

Biochemical characteristics. Carbohydrates and urea are not hydrolyzed, but arginine is hydrolyzed. 2, 3, 5-Triphenyl-tetrazolium chloride is reduced anaerobically but not aerobically. The phosphatase test is positive. "Film and spots" are not produced on either horse serum agar or egg-yolk medium. Gelatin, casein, and coagulated horse serum are not liquefied (2, 5, 7, 22). Growth is inhibited by 0.001% methylene blue (7).

Antibiotic sensitivity. This species has the same sensitivity to antibiotics as other large-

colony-forming mycoplasmas, being most sensitive to tetracyclines (20).

Storage. Stock cultures survive for years at -30 and -70 C and indefinitely in the freeze-dried state (1).

Effect on erythrocytes. Zones of alpha-type (incomplete) hemolysis develop around colonies overlaid with guinea-pig or sheep erythrocytes (2, 22); the hemolysin has been identified as hydrogen peroxide (4, 15). The colonies do not adsorb human, rat, guinea-pig, or chicken erythrocytes (12, 16).

Sensitivity to SPS and digitonin. Growth is inhibited by 5% sodium-polyanethol-sulfonate (SPS) and by 1.5% digitonin, as demonstrated by disk growth-inhibition tests on agar (8).

Electrophoretic patterns of cell proteins. The cell protein pattern is clearly distinct from the patterns of other *Mycoplasma* species of human origin, including *M. orale* and *M. faucium* (24).

Serological characteristics. Serologically distinct by disk growth-inhibition, metabolism inhibition, immunofluorescence, indirect hemagglutination, and complement-fixation tests from other *Mycoplasma* species of human source, including *M. orale* and *M. faucium*, as well as from a number of other *Mycoplasma* and *Acholeplasma* species tested (7, 9, 22). In the complement-fixation test, a marked cross-reaction between *M. buccale* and *M. orale* has been demonstrated (22). Also, sharing of antigens with other species of human origin is demonstrable by means of double immunodiffusion in agar (22).

Properties of DNA. Determinations of the guanine plus cytosine (G+C) contents range from 25% by buoyant density (18) to $26.4 \pm 0.2\%$ by T_m (9). The relative relatedness to other human *Mycoplasma* species including *M. orale*, as determined by nucleic-acid homology tests, is $\geq 10\%$ (17).

Habitat. This species is an apparently infrequent parasitic inhabitant of the human oropharynx, comprising approximately 1 to 2% of all mycoplasmas recovered from the oropharynx (7, 22); it is the predominant species of the mycoplasmal flora of the oropharynx of non-human primates (3, 6, 10, 11, 13).

Mycoplasma faucium nom. nov.

Morphology and staining characteristics. The morphology and staining characteristics are the same as for *M. buccale* (F. T. Black and E. A. Freundt 1973, unpublished data).

Gaseous requirements and absence of rever-

sion. The gaseous requirements and absence of reversion are the same as for *M. buccale* (7).

Colonial appearance. Colonies on agar medium containing 20% horse serum resemble those of *M. buccale* in size and general appearance. However, they develop somewhat more superficially and are more loosely attached to the agar surface than the colonies of other mycoplasmas (7).

Filterability characteristics. A suspension of strain DC-333 used for filtration contained 1.2×10^6 CFU per ml, and after passage through 450-, 220-, and 100-nm filters, the filtrates yielded 4×10^4 , 4×10^3 , and 0 CFU/ml, respectively (F. T. Black, 1973, unpublished data).

Growth requirements. Although growth occurs in essentially the same medium used for *M. buccale*, this species appears to be more fastidious. Fresh yeast is required (7), and L-cystein (0.1%) appears to stimulate growth significantly (R. H. Purcell 1970, unpublished data). Cholesterol is required for growth (14).

Biochemical characteristics. Carbohydrates and urea are not hydrolyzed, but arginine is (7). Triphenyl-tetrazolium chloride is reduced anaerobically, but not aerobically (3, 7). The phosphatase test is negative (F. T. Black, 1973, unpublished data). Gelatin is not liquefied (5); this species has not been tested for other proteolytic activities. Growth is inhibited by 0.001% methylene blue (7).

Effect on erythrocytes. Zones of alpha-type (incomplete) hemolysis develop around colonies overlaid with guinea-pig erythrocytes (7); the hemolysin has been identified as hydrogen peroxide (15). The colonies do not adsorb monkey, rat, or guinea-pig erythrocytes, but they do adsorb chicken erythrocytes (16).

Sensitivity to SPS and digitonin. The sensitivities are the same as for *M. buccale* (8).

Electrophoretic patterns of cell proteins. The cell protein pattern is clearly distinct from the patterns of other *Mycoplasma* species of human origin, including *M. orale* and *M. buccale* (24).

Serological characteristics. Serologically distinct by disk growth-inhibition, metabolism inhibition, indirect hemagglutination, and complement-fixation tests from other *Mycoplasma* species of human origin, including *M. orale* and *M. buccale*, as well as from several other *Mycoplasma* and *Acholeplasma* species tested (7, 8a). One-way cross relationships among strains of *M. faucium*, *M. orale*, and *M. salivarium* have been demonstrated by the complement-fixation test. Similarly, sharing of antigens with *M. orale*, *M. buccale*, *M. salivarium*, and *M. arthritidis* was revealed by double

immunodiffusion in agar (7).

Properties of the DNA. Not determined.

Habitat. This species apparently is a rare member of the normal flora of the human oropharynx, comprising less than 2% of the total number of mycoplasmas recovered (7). Occasionally it is recovered from the oropharynx of nonhuman primates (6).

REPRINT REQUESTS

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