Mycoplasma auris sp. nov., *Mycoplasma cottewii* sp. nov., and *Mycoplasma yeatsii* sp. nov., New Sterol-Requiring Mollicutes from the External Ear Canals of Goats

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Three mycoplasma strains, designated GIH^T (T = type strain), UIA^T, and VIS^T, were isolated from the external ear canals of goats and were shown to be serologically distinct from each other and from previously described *Acholeplasma*, *Entomoplasma*, *Mesoplasma*, and *Mycoplasma* species. Using light and transmission electron microscopy, we showed that the cells of these organisms were small, pleomorphic, coccoid, nonmotile, and nonhelical and that each cell was surrounded by a single cytoplasmic membrane. There was no evidence of a cell wall, and the organisms grew freely in media containing penicillin at concentrations of 1,000 U/ml or more and thallos acetate (final concentration, 1:4,000) and produced the “fried-egg” morphology typical of most mollicutes. Growth occurred both aerobically and anaerobically (as determined by the GasPak method). The ability to catabolize glucose and mannose and the ability to hydrolyze arginine varied among the three strains. All three strains required sterol for growth, and none of the strains hydrolyzed urea. The guanine-plus-cytosine contents of the DNAs of strains UIA^T, VIS^T, and GIH^T were determined to be 26.9, 27.0, and 26.6 mol%, respectively. Our data indicate that the three strains represent new *Mycoplasma* species, for which we propose the names *Mycoplasma auris*, *Mycoplasma cottewii*, and *Mycoplasma yeatsii*. The type strain of *M. auris* is UIA (= ATCC 51348 = NCTC 11731), the type strain of *M. cottewii* is VIS (= ATCC 51347 = NCTC 11732), and the type strain of *M. yeatsii* is GIH (= ATCC 51346 = NCTC 11730).

Twenty-one serologically distinct mollicutes that are not *Ureaplasma* strains have been isolated from goats and sheep, and these organisms are known variously as the “caprine-ovine mycoplasmas” or the “mycoplasmas of goats and sheep” (3, 10). Some of these mollicutes have also been isolated from other hosts, but the majority of them occur principally in these two species. A significant number of them are pathogens that have caused major economic losses worldwide in both hosts (3, 10).

During 1981 and 1982, many mycoplasmas were isolated from the external and middle-ear canals of clinically normal goats in Australia (5, 6). As many as six serologic types, including pathogenic species, were found in one ear, and up to 10⁸ CFU of mycoplasmas was recovered from a single ear swab culture. Also found in the ear canals were three previously undescribed strains, designated strains G^T (T = type strain), U^T, and V^T, which represented apparently new *Mycoplasma* species. The presence of these three strains in external ear canals (the external auditory meatus) has also been documented in the United States (8, 10), and other workers have described the presence of mycoplasmas in the ears of goats in Europe (1a) and Peru (14a). In clinically normal goat herds with a prior history of mycoplasma disease, the mycoplasmas described above and other mycoplasmas can often be isolated from the external ear canals of live goats, while other sites, such as the nares and milk, are culturally negative (7, 8). Little is known about the pathogenicity of these strains, although a

serotype similar to strain G^T has been shown to cause experimental mastitis in goats and has also been isolated from two goats with arthritis, mastitis, and pyrexia (9).

Emerging evidence indicates that the strain G^T, U^T, and V^T types of mycoplasmas described in this paper are common in the external ears of goats (5, 6, 8) and are often found in close association with the common ear mites of goats, *Psoroptes cuniculi* and *Raillietia caprae*. In Australia, the three types are prevalent in the ears of goats (4a), whereas in the United States the strain G^T type is prevalent, the strain V^T type is encountered sporadically, and the strain U^T type is rare (8a).

In this paper we characterize, describe, and redesignate strains G^T, U^T, and V^T. We demonstrate that these organisms are serologically distinct from all previously described species belonging to the genera *Acholeplasma*, *Entomoplasma*, *Mesoplasma*, and *Mycoplasma* and propose that they should be designated new *Mycoplasma* species.

MATERIALS AND METHODS

Strain designation. Previously, the mycoplasma strains described in this paper have been designated strains G^T, U^T, and V^T (5, 6, 8, 9, 10). However, these organisms were originally deposited as strains IH^T, IA^T, and IS^T, respectively, in the Mycoplasma Reference Culture Collection in Adelaide, Australia. In this study, strains G^T was the actual original strain IH^T, strain U was the actual original strain IA^T, and strain V^T was the actual original strain IS^T. For purposes of this paper, in order to consolidate the original designations, we redesignate strains G^T (= IH^T), U^T (= IA^T), and V^T (= IS^T) as strains GIH^T, UIA^T, and VIS^T, respectively.

Mycoplasmas and antisera used. Strains GIH^T, UIA^T, and VIS^T were each cloned a minimum of three times by using

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conventional filtration and single-colony pick techniques (24) prior to the study. Antisera to these three strains were prepared in rabbits by using a procedure recommended elsewhere (22). Also, hyperimmune antisera and fluorescein conjugates to the following 115 strains of mollicutes were obtained from the National Institute of Allergy and Infectious Diseases Laboratory in Frederick, Md.: *Acholeplasma axanthum* S-743^T, *Acholeplasma cavigenitalium* GP3^T, *Acholeplasma equifetale* C112^T, *Acholeplasma granularum* BTS39^T, *Acholeplasma hippikon* C1^T, *Acholeplasma laidlawii* PG8^T, *Acholeplasma modicum* PG49^T, *Acholeplasma morum* 72-043^T, *Acholeplasma multilocale* PN525^T, *Acholeplasma oculi* 19L^T, *Acholeplasma parvum* H23M^T, *Entomoplasma melaleuca* M1^T, *Entomoplasma ellychniae* ELCN-1^T, *Entomoplasma lucivorax* PIPN-2^T, *Entomoplasma luminum* PIMN-1^T, *Entomoplasma somnilux* PYAN-1^T, *Mesoplasma florum* L1^T, *Mesoplasma entomophilum* TAC^T, *Mesoplasma seiffertii* F7^T, *Mesoplasma lactucae* 831-C4^T, *Mycoplasma agalactiae* PG2^T, *Mycoplasma alkalescens* D12^T, *Mycoplasma alvi* IIsley^T, *Mycoplasma anatis* 1340^T, *Mycoplasma anseris* 1219^T, *Mycoplasma arginini* G230^T, *Mycoplasma arthritidis* PG6^T, *Mycoplasma bovigenitalium* PG11^T, *Mycoplasma bovirhinis* PG43^T, *Mycoplasma bovis* Donetta^T, *Mycoplasma bovoculi* M165/69^T, *Mycoplasma buccale* CH20247^T, *Mycoplasma californicum* ST-6^T, *Mycoplasma canadense* 275C^T, *Mycoplasma canis* PG14^T, *Mycoplasma capricolum* subsp. *capricolum* California Kid^T, *Mycoplasma capricolum* subsp. *capripneumoniae* F38^T, *Mycoplasma caviae* G122^T, *Mycoplasma caviharyngis* 117C^T, *Mycoplasma citelli* RG-2C^T, *Mycoplasma cloacale* 383^T, *Mycoplasma collis* 58B^T, *Mycoplasma columbinale* 694^T, *Mycoplasma columbinum* MMP-1^T, *Mycoplasma columborale* MMP-4^T, *Mycoplasma conjunctivae* HRC581^T, *Mycoplasma cricetuli* CH^T, *Mycoplasma cynos* H831^T, *Mycoplasma dispar* 462/2^T, *Mycoplasma edwardii* PG24^T, *Mycoplasma equigenitalium* T37^T, *Mycoplasma equirhinis* M432/72^T, *Mycoplasma fastidiosum* 4822^T, *Mycoplasma faucium* DC333^T, *Mycoplasma felifaucium* PU^T, *Mycoplasma feliminutum* Ben^T, *Mycoplasma felis* CO^T, *Mycoplasma fermentans* PG18^T, *Mycoplasma flocculare* Ms42^T, *Mycoplasma gallinaceum* DD^T, *Mycoplasma gallinarum* PG16^T, *Mycoplasma gallisepticum* PG31^T, *Mycoplasma gallopavonis* WR1^T, *Mycoplasma gateae* CS^T, *Mycoplasma genitalium* G37^T, *Mycoplasma glycophilum* 486^T, *Mycoplasma hominis* PG21^T, *Mycoplasma hyopharyngis* H3-6BF^T, *Mycoplasma hyopneumoniae* J^T, *Mycoplasma hyorhinis* BTS7^T, *Mycoplasma hyosynoviae* S16^T, *Mycoplasma indienne* 3T^T, *Mycoplasma iners* PG30^T, *Mycoplasma iowae* 695^T, *Mycoplasma leocaptivus* 3L2^T, *Mycoplasma leopharyngis* LL2^T, *Mycoplasma lipofaciens* R171^T, *Mycoplasma lipophilum* MaBy^T, *Mycoplasma maculosum* PG15^T, *Mycoplasma meleagridis* 17529^T, *Mycoplasma moatsii* MK405^T, *Mycoplasma mobile* 163K^T, *Mycoplasma molare* H542^T, *Mycoplasma muris* RIII4^T, *Mycoplasma mustelae* MX9^T, *Mycoplasma mycoides* subsp. *capri* PG3^T, *Mycoplasma mycoides* subsp. *mycoides* B3, *Mycoplasma neurolyticum* Type A^T, *Mycoplasma opalescens* MH5408^T, *Mycoplasma orale* CH19299^T, *Mycoplasma ovipneumoniae* Y98^T, *Mycoplasma oxoniensis* 128^T, *Mycoplasma penetrans* GTU54^T, *Mycoplasma phocacerebrale* 1049^T, *Mycoplasma phocarinis* 852^T, *Mycoplasma phocidae* 105^T, *Mycoplasma pirum* 70-159^T, *Mycoplasma pneumoniae* FH^T, *Mycoplasma primatum* HRC292^T, *Mycoplasma pullorum* CKK^T, *Mycoplasma pulmonis* PG34^T, *Mycoplasma putrefaciens* KS-1^T, *Mycoplasma salivarium* PG20^T, *Mycoplasma simbae* LX^T, *Mycoplasma spermatophilum* AH159^T, *Mycoplasma spumans* PG13^T, *Mycoplasma sualvi* Mayfield B^T, *Mycoplasma subdolum* TB^T, *Mycoplasma synoviae* WVU 1853^T, *Mycoplasma testudinis* 01008^T, *Mycoplasma vercundum* 107^T, bovine mycoplasma strain PG50 (bovine sero-

group 7), bovine mycoplasma strain California calf, caprine mycoplasma strain G145, and ovine mycoplasma strain 3306. In addition, another set of antisera to strains GIH^T, UIA^T, and VIS^T were obtained from the University of California, Davis. The mollicutes listed above include all of the *Acholeplasma* and *Mycoplasma* species that have been described and all members of the recently described genera *Entomoplasma* and *Mesoplasma* (26).

Culture medium and growth conditions. Liquid or solid medium B (11) supplemented with either 5, 10, or 20% (vol/vol) equine or porcine serum was used as a general-purpose medium. Penicillin G (potassium salt; 1,000 IU/ml) and thallos acetate (final concentration, 1:4,000) were used as bacterial inhibitors. Bacterial inhibitors were omitted in studies in which we specifically examined bacterial reversion. The base media used for glucose fermentation and arginine hydrolysis studies were treated as described elsewhere (12) with glucose oxidase (type II; Sigma Chemical Co., St. Louis, Mo.) and arginine decarboxylase (Sigma) before enrichments were added. For fermentation studies, medium B was supplemented with 1% (wt/vol) D-glucose or 1% (wt/vol) D-mannose and a phenol red indicator (pH 7.6). Arginine hydrolysis was determined in medium FF (11) modified by omitting glucose and adding 0.2% (wt/vol) L-arginine (pH 7.4). All cultures were incubated aerobically or anaerobically (GasPak; BBL Microbiology Systems, Cockeysville, Md.) at 37°C. For hemolysis tests, tryptose blood agar base (Difco Laboratories, Detroit, Mich.) was supplemented with 5% (vol/vol) defibrinated ovine blood. The agar used for growth inhibition studies was mycoplasma agar base (BBL) supplemented with 1% (vol/vol) PPLO serum fraction (Difco). For studies in which we examined sterol dependence in liquid media, mycoplasma broth base (BBL) was supplemented with 10% fresh yeast extract and 15% fetal bovine serum.

Erythrocyte hemadsorption. We determined the ability of strains GIH^T, UIA^T, and VIS^T to hemadsorb chicken and guinea pig erythrocytes as described elsewhere (13).

Serologic studies. Strains GIH^T, UIA^T, and VIS^T were each tested against antisera to strains GIH^T, UIA^T, and VIS^T by performing growth inhibition tests and direct and indirect immunofluorescence tests and were also tested against the antisera and fluorescein conjugates listed above, as described below. For these tests we used a standard disc growth inhibition test (2) or a modified procedure in which 4-mm agar wells were filled with antisera after the agar plates were seeded with the appropriate mycoplasma concentration and subsequently allowed to dry. The agar plates were kept at 25°C for 24 h and then incubated at 37°C until growth was optimum, usually 48 to 72 h. A direct agar plate epiimmunofluorescence procedure (14) was performed with colonies of strains GIH^T, UIA^T, and VIS^T grown on SP4 medium (27) by using fluorescein-conjugated antisera to the mollicute strains listed above. In addition, an indirect fluorescence procedure was performed with GIH^T, UIA^T, and VIS^T colonies grown on horse serum agar medium by using specific rabbit antisera to strains GIH^T, UIA^T, and VIS^T and then fluorescein-conjugated goat anti-rabbit antisera.

Absence of reversion. Strains GIH^T, UIA^T, and VIS^T were subcultured five times in solid and liquid medium B preparations that contained no antibiotics. Following each passage, agar colonies were treated with Dienes stain and examined with a stereomicroscope (17). Centrifuged liquid cultures were examined by bright-field microscopy at a magnification of ×1,250 following staining with Giemsa stain.

Filtration studies. Samples of strains GIH^T, UIA^T, and VIS^T in medium B broth that had been incubated for 48 h were

separately filtered through membrane filters (Millipore Corp., Bedford, Mass.) with average graded pore sizes of 450, 300, and 220 nm. An unfiltered sample was also examined as a control. Filtered and unfiltered samples were titrated by preparing serial 10-fold dilutions and then determining a viability count (CFUs) for each dilution (18).

Sterol dependence. We determined whether sterol was required for growth by (i) an indirect procedure based on susceptibility to digitonin-impregnated discs (25) on medium B agar containing 20% equine serum and (ii) a modified test procedure in which we compared sustained growth in serum-free and serum-containing culture media and in serum-free medium containing 0.04% polyoxyethylene sorbitan (Tween 80) (20).

Morphological studies. Centrifuged deposits from broth cultures of strains GIH^T, UIA^T, and VIS^T in the logarithmic phase of growth were examined at a magnification of $\times 1,250$ by dark-field microscopy and by transmission electron microscopy of ultrathin sections processed by a procedure described elsewhere (19).

Biochemical activity. Tests to examine the breakdown of glucose, mannose, and arginine were conducted in the media described above, while tests to examine the breakdown of urea and "film and spot" production were performed as described by Aluotto et al. (1). The ability to reduce 2,3,5-triphenyltetrazolium chloride aerobically, the ability to digest serum and casein, and the ability to produce phosphatase were determined by procedures described by Cottew (4). In general, the requirements of the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Mollicutes* (15) were followed.

Estimation of DNA base composition. To extract and prepare DNA, cells were grown in 200 ml of glucose broth and were pelleted by centrifugation at $10,000 \times g$. The DNA was extracted and purified by a procedure described elsewhere (23). To estimate base composition, DNA samples were dissolved in $0.33 \times \text{SSC}$ ($1 \times \text{SSC}$ is 150 mM NaCl plus 15 mM trisodium citrate). The melting temperature (T_m) was estimated by thermal denaturation in a recording spectrophotometer. The base composition was calculated from the following equation, as described previously (16): $G+C \text{ content} = 2.24T_m - 135.14$ (in $0.33 \times \text{SSC}$). The $G+C$ content of each DNA was calculated from the mean of two T_m estimates. *A. laidlawii* PG8 (= NCTC 10116), which has a previously reported $G+C$ content of 32.6 mol% (21), was used as a control.

RESULTS AND DISCUSSION

Serologic studies. Reciprocal testing of strains GIH^T, UIA^T, and VIS^T in both the growth inhibition and direct epiimmunofluorescence tests indicated that these three mollicutes were serologically distinct from each other. When we performed indirect immunofluorescence tests with an additional set of antisera to strains GIH^T, UIA^T, and VIS^T prepared in a separate laboratory, we found that the three strains were similarly distinct in reciprocal tests. In addition, no significant serologic cross-reactions were observed when antigens of the three caprine isolates were tested against antisera to all of the previously described *Acholeplasma*, *Entomoplasma*, *Mesoplasma*, and *Mycoplasma* species in disc growth inhibition and direct epiimmunofluorescence serologic tests. Thus, in all of the tests there were completely homologous reactions, and no cross-reactions of significance occurred.

Sterol requirement. The growth of strain GIH^T, UIA^T, or VIS^T on agar containing 20% serum was inhibited to a distance of 6 to 11 mm from the well by a digitonin-impregnated disc.

All three strains grew for only 2 passages in serum-free medium, for 1 passage in serum-free medium supplemented with Tween 80, and for all 23 passages tested in a control medium containing 15% fetal bovine serum.

Morphological studies. Examination by phase-contrast and light microscopy revealed pleomorphic coccoid forms. No evidence of motility was observed, and helical forms were not seen. Transmission electron microscopy of ultrathin sections revealed pleomorphic coccoid forms with an outer unit cytoplasmic membrane that showed no evidence of cell wall material (Fig. 1).

Growth conditions. Strains GIH^T, UIA^T, and VIS^T grew well aerobically in media supplemented with either 5, 10, or 20% equine or porcine serum. Colonies on agar had the typical "fried-egg" appearance characteristic of most mycoplasma cultures, and they readily took up Dienes stain. Colony diameters ranged from 0.5 to 1.5 mm after 48 to 72 h of incubation. Anaerobically, strain UIA^T grew equally well with all three serum concentrations in the agar, but the growth of strains GIH^T and VIS^T was sparse. A peculiar and very interesting characteristic of strain VIS^T was its ability to produce highly pigmented, intense brown to black colonies on agar, as previously described (6, 10). Pigmentation developed on agar colonies after 2 to 4 days (or more) of incubation and only in colonies that were well separated. This characteristic was first noticeable in the center of each colony, but coloration gradually became more intense and in many cases encompassed most of the colony within 4 to 7 days. Pigmentation occurred only on solid agar medium supplemented with thallous acetate. On this type of agar, the pigmentation produced by strain VIS^T colonies (assuming that pigmentation was expressed in well-isolated colonies) was usually sufficient for a presumptive diagnosis of this serologic type.

Biochemical characteristics. Strain GIH^T utilized both glucose and mannose slowly, requiring about 2 weeks for a decline in pH of 0.5 or more units. Sterol was required for growth, and arginine was hydrolyzed. Tetrazolium was reduced. Phosphatase was not produced, and urea was not hydrolyzed. Serum and casein were not digested, and film and spots were not produced.

Strain UIA^T did not ferment glucose or mannose but did hydrolyze arginine. Sterol was required for growth, and urea was not hydrolyzed. Tetrazolium was not reduced. Phosphatase was produced. Serum and casein were not digested, and film and spots were not produced.

Strain VIS^T fermented both glucose and mannose. Arginine and urea were not hydrolyzed. Sterol was required for growth. Tetrazolium was reduced. Phosphatase was not produced. Serum and casein were not digested, and film and spots were not produced.

Hemolytic activity. Strains GIH^T and VIS^T grew aerobically on ovine blood agar, producing colonies that were 0.1 to 0.25 mm in diameter after 48 to 72 h of incubation and a zone (diameter, approximately 1.5 mm) of viridans type (α) hemolysis (i.e., a greenish discoloration of the blood agar). After 4 to 7 days of incubation the lysis generally became more intense, resembling weak beta-hemolysis. Strain UIA^T grew poorly on ovine blood agar, producing barely discernible colonies that were about 0.1 mm in diameter and weak viridans type hemolytic zones that were approximately 1 mm in diameter.

Erythrocyte hemadsorption. Strain GIH^T hemadsorbed guinea pig erythrocytes but not chicken erythrocytes. Strains UIA^T and VIS^T did not hemadsorb either type of erythrocytes.

Filtration studies. An unfiltered strain GIH^T culture contained 8×10^8 CFU/ml, while following filtration through 450-, 300-, and 220-nm-pore-size filters the resulting preparations

FIG. 1. Electron photomicrographs of sections of strains UIA^T (a), VIS^T (b), and GIH^T (c) stained with uranyl acetate and Reynold lead acetate. A single cytoplasmic membrane is present in each cell. Bars = 500 nm.

contained 4×10^8 , 9×10^7 , and 2×10^5 CFU/ml, respectively. The corresponding values for strain UIA^T were 3×10^6 (unfiltered), 1×10^6 , 2×10^5 , and 0 CFU/ml, respectively, and the values for strain VIS^T were 7×10^6 (unfiltered), 5×10^6 , 2×10^6 , and 3×10^5 CFU/ml, respectively.

DNA base composition. The DNA G+C contents calculated from the means of two T_m estimates were 26.6, 26.9, and 27.0 mol% for strains GIH^T, UIA^T, and VIS^T, respectively. For *A. laidlawii* PG8, the value which we determined was 32.4 mol% (the previously reported value was 32.6 mol%).

Observations on habitat and pathogenicity. Strain UIA^T was isolated from the external ear canals of goats in Australia, as were other serologically and otherwise indistinguishable strains (5, 6). One serologically similar strain has been isolated once in the United States (10).

Strain VIS^T and other indistinguishable strains were isolated from the external ear canals of goats in Australia (5, 6). Numerous other isolates of this serotype have been obtained from the same anatomical site of goats in the United States (8a).

Strain GIH^T was isolated from the external ear canals of goats in Australia, as were numerous other serologically and otherwise indistinguishable strains (5, 6); an isolate was also obtained from a retropharyngeal lymph node. Serologically similar isolates have been obtained frequently from the external ear canals of goats in the United States, and one isolate was obtained from the same site in a calf housed in close confinement with goats harboring strain GIH^T serologic types in their ears (8a). An isolate designated GM790A, which was reported to be a serologic equivalent of strain GIH^T, was isolated from the lungs, spleens, and milk of two naturally infected goats in the United States (9). One goat was mastitic and had an elevated temperature. Inoculation of strain GM790A into the teat canals of two lactating goats resulted in abrupt termination of lactation, leading to mastitis and agalactia within 3 days.

In Australia, serologic types similar to strains GIH^T, UIA^T, and VIS^T are prevalent in the ear canals of goats (4a). In the United States, serotypes similar to strain GIH^T are common in the ear canals of goats, strain VIS^T types have been recovered occasionally, and strain UIA^T serotypes are rare (8a).

Taxonomic conclusions. The characteristic cellular morphology and size of strains GIH^T, UIA^T, and VIS^T and their typical colony form, together with their resistance to penicillin and their failure to revert to bacterial forms in the absence of penicillin, confirm that these organisms are members of the *Mollicutes*. The sterol requirement of these organisms, their nonhelical form, their atmospheric and temperature requirements, and their inability to metabolize urea place them in the genus *Mycoplasma*. The fact that the three strains are serologically distinct from each other and from all other relevant species of *Mollicutes* indicates that they should be considered separate new *Mycoplasma* species, as proposed below.

Taxonomic proposals for new species and taxonomic descriptions. (i) **Taxonomic proposals.** For strain UIA and other indistinguishable isolates we propose the name *Mycoplasma auris* sp. nov., with strain UIA (= ATCC 51348 = NCTC 11731) as the type strain.

For strain VIS and other indistinguishable isolates, we propose the name *Mycoplasma cottewii* sp. nov., with strain VIS (= ATCC 51347 = NCTC 11732) as the type strain.

For strain GIH and other indistinguishable isolates, we propose the name *Mycoplasma yeatsii* sp. nov., with strain GIH (= ATCC 51346 = NCTC 11730) as the type strain.

(ii) **Description of *Mycoplasma auris* sp. nov.** *Mycoplasma auris* (aur'is. L. gen. n. *auris*, of the ear, referring to the provenance of the organism, the ears of goats). *M. auris* is

serologically distinct from all of the other *Acholeplasma*, *Entomoplasma*, *Mesoplasma*, and *Mycoplasma* species evaluated in this study.

Colonies on agar have the fried-egg appearance characteristic of most members of the *Mollicutes*.

M. auris does not catabolize glucose or mannose, but arginine is hydrolyzed. Sterol is required for growth, and urea is not hydrolyzed. Tetrazolium is not reduced aerobically. Phosphatase is produced. Serum and casein are not digested, and film and spots are not produced on equine or porcine serum-containing media. Chicken and guinea pig erythrocytes are not hemadsorbed.

Growth is equally good under aerobic and anaerobic (GasPak) conditions. Very small colonies. Slight viridans-type hemolysis occurs on ovine blood agar incubated aerobically; beta-hemolysis occurs after further incubation.

Filterable through 300-nm-pore-size filters with some loss of viability but not filterable through 220-nm-pore-size filters.

The G+C content of the DNA is 26.9 mol%.

The type strain is UIA (= ATCC 51348 = NCTC 11731).

Many other serologic types similar to strain UIA^T have been isolated from the ears of goats in Australia. The organism has been recovered only once in the United States.

The only known habitat of *M. auris* is the external ear canals of goats. Pathogenicity is unknown.

(iii) **Description of *Mycoplasma cottewii* sp. nov.** *Mycoplasma cottewii* (cot.tew'i.i. M. L. gen. n. *cottewii*, of Cottew, named, as proposed by R. H. Leach, after G. S. Cottew, one of the coisolators of the organism). *M. cottewii* is serologically distinct from all of the other *Acholeplasma*, *Entomoplasma*, *Mesoplasma*, and *Mycoplasma* species evaluated in this study.

Colonies on agar have the fried-egg appearance characteristic of most mycoplasmas.

M. cottewii catabolizes glucose and mannose. Arginine and urea are not hydrolyzed. Tetrazolium is reduced aerobically. Phosphatase is not produced. Sterol is required for growth. Serum and casein are not digested, and film and spots are not produced on equine or porcine serum-containing media. Chicken and guinea pig erythrocytes are not hemadsorbed.

Aerobic growth is greater than growth under anaerobic (GasPak) conditions when the two methods are compared. Colonies on ovine blood agar that are incubated aerobically produce zones of viridans type hemolysis, which changes to a beta-hemolysis after additional incubation. Brown to black pigmentation, which is often intense, occurs in young, well-separated colonies on thallium acetate-containing agar medium; this characteristic can be used for presumptive diagnostic identification of *M. cottewii*.

Filterable through 220-nm-pore-size filters with some loss of viability.

The G+C content of the DNA is 27.0 mol%.

The type strain is VIS (= ATCC 51347 = NCTC 11732).

Serologic types similar to strain VIS^T are common in the ears of goats in Australia and have been recovered occasionally from the ears of goats in the United States. One isolation of a strain VIS^T serotype has been reported from the nasal sinuses of goats (6). Pathogenicity is unknown.

(iv) **Description of *Mycoplasma yeatsii* sp. nov.** *Mycoplasma yeatsii* (yeat'si.i. M. L. gen. n. *yeatsii*, of Yeats, named after F. R. Yeats, one of the original coisolators of the organism). *M. yeatsii* is serologically distinct from all of the other *Acholeplasma*, *Entomoplasma*, *Mesoplasma*, and *Mycoplasma* species evaluated in this study.

Colonies on agar have the fried-egg appearance characteristic of most mycoplasmas.

M. yeatsii utilizes glucose and mannose slowly, requiring 2

weeks or longer for a decrease in pH of 0.5 or more units. Sterol is required for growth. Arginine is hydrolyzed, and tetrazolium is reduced aerobically. Phosphatase is not produced, and urea is not hydrolyzed. Serum and casein are not digested, and film and spots are not produced on equine or porcine serum-containing media. Guinea pig erythrocytes are hemadsorbed, but chicken erythrocytes are not hemadsorbed.

Good growth occurs aerobically, but only sparse growth occurs under anaerobic (GasPak) conditions. Colonies on ovine blood agar that are incubated aerobically produce a zone of viridans type hemolysis, which changes to weak beta-hemolysis after further incubation.

Filterable through 220-nm-pore-size filters with some loss of viability.

The G+C content of the DNA is 26.6 mol%.

The type strain is GIH (= ATCC 51346 = NCTC 11730).

Many other serologically similar isolates have been identified in Australia and in the United States.

The usual habitat is the external ear canals of goats, but strain GIH^T serotypes have also been isolated from a retropharyngeal lymph node (6) and from nasal tracts, internal organs, and milk of naturally infected goats (9).

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