**Mycoplasma anseris** sp. nov. Found in Geese

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A mycoplasma designated strain 1219T (T, type strain) was isolated from the phallus of a gander in Hungary. It was assigned to the class Mollicutes, order Mycoplasmatales, on the basis of morphological, cultural, and physical studies. The base composition of its deoxyribonucleic acid was 25 mol% guanine plus cytosine. It was dependent on sterol for growth, and its growth was inhibited by digitonin. The organism was assigned to the genus *Mycoplasma* since it did not hydrolyze urea and there was no evidence of helical forms. It hydrolized arginine, but other biochemical tests were negative. Strain 1219T could not be identified as any of 77 accepted *Mycoplasma* species by growth inhibition, immunofluorescence, or metabolism inhibition tests and thus appeared to be a new species. We propose the name *Mycoplasma anseris* for this organism, for which the type strain is strain 1219.

Several *Mycoplasma* and *Acholeplasma* species have been isolated from geese during the past 10 to 12 years, including *M. anatis* (12), *M. cloacale* (5, 19), *M. gallinarum* (17), *M. gallisepticum* (6), *A. laidlawii*, and *A. axanthonum* (17, 20) together with several unclassified strains. Isolations were made, for example, from goose embryo fibroblasts, dead embryos, and the goose respiratory tract.

In this paper, we describe the characteristics of a mollicute, strain 1219T (T, type strain), which was isolated from the phallus of a goose in Hungary. We used the methods recommended by the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes (13) to establish whether this isolate represents a new species.

**MATERIALS AND METHODS**

*Mycoplasma strains.* The strains used are shown in Table 1. Strain 1219T was isolated from the phallus lymph of a gander from a flock with a history of phallus inflammation. Most reference strains were obtained from the Food and Agriculture Organization/World Health Organization Collaborating Centre for Animal Mycoplasmas, University of Aarhus, Aarhus, Denmark; exceptions were the reference strains of *M. sualvi*, *M. faucaum*, *M. hovoculi*, and *M. hominis* from the National Collection of Type Cultures, London, England; *M. genitalium*, *M. hypharyngis*, *M. muris*, and *M. pirum* from J. Tully, Frederick Cancer Research Facility, Frederick, Md.; and *M. cavipharyngis*, *M. cicituli*, *M. colis*, *M. felisfaucaum*, and *M. testudinis* from A. Hill, Medical Research Council Experimental Embryology and Teratology Unit, Carshalton, Surrey, England. *M. lipifaciens* (4), *M. glycolphimum* (10), and *M. cloacale* (3) were from the collection of J.M.B.

**Media, cultivation, and purification.** The media and conditions used to propagate most of these strains were described previously (4), although in Hungary, medium B (8) was used for some species. Attempts to grow *M. fastidiosum*, *M. genitalium*, and *M. hypharyngis* were unsuccessful. Most morphological and biochemical tests were carried out in both England and Hungary by using the same media used in earlier studies (4, 8, 9). *Mycoplasma* sp. strain 1219T was subcultured routinely at 37°C in a carbon dioxide-rich atmosphere. It was purified by filter cloning three times through filters of 450-nm average pore diameter (Nuclepore, Wallabs Inc.).

**Characterization studies.** The colony morphology of strain 1219T was examined by light microscopy, and the cell morphology was examined by light and electron microscopy (4, 18). Tests for absence of reversion were carried out in media without bacterial inhibitors. In Hungary, this was for five passages in broth, and the final passage was examined by light microscopy. In England, 10 passages in broth and also 10 on agar were carried out, each passage being examined for bacteria by blood agar culture and by light microscopy. Filterability studies were conducted by using 450- and 220-nm pore diameter membrane filters. Deoxyribonucleic acid (DNA) was extracted as described previously (4). Two preparations were made, and their guanine-plus-cytosine (G+C) contents were determined by melting temperature (14) and by buoyant density in cesium chloride (16).

Strain 1219T was examined for its susceptibility to digitonin (11, 12) and for its growth response to sterol (7). Other biochemical tests were carried out as described previously (1).

**Serological tests.** Strain 1219T was tested by the agar well modification of the growth inhibition (GI) test (2) and by the indirect fluorescent antibody (IFA) test (15) with rabbit antisera to all the 82 reference strains and serovars using sera obtained from the same sources as the corresponding strains. Homologous control tests were carried out except for the three species which we were unable to grow. In addition, antiserum prepared in rabbits against strain 1219T was titrated by the IFA test. It was subsequently used undiluted in GI tests and diluted 1 in 40 in IFA tests with the reference strains of all the avian *Mycoplasma* species and serovars, and with those mammalian species whose antiserum had given reactions in GI tests.

**RESULTS AND DISCUSSION**

Strain 1219T grew well on mycoplasma agar, producing typical fried egg colonies. Growth appeared to be better in an atmosphere of increased carbon dioxide. Giemsa-stained films showed coccoid forms, and phase-contrast microscopy also showed mainly coccoid elements but with some evidence of pleomorphism. No helical or motile forms were seen. Electron microscopy of ultrathin sections revealed a

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plasma membrane but no evidence of a cell wall (Fig. 1 and 2).

There was no evidence of reversion to bacteria after passage in media devoid of bacterial inhibitors. In filtration studies, cultures containing approximately 10^9 colonies per ml were reduced by either 86% (Hungary) or 75% (England) on passage through a 450-nm pore diameter filter. On passage in media devoid of bacterial inhibitors. In filtration when carried out in England.

The average G+C content of the DNA extracted from strain 1219^T was 26.0 mol% as determined by melting temperature and 24.7 mol% by buoyant density. The mean of all the results was 25 ± 1 mol%.

Mycoplasma sp. strain 1219^T was susceptible to 1.5% (wt/vol) digitonin, giving an inhibition zone of 8 mm. It showed a positive growth response to sterol, although it also produced scant growth on the basal medium without supplements and on basal medium without cholesterol but supplemented with palmitic acid and bovine albumin. This has been observed previously with members of the genus Mycoplasma, for example, M. anatis (7) and M. glycomophilum (10), and may be due to a low cholesterol requirement of the particular organism (7). This requirement might have been met by traces in the medium or perhaps by carry-over from the inoculum. Results of other biochemical tests are given in the species description below.

There was no serological evidence to suggest a relationship with any previously described Mycoplasma species. Of a total of 84 mammalian and avian species and serovars compared by GI with strain 1219^T, only 6 showed any sort of reaction. Of these, four were avian species and were also arginine utilizers. Immunofluorescence did not confirm a serological relationship.

![FIG. 1. Sediment of 24-h broth culture of Mycoplasma sp. strain 1219. Ultrathin section. Bar, 500 nm.](image)

![FIG. 2. Sediment of 24-h broth culture of Mycoplasma sp. strain 1219. Ultrathin section. Bar, 200 nm.](image)
From these results we conclude that strain 1219 is a new *Mycoplasma* species. It has morphological properties and a G+C content consistent with membership of the class *Mollicutes* as defined by the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes (13). It is proposed that this organism be assigned to the family *Mycoplastaceae*, genus *Mycoplasma*, on the basis of its sterol requirement and lack of urea utilization and that it be given the name *Mycoplasma anseris*.

This organism is one of three which have been found recently in geese (18) the other two being *M. cloacae* and an organism which is not yet fully characterized but which would appear to be a previously unrecognized species. A correlation has been found between the occurrence of these three organisms and cloaca and phallus inflammation in goose flocks (19), but in the absence of available mycoplasma-free geese, critical pathogenicity studies have not been undertaken.

**Description of Mycoplasma anseris sp. nov.** *Mycoplasma anseris* (an.ser’is. L. gen. n. anseris, of the goose). Thin sections showed mainly spherical particles with a triple layer membrane and no cell wall.

Cells pass through filters of 450-nm pore size but may be retained by filters of pore size of 220-nm.

Typical fried egg colonies are formed.

The organism is resistant to penicillin and thallium acetate, and there is no reversion to bacterial forms in the absence of these compounds.

 Cultures show a growth response to cholesterol, and growth is inhibited by digitonin. Arginine is utilized, but glucose and urea are not. There is no production of films or spots or phosphatase activity, no reduction of tetrazolium and no hydrolysis of esculin or arbutin.

The species is serologically distinct from other *Mycoplasma* species. The type strain was isolated from the phallus of a goose. The pathogenicity is unknown. The G+C content of the DNA is 25 mol%.

The type strain is strain 1219.

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**LITERATURE CITED**


