Mycoplasma sturni sp. nov., from the Conjunctiva of a European Starling (Sturnus vulgaris)


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Strain UCMFT (T = type strain) was isolated from the conjunctiva of a European starling (Sturnus vulgaris) with conjunctivitis. Colonies grown on conventional mycoplasma agar possessed the typical fried-egg appearance observed with many mycoplasmal species. Electron micrographs of ultrathin sections of UCMFT revealed a pleomorphic cellular morphology; the cells ranged from spherical to elliptical or flask shaped. The cell size ranged from 0.3 to 0.5 μm. Strain UCMFT grows well in a variety of mycoplasma broth formulations at 25°C, with rapid and heavy growth at 37°C. No growth occurs at 42°C. This organism ferments glucose but does not hydrolyze urea or arginine and has an absolute requirement for sterol for growth. Strain UCMFT does not hemagglutinate or hemadsorb chicken erythrocytes. The genome size is 870 kbp, and the guanine-plus-cytosine content is 31 mol%. Sequence analysis of the 16S rRNA gene demonstrated that this organism is unique and has not been described previously. Serological analysis confirmed that strain UCMFT is distinct from all previously identified Mycoplasma, Acholeplasma, Spiroplasma, Entomoplasma, and Mesoplasma species. This organism represents a new species, for which we propose the name Mycoplasma sturni. Strain UCMFT (= ATCC 51945) is the type strain of M. sturni sp. nov.

MATERIALS AND METHODS

Isolation. The new organism was isolated from a live European starling (Sturnus vulgaris) with conjunctivitis. This bird was submitted as part of an ongoing effort to monitor the incidence of conjunctivitis in house finches (Carpodacus mexicanus) and other passerines along the east coast of the United States. Although the new organism (strain UCMFT [T = type strain]) was isolated in pure culture from a clinical case of naturally occurring conjunctivitis, its role as a primary pathogen of passerines has yet to be definitively established.

On the basis of the standards set forth for the description of new species of mycoplasmas (9), this organism is a distinct, previously undescribed mycoplasma species. We propose the name Mycoplasma sturni for this new species.

FC broth and on FC agar. Later passages were also subcultured in avian mycoplasma medium SP-4 (10% horse serum, 0.25 mg of glucose per mL, Phenol Red Broth Base [Difco]) (20). Cultures were incubated routinely at 37°C unless indicated otherwise. Strain UCMFT was purified by conventional cloning techniques (18). The range of permissive temperatures was determined by incubating a clonal population in FC broth at 23, 30, 32, 35, 37, and 42°C for a maximum of 5 days.

Morphological studies. For electron microscopic studies, the organism was either pelleted from a mid-log-phase broth culture or picked as an agar plug containing a colony grown from overnight incubation of an inoculated FC agar plate. Samples were fixed in 1.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 3 mM MgCl₂ at pH 7.3. Samples were washed in 0.1 M sodium cacodylate buffer (pH 7.3) and postfixed with 2% osmium tetroxide. Specimens were dehydrated by using an ascending gradient of ethanol and then propylene oxide. They were then embedded in a resin mixture containing Araldite 502 ( Electron Microscopy Sciences, Fort Washington, Pa.) and Epon 812 (Tousimis Research Corp., Rockville, Md.). Ultrathin sections were cut with an Ultratome V (LKB-Produkter AB, Stockholm, Sweden), double stained with lead citrate and uranyl acetate, and observed with a model 300 electron microscope ( Phillips International, Eindhoven, The Netherlands).

The morphology of strain UCMFT cultivated in SP-4 broth was also examined by dark-field microscopy at a magnification of ×1,250. Strain UCMFT grown on FC agar at 37°C was observed with a stereomicroscope, and typical colonies were photographed.

Biological and biochemical studies. Strain UCMFT was serially passaged in broth culture numerous times and then plated onto solid agar, incubated, and examined for typical colony formation. The technique used to assess filtration characteristics has been described previously (18). The techniques used to assess carbohydrate fermentation and hydrolysis of arginine and urea have also been described previously (2). The hemadsorption and hemagglutination properties were determined as described previously (5).

Sterol requirement. Growth requirements for sterol were determined by the standard broth culture method (13, 19) and by a technique that was specific for determining the Tween 80 requirement (14).

Serological test. Antiserum to strain UCMFT was produced in a rabbit by a standard technique (17). Standard disc growth inhibition tests (3) were performed on agar plates with strain UCMFT and antisera to the following fermen-

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Mycoplasma cynos HR3T, Mycoplasma dispar 462T, Mycoplasma edwardii PG23T, Mycoplasma equigenitalium T37T, Mycoplasma fastidioiust 42227T, Mycoplasma felinum Tum BenT, Mycoplasma felis CO8, Mycoplasma fermentans PG18T, Mycoplasma flocculare Ms 42T, Mycoplasma gallinarum DD6T, Mycoplasma gallisepticum PG31T, Mycoplasma gallionarum WR1T, Mycoplasma genitalium G37T, Mycoplasma glycolophilum G46T, Mycoplasma hypoxianum 3T, Mycoplasma hyorhinis BHST7T, Mycoplasma immittis 4229T, Mycoplasma iowae 695T, Mycoplasma iowae PPADV, Mycoplasma leucaphygia LL2T, Mycoplasma leucjavicius R171T, Mycoplasma mohini JKI, Mycoplasma mobile 163K, Mycoplasma molate H542T, Mycoplasma musculare MX9T, Mycoplasma myocarditis subsp. mycoides PG1T, Mycoplasma mycoides subsp. mycoides U31847, Mycoplasma mycoides subsp. capri PG3T, Mycoplasma neurolyticum Type A1T, Mycoplasma ovipneumoniae Y89T, Mycoplasma ovipneumoniae 128T, Mycoplasma penetrans GTU35T, Mycoplasma phocarhinis 882T, Mycoplasma pinnun 70-159T, Mycoplasma pneumoniae FH1T, Mycoplasma pullorum CCK1T, Mycoplasma pulmonis PG34T, Mycoplasma purpureascens KS1-1T, Mycoplasma simhae LKT, Mycoplasma suavi Mayfield B, Mycoplasma synuroi WU1857T, Mycoplasma testudinis 01008T, Mycoplasma turezandrums 107T, Mycoplasma yatari GIIHT, Mycoplasma sp. strains Cal1 188, BSP, M78206, 3306, 3446, HRC589, and Utah C, Entomoplasma elychniae ELCN-1T, Entomoplasma hirii PIPS-2T, Entomoplasma liniarum PINM-1T, and Entomoplasma sp. strain BARC318. In addition, agar colonies of strain UCMT were also used in direct immunofluorescence tests (6) using individual fluorescein-conjugated antisera to various temperatures. Optimal growth appeared to occur at temperatures between 34 and 37°C. Interestingly, it failed to grow in vitro at 42°C, which is close to the normal avian body temperature. It was isolated from an eye, which, because of exposure to the environmental temperature and its thin membranous coatings, may have had a temperature below the core body temperature. Electron micrographs demonstrated that cell walls were absent and that membranes typical of members of the Mycoplasma were present (Fig. 2). They also showed that the cell size ranged from 0.3 to 0.5 μm. Log-phase broth cultures examined by either transmission electron or dark-field microscopy were homogeneous, and the cells were spherical. However, sections obtained by cutting through colonies grown on agar revealed more irregular flask-shaped and filamentous forms. The difference in cell morphology observed in the two growth matrices may have been due to different demands when the organism was growing in two different states (liquid phase and solid phase). No reversion to a walled bacterial form was observed after numerous passages in antibiotic-free broth or on agar plates.

Sterol requirement. Preliminary tests to determine the serum requirement for growth of strain UCMT showed that the organism could be maintained through continuous passage through 23 serial dilutions in medium containing fetal bovine serum but that growth did not occur in a serum-free broth or

RESULTS AND DISCUSSION

Isolation. Strain UCMT was isolated in pure culture in FC medium without antibiotics from the eye of a European starling with bilateral conjunctivitis. It grew rapidly on agar with the fried-egg colony morphology which is characteristic of mycoplasmas (Fig. 1).

Growth and morphology. The new organism grew rapidly at various temperatures. Optimal growth appeared to occur at temperatures between 34 and 37°C. Interestingly, it failed to grow in vitro at 42°C, which is close to the normal avian body temperature. It was isolated from an eye, which, because of exposure to the environmental temperature and its thin membranous coatings, may have had a temperature below the core body temperature. Electron micrographs demonstrated that cell walls were absent and that membranes typical of members of the Mycoplasmatas were present (Fig. 2). They also showed that the cell size ranged from 0.3 to 0.5 μm. Log-phase broth cultures examined by either transmission electron or dark-field microscopy were homogeneous, and the cells were spherical. However, sections obtained by cutting through colonies grown on agar revealed more irregular flask-shaped and filamentous forms. The difference in cell morphology observed in the two growth matrices may have been due to different demands when the organism was growing in two different states (liquid phase and solid phase). No reversion to a walled bacterial form was observed after numerous passages in antibiotic-free broth or on agar plates.

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**FIG. 1.** Mycoplasma sturni colonies grown on FC agar. Bar = 100 μm.
in a serum-free medium supplemented with 0.04% polysorbate 80 (Tween 80) (14). A standard cholesterol test, in which growth yields in various broth formulations were determined, indicated that the organism had a definite requirement for cholesterol (Table 1).

**Biological and biochemical properties.** Strain UCMFT rapidly catabolized glucose but did not hydrolyze arginine or urea. This organism failed to bind chicken erythrocytes in both hemadsorption and hemagglutination tests. Unfiltered broth cultures typically had a titer of $10^8$ color-changing units/ml. Filtration through membranes with average pore diameters of 450 and 300 nm also yielded titers of $10^8$ color-changing units/ml, while filtration through a 220-nm-pore-diameter membrane filter yielded no viable organisms.

**Serological tests.** Growth inhibition tests performed with antisera to all previously recognized fermentative species in the genus *Mycoplasma* (see above) revealed that strain UCMFT was serologically distinct. These findings were confirmed by the results of direct agar plate immunofluorescence tests.

**Genomic analysis.** When BLAST and FASTA (GCG) were used, a search of the 16S rRNA nucleotide sequences in the GenBank databases did not reveal any sequence identical to the sequence determined for *Mycoplasma sturni*. However, on the basis of homology to various mycoplasmal 16S rRNA genes, it appears that *Mycoplasma sturni* belongs to the *Mycoplasma hominis* clade. An analysis of the 16S rRNA gene demonstrated that strain UCMFT is most closely related to *Mycoplasma gallinaceum* (level of homology, 93%), *Mycoplasma coragypsi* (92.1%), *Mycoplasma felis* (92%), and *Mycoplasma synoviae* (91%). The pattern of bands resulting from arbitrary primer PCR amplification was distinct from the patterns obtained for the other mycoplasmas examined.

As determined by pulsed-field gel electrophoresis, the average total genome size of strain UCMFT was 870 kbp. The G+C base composition was 31 mol%. This value was based on the values obtained for the two reference organisms, *Mycoplasma gallisepticum* and *Micrococcus lysodeikticus*, which were consistent with values determined previously (36 and 72 mol%, respectively).

![FIG. 2. Ultrathin section of a Mycoplasma sturni culture viewed and photographed with an electron microscope. Bar = 100 nm.](image)
The properties of strain UCMFT\textsuperscript{T} described in this paper fulfill the criteria for species descriptions of members of the class \textit{Mollicutes} (9). This organism does not have a cell wall, is filterable, fails to revert to walled bacteria when it is grown in the absence of antibiotics, is resistant to penicillin, and produces typical fried-egg colonies on agar. A growth requirement for serum or cholesterol, an optimum growth temperature of 37°C, and a genome size of 870 kbp place this organism in the order \textit{Mycoplastales}. The inability of strain UCMFT\textsuperscript{T} to hydrolyze urea indicates that it belongs in the genus \textit{Mycoplasma}. The lack of serological relatedness to previously described fermentative mycoplasma species indicates that this organism represents a new \textit{Mycoplasma} species. We propose the name \textit{Mycoplasma sturni} for strain UCMFT\textsuperscript{T}.

The taxonomic description below summarizes the properties of the new species.

**Description of \textit{Mycoplasma sturni} sp. nov.** \textit{Mycoplasma sturni} (stur'ni. M. L. n. \textit{Sturnus}, a genus of birds; M. L. gen. n. \textit{sturni}, of the genus \textit{Sturnus}, the genus of the bird from which the organism was isolated). Most cells are coccoid and have sizes ranging from 300 to 500 nm, but some irregular flask-shaped and filamentous forms also occur. All cells lack a cell wall. Colonies on solid medium usually have a fried-egg appearance. Chemoorganotroph. Grows rapidly in broth medium, with acid production from glucose. Does not hydrolyze arginine or urea. Cholesterol or serum is required for growth.

The optimum temperature for growth is 34 to 37°C, and growth does not occur at 42°C.

Serologically distinct from previously described \textit{Mycoplasma} species.

Isolated from ocular tissues of a starling (\textit{S. vulgaris}) with conjunctivitis, but it has not been determined whether the organism plays a pathogenic role in avian disease.

The G+C content of the DNA is 31 mol\%, and the genome size, as determined by pulsed-field gel electrophoresis, is 870 kbp.

The type strain is strain UCMF (= ATCC 51945).

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**REFERENCES**