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


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Strain UCMF (T = type strain) was isolated from the conjunctivita 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 UCMF 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 UCMF grows well in a variety of mycoplasma broth formulations at 25°C, with rapid and high 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 UCMF 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 UCMF 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 UCMF (= ATCC 51945) is the type strain of *M. sturni* sp. nov.

Members of the class Mollicutes are prokaryotic organisms which are devoid of cell wall material. Although members of the genera *Ureaplasma* and *Acholeplasma* have been isolated from avian hosts, the majority of isolates belonging to the class Mollicutes are members of the genus *Mycoplasma* (10). In this paper, we describe the isolation and characterization of a novel species of mycoplasma obtained from the conjunctivita of an adult 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 UCMF [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.

**MATERIALS AND METHODS**

Isolation. The new organism was isolated from a live European starling submitted to the Northeastern Research Center for Wildlife Diseases at the University of Connecticut. The bird had severe bilateral conjunctivitis with mucous discharge. Moderate blepharitis was noted, and the upper and lower eyelids of the left eye were crusted closed.

Swabs of the left and right conjunctival sacs were used to inoculate fortified commercial (FC) broth (11), incubated at 37°C for 48 h, and then plated onto FC agar, which were incubated in a humidified environment at 37°C until colonies formed on agar plates, which were incubated in a humidified environment at 37°C until colonies formed on agar plates.

**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 (Ted Pella, Inc., Redding, Calif.). 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 UCMF cultured in SP-4 broth was also examined by dark-field microscopy at a magnification of ×1,250. Strain UCMF grown on FC agar at 37°C was observed with a stereomicroscope, and typical colonies were photographed.

**Biological and biochemical studies.** Strain UCMF 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 of M. sturni (14).

Mycoplasma cynos HR31T, Mycoplasma dispar 4622T, Mycoplasma edwardii PG23T, Mycoplasma equigenitalium T37T, Mycoplasma fastidiosum 45227, Mycoplasma fermentans PG18T, Mycoplasma flocculare Ms 42T, Mycoplasma gallinaceum DD1, Mycoplasma gallisepticum PG31T, Mycoplasma gallopavonis WR1T, Mycoplasma genitalium G37T, Mycoplasma glycophillum 486T, Mycoplasma hominis 37T, Mycoplasma hyorhinis HT3T, Mycoplasma imitans 4229T, Mycoplasma iowae 695, Mycoplasma iowae PPAAV1, Mycoplasma leucophaeae 3L2T, Mycoplasma leophaggiae LL2T, Mycoplasma leopodium R171T, Mycoplasma micros MK 405T, Mycoplasma mobile 163K1T, Mycoplasma nodae HS42T, Mycoplasma mycoides subsp. arvalis HRC689, and Utah C, Mycoplasma mycoides subsp. capri PG3T, Mycoplasma mycoides subsp. mycoides U30847, Mycoplasma mycoides subsp. capri PG3T, Mycoplasma neurolipticum Type A1T, Mycoplasma ovis pneumoniae Y98T, Mycoplasma ovis pneumoniae 128T, Mycoplasma penetrans GTUS5T, Mycoplasma phocarhinis 882T, Mycoplasma pinnun 70-159T, Mycoplasma pneumoniae FH1T, Mycoplasma pullorum CCK1T, Mycoplasma pullorum PG34T, Mycoplasma purpureus KS-1T, Mycoplasma simiae LSXT, Mycoplasma suaviis Mayfield B2T, Mycoplasma syringae WUV 1853T, Mycoplasma testudinis 01008T, Mycoplasma virens 107T, Mycoplasma vestita GIIT, Mycoplasma sp. strain Cali 188, BSP, M7806, 3306, 3446, HRC589, and Utah C, Entomoplasma elychniae ELMN-1T, Entomoplasma hirasa EPN52T, Entomoplasma luminum PNM-1T, and Entomoplasma sp. strain BARC 318. In addition, agar colonies of strain UCMFT were also used in direct immunofluorescence tests (6) using individual fluorescein-conjugated antisera to each of the type strains listed above.

**FIG. 1.** Mycoplasma sturni colonies grown on FC agar. Bar = 100 μm.

**RESULTS AND DISCUSSION**

**Isolation.** Strain UCMFT 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 Mycoplasmatales 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 flasks-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 UCMFT 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 kit (Pharmacia Biotech., Inc., Piscataway, N.J.) and an automated laser sequence (Pharmacia). Homology searches were accomplished by using BLAST (1) and FASTA (CCG) with nonredundant nucleotide databases.

**Nucleotide sequence accession number.** The nucleotide sequence of the strain UCMFT 16S rRNA gene determined in this study has been deposited in the GenBank database under accession number U22013.
in a serum-free medium supplemented with 0.04% polyoxyethylene sorbitan (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).

### TABLE 1. Growth response of strain UCMFT to cholesterol

<table>
<thead>
<tr>
<th>Supplement(s) added to serum-free base medium</th>
<th>Cholesterol concn (µg/ml)</th>
<th>Amt of protein (mg/100 ml)*</th>
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<tr>
<td>SP-4 medium</td>
<td>0 (control)</td>
<td>13.00</td>
</tr>
<tr>
<td>1% Bovine serum fraction medium</td>
<td>0 (control)</td>
<td>3.76</td>
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<tr>
<td>None</td>
<td>0</td>
<td>IG*</td>
</tr>
<tr>
<td>Albumin (1%), Tween 80 (0.01%), and palmitic acid (10 µg/ml)</td>
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<td>1.69</td>
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<tr>
<td></td>
<td>5</td>
<td>2.40</td>
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<td></td>
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<td>2.76</td>
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<tr>
<td></td>
<td>20</td>
<td>2.83</td>
</tr>
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</table>

*Cellular protein yields from mycoplasmas sedimented from 100-ml quantities of control broth or broth media containing various supplements. Each bottle received a 1% inoculum of strain UCMFT in bovine serum fraction broth, and cultures were harvested after 3 days of incubation at 37°C.  

*IG, insufficient growth.*

**FIG. 2.** Ultrathin section of a *Mycoplasma sturni* culture viewed and photographed with an electron microscope. Bar = 100 nm.
The properties of strain UCMF\textsuperscript{T} described in this paper fulfill the criteria for species descriptions of members of the class 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 Mycoplasmatales. The inability of strain UCMF\textsuperscript{T} to hydrolyze urea indicates that it belongs in the genus Mycoplasma. The lack of serological relatedness to previously described fermentative mycoplasma species indicates that this organism represents a new Mycoplasma species. We propose the name Mycoplasma sturni for strain UCMF\textsuperscript{T}.

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

**Description of Mycoplasma sturni sp. nov.** Mycoplasma sturni (stur’ni. M. L. n. Sturnus, a genus of birds; M. L. gen. n. sturni, of the genus 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 Mycoplasma species.

Isolated from ocular tissues of a starling (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\textsuperscript{T} (= ATCC 51945).

**ACKNOWLEDGMENTS**

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**REFERENCES**