Mycoplasma ellychniae sp. nov., a Sterol-Requiring Mollicute from the Firefly Beetle *Ellychnia corrusca*

JOSEPH G. TULLY,^{1*} DAVID L. ROSE,¹ KEVIN J. HACKETT,² ROBERT F. WHITCOMB,² PATRICIA CARLE,³
JOSEPH M. BOVÉ,³ DAVID E. COLFLESH,⁴ AND DAVID L. WILLIAMSON⁴

Mycoplasma Section, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Frederick Cancer Research Facility, Frederick, Maryland 21701¹; Insect Pathology Laboratory, United States Department of Agriculture, Beltsville, Maryland 20705²; Laboratoire de Biologie Cellulaire et Moléculaire, Institut Nationale de Recherche Agronomique, Pont-de-la-Maye, France³; and Department of Anatomical Sciences, State University of New York, Stony Brook, New York 11794⁴

Strain ELCN-1^T (T = type strain), which was isolated from the hemolymph of the firefly beetle *Ellychnia corrusca* (Coleoptera:Lampyridae) in Maryland, was shown to be a sterol-requiring mollicute. Electron and dark-field microscopy showed that the organism consisted of small, nonhelical, nonmotile, pleomorphic coccoid cells. Individual cells were surrounded by a single cytoplasmic membrane, but no evidence of a cell wall was observed. The organism grew well in SP-4 broth medium containing fetal bovine serum, but failed to grow in formulations containing horse serum or bovine serum fraction supplements. Growth on solid media occurred only when agar cultures were incubated aerobically or in an atmosphere containing 5% carbon dioxide. Strain ELCN-1^T catabolized glucose but did not hydrolyze arginine or urea. The optimum temperature for growth was 30°C, while multiplication occurred over a temperature range of 18 to 32°C. Growth was not observed at 37°C. The genome of strain ELCN-1^T was determined to be about 585 megadaltons. The guanine-plus-cytosine content of the deoxyribonucleic acid was found to be 27.5 mol%. This organism was serologically unrelated to the type strains of previously described *Mycoplasma* species and to 18 other unclassified sterol-requiring isolates cultivated from various animal, plant, or insect sources. This is the first documented insect-derived species in the genus *Mycoplasma*. Strain ELCN-1 (= ATCC 43707) is the type strain of *Mycoplasma ellychniae* sp. nov.

The presence of wall-less procaryotes (class *Mollicutes*) in the microbial flora of insects and other arthropods first became a recognized possibility with the 1967 discovery of mycoplasma-like organisms in plants infected with the leafhopper-borne mulberry dwarf disease agent (12). Although wall-less procaryotes have yet to be cultivated axenically from infected vectors or plants with mulberry disease or from any of the more than 300 other known mycoplasma-like organism-associated diseases, infections caused by mycoplasma-like organisms have been associated consistently with specific insect vectors (22). By the early 1970s, two other important observations provided substantial evidence of insect association with mollicutes. Cultivation and characterization of the first helical mollicute (*Spiroplasma citri*) from stubborn-diseased citrus plants (9, 26) had occurred, and the organism was shown to be transmissible to citrus by experimentally infected leafhoppers (11). In addition, the then uncultivated corn stunt spiroplasma was shown to be transmitted by leafhoppers (see review in reference 34). Although the role of insects as vectors of plant-pathogenic spiroplasmas was established in these studies, it remained for Clark and colleagues to demonstrate that a diversity of insects harbored an extraordinarily rich spiroplasma flora (5, 18).

The association of other mollicutes with insects followed the discovery of spiroplasmas in these hosts. The presence of nonhelical mollicutes on plant surfaces was first well documented in 1979 (13, 23), when organisms with the general features of both *Acholeplasma* and *Mycoplasma* species were isolated from vegetative and floral plant parts. Some of these isolates were serologically related to known

acholeplasmas (*Acholeplasma axanthum*, *Acholeplasma oculi*), while others were subsequently found to represent a new species (e.g., *Acholeplasma florum*) clearly associated with and known only from plant hosts (21). The occurrence of acholeplasmas in insects was established when Clark and colleagues (6) reported the isolation of sterol-nonrequiring mollicutes from the guts of five different insect species. Some of these isolates, including representatives from a deerfly (*Chrysops discalis*) and a vespid wasp (*Monobia quadridens*) and strains recovered from other insects (32), were identified as *A. florum*. These observations confirmed a direct ecological link between acholeplasmas on surfaces of plants or flowers and an insect reservoir. More recently, other acholeplasmas isolated from insect guts have turned out to be new species (e.g., *Acholeplasma entomophilum*) (31) or putative new species (30).

The possibility that sterol-requiring *Mycoplasma* species may, like acholeplasmas, be specifically associated with insect-plant interactions has been suspected but not clearly established previously (30). A number of putative new species in the genus *Mycoplasma* have been cultivated from plant sources and are in various stages of characterization (30). Recently, 30 mollicutes isolated by Clark and colleagues from the guts or hemolymphs of a wide range of insect species were examined (32); 5 of these strains were found to be sterol-requiring, nonhelical mollicutes. In this paper we describe the detailed taxonomic features of strain ELCN-1^T (T = type strain), which was isolated from hemolymph of the firefly beetle *Ellychnia corrusca*, and propose that this organism be assigned to the genus *Mycoplasma*. This is the first species in this genus to be clearly shown to be specifically associated with insects.

* Corresponding author.

TABLE 1. Mollicute strains and antisera tested

Strain	Source ^a
<i>Mycoplasma agalactiae</i> PG2 ^T	NIH
<i>Mycoplasma alkalescens</i> D12 ^T	MS-LMM
<i>Mycoplasma alvi</i> Ilsey ^T	MS-LMM
<i>Mycoplasma anatis</i> 1340 ^T	NIH
<i>Mycoplasma anseris</i> 1219 ^T	MS-LMM
<i>Mycoplasma arginini</i> G230 ^T	NIH
<i>Mycoplasma arthritis</i> PG6 ^T	NIH
<i>Mycoplasma bovigenitalium</i> PG11 ^T	NIH
<i>Mycoplasma bovirhinis</i> PG43 ^T	NIH
<i>Mycoplasma bovis</i> Donetta ^T	MS-LMM
<i>Mycoplasma bucculi</i> M165/69 ^T	MS-LMM
<i>Mycoplasma buccale</i> CH20247 ^T	NIH
<i>Mycoplasma californicum</i> ST-6 ^T	MS-LMM
<i>Mycoplasma canadense</i> 275C ^T	MS-LMM
<i>Mycoplasma canis</i> PG14 ^T	NIH
<i>Mycoplasma capricolum</i> California Kid ^T	MS-LMM
<i>Mycoplasma caviae</i> G122 ^T	MS-LMM
<i>Mycoplasma cavipharyngis</i> 117C ^T	MS-LMM
<i>Mycoplasma citelli</i> RG-2C ^T	MS-LMM
<i>Mycoplasma cloacale</i> 383 ^T	MS-LMM
<i>Mycoplasma collis</i> 58B ^T	MS-LMM
<i>Mycoplasma columbinasale</i> 694 ^T	MS-LMM
<i>Mycoplasma columbinum</i> MMP-1 ^T	MS-LMM
<i>Mycoplasma columborale</i> MMP-4 ^T	MS-LMM
<i>Mycoplasma conjunctivae</i> HRC581 ^T	MS-LMM
<i>Mycoplasma cricetuli</i> CH ^T	MS-LMM
<i>Mycoplasma cynos</i> H831 ^T	MS-LMM
<i>Mycoplasma dispar</i> 462/2 ^T	MS-LMM
<i>Mycoplasma edwardii</i> PG24 ^T	MS-LMM
<i>Mycoplasma equigenitalium</i> T37 ^T	MS-LMM
<i>Mycoplasma equirhinis</i> M432/72 ^T	MS-LMM
<i>Mycoplasma fastidiosum</i> 4822 ^T	MS-LMM
<i>Mycoplasma faucium</i> DC333 ^T	NIH
<i>Mycoplasma felifaucium</i> PU ^T	MS-LMM
<i>Mycoplasma feliminutum</i> Ben ^T	MS-LMM
<i>Mycoplasma felis</i> CO ^T	MS-LMM
<i>Mycoplasma fermentans</i> PG18 ^T	NIH
<i>Mycoplasma flocculare</i> Ms42 ^T	MS-LMM
<i>Mycoplasma gallinaceum</i> DD ^T	MS-LMM
<i>Mycoplasma gallinarum</i> PG16 ^T	NIH
<i>Mycoplasma gallisepticum</i> PG31 ^T	NIH
<i>Mycoplasma gallopavonis</i> WR1 ^T	MS-LMM
<i>Mycoplasma gateae</i> CS ^T	MS-LMM
<i>Mycoplasma genitalium</i> G37 ^T	MS-LMM
<i>Mycoplasma glycyphilum</i> 486 ^T	MS-LMM
<i>Mycoplasma hominis</i> PG21 ^T	NIH
<i>Mycoplasma hyopharyngis</i> H3-6BF ^T	MS-LMM
<i>Mycoplasma hyopneumoniae</i> J ^T	MS-LMM
<i>Mycoplasma hyorhinis</i> BTS7 ^T	NIH
<i>Mycoplasma hyosynoviae</i> S16 ^T	MS-LMM
<i>Mycoplasma iners</i> PG30 ^T	NIH
<i>Mycoplasma iowae</i> 695 ^T	MS-LMM
<i>Mycoplasma lipofaciens</i> R171 ^T	MS-LMM
<i>Mycoplasma lipophilum</i> MaBy ^T	NIH
<i>Mycoplasma maculosum</i> PG15 ^T	NIH
<i>Mycoplasma meleagridis</i> 17529 ^T	NIH
<i>Mycoplasma moatsii</i> MK405 ^T	MS-LMM
<i>Mycoplasma mobile</i> 163K ^T	MS-LMM
<i>Mycoplasma molare</i> H542 ^T	MS-LMM
<i>Mycoplasma muris</i> RIII4 ^T	MS-LMM
<i>Mycoplasma mustelae</i> MX9 ^T	MS-LMM
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> B3	MS-LMM
<i>Mycoplasma mycoides</i> subsp. <i>capri</i> PG3 ^T	MS-LMM
<i>Mycoplasma neurolyticum</i> Type A ^T	NIH
<i>Mycoplasma opalescens</i> MH5408 ^T	MS-LMM
<i>Mycoplasma orale</i> CH19299 ^T	NIH
<i>Mycoplasma ovipneumoniae</i> Y98 ^T	MS-LMM
<i>Mycoplasma pirum</i> 70-159 ^T	MS-LMM

Continued

TABLE 1—Continued

Strain	Source ^a
<i>Mycoplasma pneumoniae</i> FH ^T	NIH
<i>Mycoplasma primatum</i> HRC292 ^T	MS-LMM
<i>Mycoplasma pullorum</i> CKK ^T	MS-LMM
<i>Mycoplasma pulmonis</i> PG34 ^T	NIH
<i>Mycoplasma putrefaciens</i> KS-1 ^T	MS-LMM
<i>Mycoplasma salivarium</i> PG20 ^T	NIH
<i>Mycoplasma spumans</i> PG13 ^T	NIH
<i>Mycoplasma sualvi</i> Mayfield B ^T	MS-LMM
<i>Mycoplasma subdolum</i> TB ^T	MS-LMM
<i>Mycoplasma synoviae</i> WVU 1853 ^T	MS-LMM
<i>Mycoplasma testudinis</i> 01008 ^T	MS-LMM
<i>Mycoplasma verecundum</i> 107 ^T	MS-LMM
<i>Mycoplasma</i> sp. strain California calf (joint) ^b	MS-LMM
<i>Mycoplasma</i> sp. strain B5P (bovine joint)	MS-LMM
<i>Mycoplasma</i> sp. strain melaleuca (plant)	MS-LMM
<i>Mycoplasma</i> sp. strain 831-C4 (lettuce)	MS-LMM
<i>Mycoplasma</i> sp. strain PPAV (apple)	MS-LMM
<i>Mycoplasma</i> sp. strain F38 (caprine lung)	MS-LMM
<i>Mycoplasma</i> sp. strain M7806 (human abscess)	MS-LMM
<i>Mycoplasma</i> sp. strain 3306 (ovine genitalia)	MS-LMM
<i>Mycoplasma</i> sp. strain M2851 (equine uterus)	MS-LMM
<i>Mycoplasma</i> sp. strain S5251 (seal lung)	MS-LMM
<i>Mycoplasma</i> sp. strain HRC291 (primate throat)	MS-LMM
<i>Mycoplasma</i> sp. strain 3446 (bovine fetus)	MS-LMM
<i>Mycoplasma</i> sp. strain G145 (goat leg)	MS-LMM
<i>Mycoplasma</i> sp. strain B689 (dog throat)	MS-LMM
<i>Mycoplasma</i> sp. strain GM257A (caprine ear)	MS-LMM
<i>Mycoplasma</i> sp. strain PYAN-1 (firefly gut)	MS-LMM
<i>Mycoplasma</i> sp. strain PIMN-1 (firefly gut)	MS-LMM
<i>Mycoplasma</i> sp. strain PIPN-2 (firefly gut)	MS-LMM
<i>Acholeplasma axanthum</i> S743 ^T	MS-LMM
<i>Acholeplasma entomophilum</i> TAC ^T	MS-LMM
<i>Acholeplasma equifetale</i> C112 ^T	MS-LMM
<i>Acholeplasma florum</i> L1 ^T	MS-LMM
<i>Acholeplasma granularum</i> BTS39 ^T	MS-LMM
<i>Acholeplasma hippikon</i> C1 ^T	MS-LMM
<i>Acholeplasma laidlawii</i> PG8 ^T	MS-LMM
<i>Acholeplasma modicum</i> PG49 ^T	MS-LMM
<i>Acholeplasma morum</i> 72-043 ^T	MS-LMM
<i>Acholeplasma oculi</i> 19L ^T	MS-LMM
<i>Acholeplasma parvum</i> H23M ^T	MS-LMM

^a NIH, National Institutes of Health reference reagent; MS-LMM, reagent prepared by Mycoplasma Section, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Frederick, Md.

^b The information in parentheses indicates the source of a strain.

MATERIALS AND METHODS

Mycoplasma strains. The techniques employed in the primary isolation of mollicutes from insect tissues have been described previously (20, 32). Strain ELCN-1^T was isolated and cultivated by T. B. Clark from the hemolymph of an *E. corrusca* firefly beetle collected in Maryland. Preliminary analyses of growth requirements and serological features of this organism have been described previously (32). Strain ELCN-1^T was purified by conventional filtration cloning techniques (28). The type strains of previously described *Mycoplasma* species, as well as a large cluster of currently unclassified, nonhelical, sterol-requiring mollicutes of diverse animal, plant, and insect origins, were also used in various parts of this study (Table 1).

Culture medium and cultivation techniques. Strain ELCN-1^T was grown in primary culture on M1D medium (33) at 30°C, and after three to five early broth passages the organism was lyophilized. For characterization studies, a vial of the dried stock culture was revived and passaged twice in SP-4 broth (33) at 30°C before purification procedures were

undertaken. Following filtration cloning, a triply cloned line was designated strain ELCN-1^T; this strain was used in all subsequent characterization work. Other culture medium formulations used included the following: the Edward formulation of conventional 20% horse serum mycoplasma broth (14); serum fraction broth containing 1% bovine serum fraction (29); and serum-free media supplemented with fatty acid mixtures (31). A solid medium of each of these formulations was made by adding 0.8% Noble agar (Difco Laboratories, Detroit, Mich.). Agar cultures were incubated at 30°C either aerobically with 5% carbon dioxide in a GasPak system (BBL Microbiology Systems, Cockeysville, Md.) or under anaerobic conditions (hydrogen GasPak system).

For measuring the temperature requirements for growth of strain ELCN-1^T, a series of 10-fold dilutions of the organism in SP-4 broth was prepared. One series of the diluted mycoplasma was incubated at each of six temperatures (10, 18, 25, 30, 32, and 37°C). The relative number of organisms, measured by noting color changes (red to yellow) and turbidity in the highest dilution of SP-4 broth (color-changing units per milliliter), was recorded after incubation for 3 weeks. Early passages of strain ELCN-1^T were also grown on SP-4 medium devoid of penicillin and other antibiotics for at least five consecutive passages. At each subculture, the organism was plated onto conventional blood agar and incubated aerobically at 37°C. After 2 to 10 days, the plates were examined for bacterial colonies and evidence of reversion.

Morphological studies. Cells of strain ELCN-1^T from SP-4 broth cultures in the logarithmic phase were examined by dark-field microscopy, using a magnification of $\times 1,250$. For electron microscopic examination, the organism was grown in approximately 10 ml of broth and pelleted by centrifugation. The cells were then fixed for 2 h in 3% glutaraldehyde, postfixed in 1% osmium tetroxide for 1 h, dehydrated in acetone, embedded in Epon-araldite, sectioned, and stained with 1% aqueous uranyl acetate and Reynold lead citrate.

Sterol requirement. Sterol requirements for growth were assayed by using a broth culture method, which was slightly modified from the method described previously (25). Cultures were incubated at 30°C rather than 37°C, and protein assays were performed with a Bio-Rad kit (Bio-Rad Laboratories, Richmond, Calif.).

Tests for biological and biochemical properties. The procedures used for demonstrating carbohydrate fermentation and arginine and urea hydrolysis have been described previously (1). Filtration characteristics were measured in SP-4 broth by using techniques described previously (28). The procedures used for the hemadsorption assay (16) and film and spot reaction (15) also have been described previously.

Serological tests. Antiserum to strain ELCN-1^T was raised in rabbits. Hyperimmune antisera to all previously described *Mycoplasma* species and to 18 other unclassified mycoplasmas (Table 1) came from the reference collection maintained at the National Institute of Allergy and Infectious Diseases laboratory in Frederick, Md. These antisera and strain ELCN-1^T were tested in a standard disk growth inhibition test (8), in which we used SP-4 agar plates and aerobic incubation at 30°C. In addition, direct plate immunofluorescent tests (17) were performed on strain ELCN-1^T colonies on SP-4 agar medium by using fluorescein-conjugated antisera to the mycoplasmas listed in Table 1. Finally, disk growth inhibition tests with strain ELCN-1^T were performed with antisera to the 11 recognized *Acholeplasma* species (31).

Genomic analysis. Techniques for extraction and purifica-

FIG. 1. Colonies of strain ELCN-1^T on SP-4 agar medium after 3 days of incubation at 30°C under aerobic conditions. Bar = 100 μ m.

tion of chromosomal deoxyribonucleic acid (DNA) from mollicutes have been described previously (3). A method for determining genome size by measuring the renaturation kinetics of denatured DNA has also been described previously (2). The guanine-plus-cytosine content of purified DNA from strain ELCN-1^T was assayed by the following three techniques: buoyant density, melting temperature, and high-performance liquid chromatography techniques (4). Purified DNA from *S. citri* (genome, approximately 1,000 megadaltons; base composition, 26 mol% guanine plus cytosine) was used as a reference DNA in all procedures.

RESULTS AND DISCUSSION

Cultural and morphological properties. Strain ELCN-1^T grew only in SP-4 or M1D medium or on solid media prepared from these formulations. No evidence of multiplication occurred in conventional mycoplasma media containing horse serum (Edward formulation) or bovine serum fraction supplements. Growth occurred over a temperature range of 18 to 32°C, with optimum growth observed at 30°C. No growth was recorded during a 3-week period in broth media incubated at 10 or 37°C. Colonies of strain ELCN-1^T on SP-4 agar exhibited a typical fried-egg morphology (Fig. 1). Growth on solid medium was observed on agar plates incubated aerobically at 30°C or in a GasPak system under a carbon dioxide atmosphere. No growth occurred on agar media under anaerobic conditions.

Logarithmic-phase cultures of strain ELCN-1^T in SP-4 medium examined by dark-field microscopy contained numerous pleomorphic coccoid forms and short branching filaments. No evidence of helical forms was observed, and filamentous elements were nonmotile. The cells of the organism sedimented from broth cultures and examined by electron microscopic techniques were predominately pleo-

FIG. 2. Electron micrograph of a sectioned and stained cell pellet of strain ELCN-1^T. Sections were stained with 2% aqueous uranyl acetate and Reynold lead citrate. The arrow indicates the unit membrane. Bar = 100 nm.

morphic coccoid cells (Fig. 2). No evidence of a cell wall was observed in these preparations; representative round cells were about 200 to 300 nm in diameter and were surrounded by a single cytoplasmic membrane.

Sterol requirement. The response of strain ELCN-1^T to additions of cholesterol to serum-free SP-4 medium is shown in Table 2. The SP-4 medium control flask (containing serum) appeared to reach maximum growth after 2 days of incubation at 30°C, and cells were removed by centrifugation for a protein assay. After incubation for 5 days, growth was not apparent in the base broth alone or in base broth containing fatty acid supplements. However, at this time, enhanced growth (measured by increasing levels of protein) was detected when 5 to 20 µg of cholesterol per ml was included in the base broth. Similar responses (data not shown) were observed when various amounts (1 to 17%) of fetal bovine serum were added to the SP-4 base broth.

Biochemical and biological properties. Strain ELCN-1^T fermented glucose with the production of acid and a consequent decline in the pH of the culture medium. No evidence of arginine or urea hydrolysis was observed. Strain ELCN-1^T did not produce the film and spot reaction, and colonies of the organism on an agar medium did not hemadsorb guinea pig erythrocytes. Passage of broth cultures of strain ELCN-1^T through 450- or 300-nm membrane filters did not reduce the viable cell titer (10⁸ color-changing units per ml). The titer of a broth culture filtrate obtained after passage through a 220-nm membrane was reduced about 10-fold (to 10⁷ color-changing units per ml), while the 100-nm membrane filtrate was free of viable cells.

Serological tests. Growth inhibition and plate immunofluorescent tests, which were performed with antisera or conjugates prepared to the mycoplasmas and achleplasmas listed in Table 1, indicated that strain ELCN-1^T was not related serologically to previously described species in the

two genera or to a group of 18 unclassified, nonhelical, sterol-requiring mollicutes that probably represent putative new species in the genus *Mycoplasma*.

Genome size and DNA base composition. The renaturation kinetics of strain ELCN-1^T DNA indicated that the genome molecular weight of the organism was approximately 585 × 10⁶ (average of five determinations [504, 525, 606, 621, and 669 megadaltons]). The values for the base composition (guanine-plus-cytosine content) of the DNA of strain ELCN-1^T were 27.5, 27.3, and 27.9 mol% for the buoyant density, melting temperature, and high-pressure liquid chromatography techniques, respectively.

Habitat. The single strain described here was isolated directly from the hemolymph of the firefly beetle *E. corrugata*. Most mollicutes cultivated from insects have been isolated from guts (5, 6, 18, 30, 32); these organisms are rarely if ever pathogenic. Certain mollicutes that establish natural infections in insect hemolymphs, such as *Spiroplasma melliferum* (7) and *Spiroplasma apis* (24), eventually kill their hosts. Whether strain ELCN-1^T is pathogenic for firefly beetles or other insects has not been determined. Occurrence of the organism in the hemolymph suggests that the relationship of strain ELCN-1^T and its insect host may be specific and unique. Additional isolations of organisms related to strain ELCN-1^T will be required to confirm this suspected specificity.

Little information is currently available on the frequency of infection of these newly recognized, nonhelical, sterol-requiring mycoplasmas in their insect hosts. Current data suggest that achleplasmas occur much more frequently than mycoplasmas in the gut contents of insects (32), but this observation may reflect a bias induced by the insect hosts examined. However, at least six other nonhelical, sterol-requiring mollicutes have been isolated from insect or plant sources by other investigators (see review in reference 30).

Two previous reports of the isolation of *Mycoplasma* species from arthropods should also be noted. In one report, Shifrine and colleagues (27) documented the isolation of *Mycoplasma mycoides* subsp. *mycoides* from ticks collected on cattle with contagious bovine pleuropneumonia. However, the authors could not demonstrate transmission of the bovine disease with mycoplasma-infected ticks. More recently, Cottew and Yeats (10) reported the occurrence of other *Mycoplasma* species in mites found in the ears of clinically normal goats and suggested that these infected arthropods might represent an important reservoir for subsequent mycoplasmal infection in goats.

The properties described here for strain ELCN-1^T fulfill the essential criteria (19) for species in the class *Mollicutes*, including the absence of a cell wall, filterability, lack of reversion to walled bacteria when organisms are grown in antibiotic-free media, penicillin resistance, and production

TABLE 2. Growth response of strain ELCN-1^T to cholesterol

Supplement(s) added to serum-free base medium	Cholesterol concn (µg/ml)	Amt of protein (mg/100 ml)
17% fetal bovine serum (control)	0	2.80
No supplement	0	IG ^a
1% albumin, 0.01% Tween 80, and 10 µg of palmitic acid per ml	0	IG
	1	IG
	5	0.23
	10	0.90
	20	1.98

^a IG, Insufficient growth.

of typical colony forms on agar. The growth requirement for sterol or serum and the lack of helicity place this organism in the family *Mycoplasmataceae*. The inability of strain ELCN-1^T to hydrolyze urea mandates its assignment to the genus *Mycoplasma*. Finally, a serological comparison of strain ELCN-1^T with an extensive collection of all previously described *Mycoplasma* species and with other unclassified strains that probably represent new species in the genus *Mycoplasma* demonstrated that the new insect isolate is unrelated to other organisms in the genus. Therefore, we propose the name *Mycoplasma ellychniae* for strain ELCN-1^T and similar organisms. The taxonomic description below summarizes the properties of the species.

***Mycoplasma ellychniae* sp. nov.** *Mycoplasma ellychniae* (el.lych'ni.äe. N.L. n. *Ellychnia*, a genus of firefly beetles; N.L. gen. n. *ellychniae*, of *Ellychnia*, from which the organism was first isolated). Cells are nonhelical, pleomorphic filaments, with some branching; small coccoid forms (varying in diameter from 200 to 300 nm) also occur. Cells lack true cell walls. Nonmotile. Colonies on solid medium containing 0.8% Noble agar usually have the appearance of fried eggs. Chemoorganotroph. Acid produced from glucose. Does not hydrolyze arginine or urea. Film and spot reaction negative.

Does not hemadsorb guinea pig erythrocytes.

Cholesterol or serum is required for growth.

Temperature range for growth, 18 to 32°C, with optimum growth at 30°C.

Serologically distinct from previously described *Mycoplasma* species. Isolated from the hemolymph of the firefly beetle *E. corrusca*. Pathogenicity for insects has not been determined.

The genome averages 585 megadaltons. The guanine-plus-cytosine content of the DNA is 27.5 ± 1 mol%, as determined by the buoyant density method.

The type strain is strain ELCN-1 (= ATCC 43707).

LITERATURE CITED

- Aluotto, B. B., R. G. Wittler, C. O. Williams, and J. E. Faber. 1970. Standardized bacteriologic techniques for characterization of *Mycoplasma* species. *Int. J. Syst. Bacteriol.* 20:35-58.
- Carle, P., and J. M. Bové. 1983. Genome size determination. *Methods Mycoplasmol.* 1:309-311.
- Carle, P., C. Saillard, and J. M. Bové. 1983. DNA extraction and purification. *Methods Mycoplasmol.* 1:295-299.
- Carle, P., C. Saillard, and J. M. Bové. 1983. Determination of guanine plus cytosine content of DNA. *Methods Mycoplasmol.* 1:301-308.
- Clark, T. B. 1982. Spiroplasmas: diversity of arthropod reservoirs and host-parasite relationships. *Science* 217:57-59.
- Clark, T. B., J. G. Tully, D. L. Rose, R. Henegar, and R. F. Whitcomb. 1986. Acholeplasmas and similar nonsterol-requiring mollicutes from insects: missing link in microbial ecology. *Curr. Microbiol.* 13:11-16.
- Clark, T. B., R. F. Whitcomb, J. G. Tully, C. Mouches, C. Saillard, J. M. Bové, H. Wróblewski, P. Carle, D. L. Rose, and D. L. Williamson. 1985. *Spiroplasma melliferum* sp. nov., a new species from the honeybee (*Apis mellifera*). *Int. J. Syst. Bacteriol.* 35:296-308.
- Clyde, W. A., Jr. 1983. Growth inhibition tests. *Methods Mycoplasmol.* 1:405-410.
- Cole, R. M., J. G. Tully, T. J. Popkin, and J. M. Bové. 1973. Ultrastructure of the agent of citrus "stubborn disease." *J. Bacteriol.* 115:367-386.
- Cottew, G. S., and F. R. Yeats. 1982. Mycoplasmas and mites in the ears of clinically normal goats. *Aust. J. Vet. Sci.* 59:77-81.
- Daniels, M. J., P. G. Markham, B. M. Meddins, A. K. Plaskitt, R. Townsend, and M. Bar-Joseph. 1973. Axenic culture of a plant pathogenic spiroplasma. *Nature (London)* 244:523-524.
- Doi, Y., M. Teranaka, K. Yora, and H. Asuyama. 1967. Mycoplasma- or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or Paulownia witches' broom. *Ann. Phytopathol. Soc. Jpn.* 33:259-266.
- Eden-Green, S., and J. G. Tully. 1979. Isolation of *Acholeplasma* spp. from coconut palms affected by lethal yellowing disease in Jamaica. *Curr. Microbiol.* 2:311-316.
- Edward, D. G. ff. 1947. A selective medium for pleuropneumonia-like organisms. *J. Gen. Microbiol.* 1:238-243.
- Freundt, E. A. 1983. Film and spot production. *Methods Mycoplasmol.* 1:373-374.
- Gardella, R. S., and R. A. Del Giudice. 1983. Hemagglutination, hemadsorption, and hemolysis. *Methods Mycoplasmol.* 1:379-384.
- Gardella, R. S., R. A. Del Giudice, and J. G. Tully. 1983. Immunofluorescence. *Methods Mycoplasmol.* 1:431-439.
- Hackett, K. J., and T. B. Clark. 1989. The ecology of spiroplasmas, p. 113-199. *In* R. F. Whitcomb and J. G. Tully (ed.), *The mycoplasmas*, vol. 5. Academic Press, Inc., New York.
- International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Mollicutes*. 1979. Proposal of minimal standards for descriptions of new species of the class *Mollicutes*. *Int. J. Syst. Bacteriol.* 29:172-180.
- Markham, P. G., T. B. Clark, and R. F. Whitcomb. 1983. Culture techniques for spiroplasmas from arthropods. *Methods Mycoplasmol.* 2:217-223.
- McCoy, R. E., H. G. Basham, J. G. Tully, D. L. Rose, P. Carle, and J. M. Bové. 1984. *Acholeplasma florum*: a new species isolated from plants. *Int. J. Syst. Bacteriol.* 34:11-15.
- McCoy, R. E., A. Caudwell, C. J. Chang, T. A. Chen, L. N. Chiykowski, M. T. Cousin, J. L. Dale, G. T. N. de Leeuw, D. A. Golino, K. J. Hackett, B. C. Kirkpatrick, R. Marwitz, H. Petzold, R. C. Sinha, M. Sugiura, R. F. Whitcomb, I. L. Yang, B. M. Zhu, and E. Seemüller. 1989. Plant diseases associated with mycoplasma-like organisms, p. 545-640. *In* R. F. Whitcomb and J. G. Tully (ed.), *The mycoplasmas*, vol. 5. Academic Press, Inc., New York.
- McCoy, R. E., D. S. Williams, and D. L. Thomas. 1979. Isolation of mycoplasmas from flowers, p. 75-80. *In* R. E. McCoy and H. Su (ed.), *Proceedings of the Republic of China-United States Cooperative Science Seminar, Symposium Series 1*. National Science Council, Taipei, Taiwan.
- Mouches, C., J. M. Bové, J. G. Tully, D. L. Rose, R. E. McCoy, P. Carle-Junca, M. Garnier, and C. Saillard. 1983. *Spiroplasma apis*, a new species from the honey bee (*Apis mellifera*). *Ann. Inst. Pasteur (Paris)* 134A:383-397.
- Razin, S., and J. G. Tully. 1970. Cholesterol requirements of mycoplasmas. *J. Bacteriol.* 102:306-310.
- Saglio, P., M. L'Hospital, D. Lafèche, G. Dupont, J. M. Bové, J. G. Tully, and E. A. Freundt. 1973. *Spiroplasma citri* gen. and sp. n.: a mycoplasma-like organism associated with "stubborn" disease of citrus. *Int. J. Syst. Bacteriol.* 23:191-204.
- Shifrine, M., K. P. Bailey, and S. S. Stone. 1972. Contagious bovine pleuropneumonia: isolation of *Mycoplasma mycoides*, var. *mycoides* from ticks collected from infected cattle and infection attempts using these ticks. *Bull. Epizoot. Dis. Afr. (Spec. Issue)* 20:43-45.
- Tully, J. G. 1983. Cloning and filtration techniques for mycoplasmas. *Methods Mycoplasmol.* 1:173-177.
- Tully, J. G. 1984. The family *Acholeplasmataceae*, genus *Acholeplasma*, p. 781-787. *In* N. R. Krieg and J. M. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
- Tully, J. G. 1989. Class *Mollicutes*: new perspectives from plant and arthropod studies, p. 1-31. *In* R. F. Whitcomb and J. G. Tully (ed.), *The mycoplasmas*, vol. 5. Academic Press, Inc., New York.
- Tully, J. G., D. L. Rose, P. Carle, J. M. Bové, K. J. Hackett, and

- R. F. Whitcomb.** 1988. *Acholeplasma entomophilum* sp. nov. from gut contents of a wide range of host insects. *Int. J. Syst. Bacteriol.* **38**:164–167.
32. **Tully, J. G., D. L. Rose, R. F. Whitcomb, K. J. Hackett, T. B. Clark, R. B. Henegar, E. Clark, P. Carle, and J. M. Bové.** 1987. Characterization of some new insect-derived acholeplasmas. *Isr. J. Med. Sci.* **23**:699–703.
33. **Whitcomb, R. F.** 1983. Culture media for spiroplasmas. *Methods Mycoplasma* **1**:147–158.
34. **Whitcomb, R. F.** 1989. Biology of *Spiroplasma kunkelii*, p. 487–544. *In* R. F. Whitcomb and J. G. Tully (ed.), *The mycoplasmas*, vol. 5. Academic Press, Inc., New York.