**Spiroplasma syrphidicola** sp. nov., from a Syrphid Fly (Diptera: Syrphidae)

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**Spiroplasma** sp. strain EA-1T (T = type strain) (subgroup VIII-1), which was isolated from the syrphid fly *Eristalis arbustorum*, was serologically distinct from other *Spiroplasma* species, groups, and subgroups. The cells of this strain, as revealed by dark-field light microscopy, were short, helical, and motile. An electron microscopic examination revealed wall-less cells delimited by a single membrane. The unusually small cells passed through 220-nm filter pores with no reduction in titer. The organisms grew well in SM-1, M1D, and SP-4 liquid media. Growth also occurred in conventional horse serum medium and 1% serum fraction medium. Strain EA-1T grew at temperatures between 10 and 41°C, and optimum growth occurred at 32°C. The doubling time at the optimal temperature was 1.0 h. The strain catalyzed glucose and hydrolyzed arginine but did not hydrolyze urea. The guanine-plus-cytosine content of the DNA was 30 ± 1 mol%. The genome size was about 1,230 kbp. Strain EA-1 ( = ATCC 33826), which represents subgroup VIII-1, is designated the type strain of a new species, *Spiroplasma syrphidicola*.

In 1987, Tully et al. (24) published a revised informal classification of the genus *Spiroplasma* that increased the number of groups from 11 to 23. In addition, eight subgroups of group I were recognized. Two more groups have since been added (9, 31), and eight other strains representing putative species have been discovered (23, 24). Subgroups of groups VIII (8) and XVI (1) have also been proposed. Since the initial proposal of combined genomic-serological criteria for spiroplasma groups (13), these clusters have been considered putative species, under the assumption that they represent groups of strains that exhibit little or no appreciable intergroup DNA-DNA homology. In all of the cases that have been examined, DNA-DNA hybridization studies have confirmed this view, so that today, as represented by clusters of organisms that have putative species status, but have not been given formal recognition. In 1987, an ad hoc committee appointed by the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes proposed criteria (27) for designation of spiroplasma groups. Under some circumstances, subgroups can be designated species (11).

Three spiroplasma groups have been divided into subgroups. Group I contains eight subgroups (3, 33), and group XVI contains three subgroups (1). In 1993, Gasparich et al. (8) subdivided group VIII into three subgroups. Initially, group VIII strain EA-1T (T = type strain), which was isolated from a syrphid fly, fit the criteria used to classify spiroplasma strains into groups (27). However, a large number of spiroplasma isolates obtained from dipterous insects, including horseflies and deerflies (Diptera: Tabanidae), have been found to cross-react significantly with both group VIII strain EA-1T and strain DF-1, which in previous studies was placed in group XVII (24). One of these strains, TAAS-1, which was isolated from a horsefly, was used to determine the levels of relatedness of the three strains on the basis of serological, guanine-plus-cytosine (G+C) content, and DNA-DNA hybridization data (8). Serologically, strains EA-1T, DF-1, and TAAS-1 shared antigenic determinants. Although strains EA-1T and DF-1 failed to cross-react reciprocally in deformation and metabolism inhibition tests, antisera to strain TAAS-1 cross-reacted weakly with both strains. In addition, strains EA-1T, DF-1, and TAAS-1 had similar G+C contents (30 ± 1 mol%), and strains DF-1 and EA-1T exhibited levels of DNA-DNA homology (under high-stringency conditions) of 33 to 48%, while strain TAAS-1 exhibited levels of homology of 42 and 67% with strains DF-1 and EA-1T, respectively (8). These data support placing these three strains in three group VIII subgroups. Strain EA-1T represents subgroup VIII-1, strain DF-1 represents subgroup VIII-2, and strain TAAS-1 represents subgroup VIII-3. As a result of these nomenclatural changes, group XVII, which was originally proposed for strain DF-1, is now vacant (8).

In this paper, we describe the results of characterization studies that established that strain EA-1T ( = ATCC 33826) represents a new spiroplasma species. We propose the name *Spiroplasma syrphidicola* for this organism.

**MATERIALS AND METHODS**

**Spiroplasma** strains. Strain EA-1T was isolated by standard techniques (15) from the hemolymph of a syrphid fly (*Eristalis arbustorum*). Some of the genomic and serological features of this organism have been described previously (24). Strain EA-1T was purified by conventional filtration cloning techniques (21). Representative strains of all previously recognized groups and subgroups (9, 24, 31), including the type strains of previously recognized species (25, 30) and eight putative groups (23, 30), were also used in this study.
Culture medium and cultivation techniques. The primary culture of strain EA-1T was grown in SM-1 liquid medium (26) at 30°C. After several broth passages, the isolate was lyophilized. For characterization studies, the dried cultures were revived and passed twice in SM-1 broth (20) at 30°C before cloning. Following filtration cloning, a triply cloned line was designated strain EA-1T and used in characterization studies. Other media used included M1D medium (26), SP-4 medium (26), and serum-fraction broth supplemented with 1% bovine serum fraction (20). Solid media were prepared by adding Noble agar (Difco Laboratories, Detroit, Mich.) to a final concentration of 1.6 or 2.25%. Agar cultures were incubated at 30°C either aerobically in the presence of 5% carbon dioxide (GasPak system; BBL, Microbiology Systems, Cockeysville, Md.) or anaerobically (Hydrogen GasPak system). Temperature requirements for growth were assessed as described previously (14).

Morphological studies. Cells of strain EA-1T in a broth culture in the logarithmic phase were examined at a magnification of ×1,250 by dark-field microscopy. For electron microscopy, cells were grown in approximately 20 ml of broth and pelleted by centrifugation. The pelleted cells were fixed for 2 h in 3% glutaraldehyde, postfixed for 1 h in 1% osmium tetroxide, dehydrated in acetone, embedded in Epon, sectioned, and stained with 1% aqueous uranyl acetate and Reynolds's lead citrate.

Sterol requirement. Sterol requirements for growth were determined by a standard broth culture method (17, 20) and by a modified method based on sustained passage in sterol-free media (18).

Tests for biological and biochemical properties. The procedures used to study carbohydrate fermentation and arginine and urea hydrolysis have been described previously (2). Filtration characteristics were determined in M1D broth by previously described techniques (20).

Serological tests. Antiserum to strain EA-1T was raised in rabbits as described previously (34). Hyperimmune antisera to all previously described Spiroplasma species, groups, putative groups (23, 30), and subgroups were obtained from reference collections at the Beltsville Agricultural Research Center and the National Institute of Allergy and Infectious Diseases laboratory in Frederick, Md. Strain EA-1T and these antisera were tested reciprocally by performing metabolism inhibition and deformation tests as described previously (32, 34).

Genomic analysis. Techniques for extracting and purifying mollicute chromosomal DNA have been described previously (5). The G+C content of purified strain EA-1T DNA was determined as described previously (6). The genome size was also determined as described previously (4). Purified DNA from Spiroplasma citri (genomic size, approximately 1,000 mDa; G+C content, 26 ± 1 mol%) was used as a reference in all procedures (4).

RESULTS AND DISCUSSION

Cultural and morphological properties. Strain EA-1T grew well in SM-1, M1D, and SP-4 media. This strain also grew in conventional mycoplasma medium containing horse serum (the Edward formulation) and bovine serum fraction medium. Growth occurred at temperatures between 10 and 41°C, optimum growth occurred at 32°C. No growth was observed during 3 weeks of incubation in broth media at 5 or 43°C. The doubling times at 10, 15, 20, 25, 30, 32, 37, and 41°C were 52.7, 16.7, 10.2, 7.1, 3.1, 1.9, 1.0, 1.2, and 1.7 h, respectively. The colonies of strain EA-1T on solid horse serum medium containing 2.25% agar grew under anaerobic conditions (Fig. 1) had irregular margins, and nearby satellite colonies were present. Diffuse zones of growth within the agar were observed at a Noble agar concentration of 1.6%.

When logarithmic-phase cultures of strain EA-1T in M1D medium were examined by dark-field microscopy, we observed numerous short motile filaments. When cells of the organism were examined by electron microscopy, we observed filamentous cells and there was no evidence of a cell wall (Fig. 2). The representative cells examined were surrounded by a single cytoplasmic membrane.

Sterol requirement. Table 1 shows the response of strain EA-1T to additions of cholesterol to serum-free SP-4 medium. No growth occurred in the base broth alone; however, growth was enhanced by the presence of serum fraction or by the presence of 5 to 20 μg of cholesterol per ml. Also, strain EA-1T failed to grow in sustained passage in sterol-free media (18).

Biochemical and biological properties. Strain EA-1T produced acid from glucose and hydrolyzed arginine but not urea. Passage of broth cultures of strain EA-1T through membrane filters with pore sizes of 450, 300, and 220 nm did not reduce the viable cell titers. The organisms did not pass through filters with 100-nm pores.

Serological tests. Metabolism inhibition and spiroplasma deformation tests performed with antisera to previously described spiroplasma species, groups, subgroups, and putative species revealed (Table 2) that strain EA-1T was not serologically related to other Spiroplasma representative or type strains. Reciprocal cross-reactions were observed only with representatives of other subgroups of group VIII (Table 2). A one-way, low-titer cross-reaction was observed in deformation tests in which strain EA-1T (as the antigen) was tested against antisera to strain N525, a representative of subgroup 1-F. No cross-reactions were observed in metabolism inhibition tests. The results of previous growth inhibition tests (30), which were not performed for all crosses, confirm the unique position of strain EA-1T (24).

Genome size and DNA base composition. The genome size of strain EA-1T was approximately 1,230 kbp, as determined by pulsed-field electrophoresis (4). The genome size was deduced by using mobilized linear nonrestricted DNA, as well as from the sum of the sizes of the restriction fragments obtained after NaiI (1,150 kbp) digestion. The base composition (G+C content) of strain EA-1T DNA, as determined by buoyant density, melting temperature, and high-performance liquid chromatography methods (6), was 30 ± 1 mol%.

Habitat. The strain described in this paper was isolated from the hemolymph of the syrphid fly E. arbutorum. Most mollicutes isolated from insects have been isolated from guts (10). Some mollicutes that reside in insect hemolymph, such as Spiroplasma melliferarum (7) and Spiroplasma apis (16), reduce the longevity of the host. Strain EA-1T is not known to be pathogenic to its insect host. This strain is serologically related (at different levels) to a large number of isolates obtained from the guts of various species of tabanid flies (Diptera: Tabanidae) (28).

The properties of strain EA-1T described in this paper fulfill
the following proposed criteria (12) for descriptions of species belonging to the class Mollicutes (22): absence of a cell wall, filterability, lack of reversion to walled bacteria when organisms are grown in antibiotic-free media, and penicillin resistance. The helicity and motility of strain EA-1T and its inability to utilize urea place this organism in the family Spiroplasmataceae (19). Finally, the results of a serological comparison of strain EA-1T with other Spiroplasma species and other unclassified spiroplasma strains that represent putative species demonstrate the uniqueness of this new spiroplasma species. Therefore, we propose the name *Spiroplasma syrphidicola* for this organism.

**Description of *Spiroplasma syrphidicola* sp. nov.** *Spiroplasma syrphidicola* (syr phi di’co la. M. L. pl. n. Syrphidae, a family of

<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>
</tr>
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<tbody>
<tr>
<td>Serum fraction</td>
<td>0</td>
<td>1.54</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0.20</td>
</tr>
<tr>
<td>Palmitic acid (10 µg/ml) and albumin (1%)</td>
<td>0</td>
<td>0.42</td>
</tr>
<tr>
<td>Albumin (1%), Tween 80 (0.01%), and palmitic acid (10 µg/ml)</td>
<td>0</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.64</td>
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</table>
flies [nomenclatural name]; L. n. cola, dweller, inhabitant; M. L. m. n. syphridicola, inhabitant of syrphid flies, the insects from which the organism was obtained]. Cells are helical, motile filaments that lack a cell wall. Colonies on solid medium containing 2.25% Noble agar are irregular with satellites and diffuse and never have a fried-egg appearance. Growth on solid medium containing 1.6% Noble agar is diffuse. Chemoorganotroph. Acid is produced from glucose. Hydrolyzes arginine but not urea. Growth on solid medium has been determined.

**Table 2. Serological reactions and cross-reactions of strain EA-1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>Antiserum</th>
<th>Antigen</th>
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<tbody>
<tr>
<td>I-7</td>
<td>N525</td>
<td>20°</td>
<td>0 (10,240)*</td>
</tr>
<tr>
<td>VIII-1</td>
<td>EA-1T</td>
<td>2,560°</td>
<td>2,560</td>
</tr>
<tr>
<td>VIII-2</td>
<td>DF-1</td>
<td>&lt;20</td>
<td>&lt;20 (10,240)</td>
</tr>
<tr>
<td>VIII-3</td>
<td>TAAAS-1</td>
<td>20</td>
<td>20 (2,560)</td>
</tr>
<tr>
<td>All other</td>
<td>All others</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

* Reciprocal of the endpoint in the deformation test in which the antigen was tested with the homologous antiserum.
* Homologous titer of antiserum against which strain EA-1 cross-reacted when it was used as the antigen in heterologous tests.
* Reciprocal of the endpoint in the metabolism inhibition test in which antigen was tested with the homologous antiserum.
* Homologous titer of strain EA-1 in the test system.

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**Acknowledgments**

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**References**