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 short 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 catabolized 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 a result of these nomenclatural changes, group XVII, which was originally proposed for strain DF-1, is now vacant (8).

Sero- and serological features of this organism have been described previously (24). 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.

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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 passaged 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 (25), 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). Sensitivity to antibiotics was determined by a disk diffusion assay (30). The methods 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. Antisera 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 cini (genotype, 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, 13.1, 10.8, 12, and 1.7 h, respectively. The colonies of strain EA-1T on solid horse serum medium containing 2.25% agar grown 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 motile cells 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, 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-tier cross-reaction was observed in deformation tests in which strain EA-1T (as the antigen) was tested against antiserum to strain N525, a representative of subgroup I-7. 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 kb, 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 NsoI (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. arbustorum. Most mollicutes isolated from insects have been isolated from guts (10). Some mollicutes that reside in insect hemolymph, such as Spiroplasma melliferum (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: Tabani-
FIG. 2. Electron micrograph of cells of strain EA-1T. Sections were stained with 2% aqueous uranyl acetate and Reynold’s lead citrate. The arrows indicate the unit membrane.

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>
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<tr>
<td>Serum fraction</td>
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</tr>
<tr>
<td>None</td>
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<td>0.20</td>
</tr>
<tr>
<td>Palmitic acid (10 µg/ml) and albumin (1%)</td>
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<tr>
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<td>0.39</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.67</td>
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flies [nomenclatural name]; L. n. _cola_, dweller, inhabitant; M. L. m. _n. syrophicidula_, inhabitant of syphid flies, the insects from which the organism was isolated. Cells are helical, motile and 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.

Chemoanorganotroph. Acid is produced from glucose. Hydrolyzes arginine but not urea.

Cholesterol or serum is required for growth.

The temperature range for growth is 19 to 41°C. Optimum growth occurs at 32°C, with a doubling time of 1.0 h.

Serologically distinct from previously described _Spiroplasma_ species and putative groups. Isolated from the hemolymph of _Monobia quadridens_.

The G+C content of the DNA is 30 ± 1 mol%, as determined by the buoyant density method. The genome size is approximately 1,230 kbp.

The type strain is strain EA-1 (= ATCC 33826).

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REFERENCES


