Spiroplasma leptinotarsae sp. nov., a Mollicute Uniquely Adapted to Its Host, the Colorado Potato Beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

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Spiroplasma strain LD-1^T (T = type strain), which was isolated from the gut of a Colorado potato beetle (*Leptinotarsa decemlineata*) larva collected in Maryland, was serologically distinct from other spiroplasmas. Similar isolates were obtained from other *L. decemlineata* specimens collected in various parts of North America, in Poland, and in other eastern European countries and from *Leptinotarsa texana* specimens collected in Texas. Cells of strain LD-1^T, which in early passages were spiral, exhibited exceptionally rapid translational motility. This rapid motility and the spiral shape were lost after extended passage in culture. The organism required serum for growth. Originally isolated in coculture with insect cells in DCCM medium, strain LD-1^T adapted to several media in the absence of cocultured cells. Use of anaerobic conditions allowed primary isolation in a variety of media. The organism did not grow in serum-free media containing 2% serum fraction. Optimal growth in M1D medium occurred at 30 to 37°C (doubling time, 7.2 h). On solid M1D medium containing 2.0% Noble agar (pH 6.25) at 30°C, strain LD-1^T produced discrete colonies with numerous satellites. Strain LD-1^T hydrolyzed arginine, but did not utilize urea; there was evidence of weak fermentation of glucose. The guanine-plus-cytosine content of the DNA was determined to be 25 ± 1 mol%, and the genome size was 1,085 kb. The results of extensive studies of the ecology of this spiroplasma suggest that it is host specific for *Leptinotarsa* beetles. Strain LD-1 (= ATCC 43213) is designated the type strain of a new species, *Spiroplasma leptinotarsae*.

The Colorado potato beetle spiroplasma (CPBS) was first observed by Clark in 1982 in samples of gut fluid from larval and adult *Leptinotarsa decemlineata* beetles collected in Maryland (5). While other spiroplasmas are helical (with coils of equal amplitude), the CPBS has spiral coils (coils of increasing amplitude) (Fig. 1A), a characteristic that may be responsible for the high degree of translational motility of this organism (9).

Typically, spiroplasmas can be isolated aerobically in a number of growth media (16). However, primary isolates of the CPBS have been obtained only by using aerobic conditions with cocultured insect cells (13) or anaerobic conditions supplied by a BBL GasPak system (20). Aerobically maintained cocultured isolates that were passaged 20 to 40 times adapted to aerobic culture in insect cell-free growth media having low pH values (14, 20).

The CPBS shows every evidence of being host specific for the genus *Leptinotarsa*. It is present in overwintering adults and has been isolated consistently (9) from the guts of adult and larval specimens of *L. decemlineata*, where it attaches to midgut microvilli (11) and reaches high titers (5). The CPBS was also isolated from the guts of adult specimens of *Leptinotarsa texana* collected in Hidalgo County, Texas, but was absent from specimens of *Doryphora quadrisignata* (a member of the same

tribe [Doryphorini] as the genus *Leptinotarsa*) collected in Brazil (10). The CPBS does not persist when it is fed to or inoculated into other insects (9, 18).

In the current study, CPBS isolates formed a tight serological cluster as determined by spiroplasma deformation (36) and metabolism inhibition (34) tests. The results of a new test (the spiroplasma motility inhibition test), which was designed specifically to determine intragroup serological variation among isolates of this spiroplasma, suggested that two (or more) serovars were present; at least one serovar was distributed in northern North America (Canada south to North Carolina) and at high altitudes in Poland, and another serovar was distributed near the southern boundary of the Colorado potato beetle range in North America (Texas and New Mexico) and at low altitudes in Poland (12). Four genovars corresponded in distribution to the two serovars.

Strain LD-1^T (T = type strain) was designated the representative of spiroplasma group XX in a revised classification (30) of spiroplasma groups. Of particular significance is the biological control potential (15) of this spiroplasma for the Colorado potato beetle, an important economic pest of solanaceous crops (10). The attachment of this spiroplasma to midgut microvilli is also of interest (11) and may serve as a model for understanding spiroplasma-insect interactions. In this paper we report results of studies undertaken to fulfill proposed criteria (17) for descriptions of new species of the class *Mollicutes*. The results of these studies support designation of group XX strain LD-1 (= ATCC 43213) as the type strain of a new species of the genus *Spiroplasma*.

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FIG. 1. CPBS. (A and B) Electron micrographs of the CPBS in gut fluid, showing the spiral form (A), and strain LD-1^T in a DCCM broth culture, showing the absence of a cell wall and the unit cytoplasmic membrane (B). (C and D) Dark-field micrographs of strain LD-1^T, showing a large medusa-like aggregation of cells in an aerobic DCCM broth culture (C) and colonies on M1D medium containing 2.0% Noble agar (pH 6.25) after 4 days of incubation at 30°C in an aerobic environment (D).

MATERIALS AND METHODS

Spiroplasma strains. Techniques for isolating spiroplasmas from insect guts and hemocoel have been described previously (9, 24). Strain LD-1^T was isolated

from the gut of a larval Colorado potato beetle (*L. decemlineata* [Coleoptera: Chrysomelidae]) collected in Maryland (13). Serologically related isolates (which are collectively termed CPBSs) were obtained (10–12, 20) from the gut contents of *L. decemlineata* larvae and adults collected in Alberta, Canada (isolate Can-1),

and in various parts of the United States including Michigan (isolates Ari-1 and Ves-1), Maryland (strain LD-1^T and many other isolates), North Carolina (isolate NC-1), and New Mexico (isolate Mex-1). Isolates Tex-2 and Tex-3 were obtained from *L. texana* adults collected in Texas. In Europe, spiroplasmas were observed in the guts of potato beetles collected in Poland (in 1987), Germany (in 1987), and in parts of the former USSR, including Ukraine and Byelorussia (in 1988 and 1989) (21, 22). CPBSs were isolated from beetles collected in Poland at Olkusz (isolates JL32 and JL26), Karowice (isolates JL17 and JL22), Pszczyna (isolates JL5, JL3, and JL2), and Lublin (isolate JL9) (12).

Strain LD-1^T was purified by conventional filtration-cloning procedures (25). Representative strains (7, 28–30, 33) of previously recognized groups and subgroups, including type strains of previously recognized species and representatives of established and putative new groups (28, 30, 33), were cultivated for comparative purposes.

Culture medium and cultivation techniques. Primary isolation of isolate CPBS-1 was accomplished by coculture with *Diabrotica* insect cell line IPLB-DU182E in DCCM medium (13). Other isolates were obtained by using aerobic cocultures or by using DCCM medium and anaerobic conditions without cocultured cells (10, 12, 20). At passage 22, isolate CPBS-1 adapted to aerobic cell-free culture in DCCM medium (8) and was cloned by previously described procedures (25); the resulting clone was designated strain LD-1^T. Because the CPBS is a fastidious organism, no other isolates were cloned. DCCM medium was used for most characterization tests; cultures were maintained at 26°C. Other broth media used in this study included M1D medium (16, 31) (pH 6.0 to 7.4), SP-4 medium (16, 31) (pH 6.25 to 7.4), and serum fraction broth containing 1% bovine serum fraction (26). Solid formulations of M1D medium (pH 6.0) and SP-4 medium (pH 6.5) were prepared by adding Noble agar (Difco Laboratories, Detroit, Mich.) to final concentrations of 0.8 and 2.0%, respectively. Cultures on solid media were incubated at 30°C either aerobically or anaerobically in a BBL anaerobic GasPak system (Becton Dickinson and Co., Cockeysville, Md.) containing 25 to 35% H₂, 4 to 7% CO₂, and <1% O₂. Additional broth formulations (see below) were used for sterol and biochemical tests.

Temperature requirements. Temperature requirements for growth were determined by preparing 10-fold dilutions of strain LD-1^T in M1D broth containing phenol red indicator and DCCM broth containing bromocresol purple indicator. The dilution series were incubated at 5, 10, 15, 20, 25, 30, 32, 37, 41, and 43°C. Growth of the organisms was determined by observing indicator changes in the media and by microscopically examining the cultures during a 45-day observation period. Doubling times at each temperature were determined by observing the time required for acidification of culture media and by fitting the temperature-growth curve to a logistic growth equation (19).

Morphological studies. The morphology of strain LD-1^T cells in cultures in the logarithmic growth phase was monitored by dark-field microscopy (magnification, ×1,250). Electron microscopic techniques for spiroplasmas have been described previously (32). The strain was grown in 20 ml of DCCM broth and pelleted by centrifugation. The pelleted cells were fixed for 2 h in 3% glutaraldehyde, postfixed in 1% osmium tetroxide for 1 h, dehydrated in acetone, embedded in Epon, sectioned, and stained with 1% aqueous uranyl acetate and Reynold's lead citrate.

Sterol requirement. Sterol requirements for growth were determined by a modification of standard broth culture methods (24, 26). The modification was necessary because strain LD-1^T could not be grown in serum-free media. To circumvent this problem, we used a series of hybrid media consisting of combinations (1:1 to 1:72) of DCCM medium supplemented with 10% fetal bovine serum (DCCM medium) and serum-free DCCM medium containing (per liter) 20 mg of cholesterol and 12.7 g of bovine serum albumin as a cholesterol carrier (DC medium). Since the concentration of cholesterol in fetal bovine serum (Hyclone Laboratories, Inc., Logan, Utah) was approximately 1 mg/ml, the concentrations of cholesterol in the DCCM medium-DC medium mixtures were as follows: 1:1 mixture, 55 mg/liter; 1:3 mixture, 33 mg/liter; 1:9 mixture, 20 mg/liter; 1:18 mixture, 15 mg/liter; 1:36 mixture, 12 mg/liter; and 1:72 mixture, 11 mg/liter.

Tests for biological, biochemical, and serological properties. Procedures for determining carbohydrate fermentation, arginine hydrolysis, and urea utilization have been described previously (1). However, because the CPBS did not grow in serum fraction medium or in serum-free DCCM medium containing 1% serum fraction, 0.5% glucose, and 0.21% arginine (DSFGA medium), modifications were necessary. A DCCM base medium containing 10% fetal bovine serum (DBF medium) was used. DBF medium contained (i) moderate levels of sugar (approximately 1,280 mg of sugar [Difco Laboratories, Detroit, Mich.] per liter, 850 mg of TC-Yeastolate per liter, 280 mg of Phytone peptone [BBL] per liter, and 150 mg of fetal bovine serum [Hyclone Laboratories] per liter; all sugars may not have been metabolizable by the spiroplasma) and (ii) arginine (approximately 1,225 mg of arginine from Gibco-BRL Grace's medium [800 mg/liter] per liter, 250 mg of fetal bovine serum per liter, and 175 mg of peptides per liter; much of the amino acid in the serum and peptides, which included Phytone peptone [Oxoid U.S.A., Inc., Columbia, Md.], Liver Digest, and TC-Yeastolate, was in the form of protein and may not have been entirely available to the spiroplasma). To prepare three variants of DBF medium, 5 g of glucose per liter (DBFG medium), 2.1 g of arginine per liter (DBFA medium), or glucose and arginine (DBFGA medium) were added. Because, as estimated by acidification of the media, strain LD-1^T only weakly fermented glucose in all of the variants, efforts were made to

determine if this organism could acidify M1D medium (pH 7.4, 6.85, and 6.45) and DCCM medium (pH 6.45) under aerobic and anaerobic conditions. Urea utilization was tested in a DBF medium variant containing 2 g of glucose per liter plus 10 g of urea per liter (DBFGU medium). Bromocresol purple (0.002%) was used as a growth indicator in all media.

Filtration characteristics (25) were determined in DCCM broth. Titers of filtered cells were determined by preparing 10-fold dilution series in microtiter plates (96 wells; 0.3 ml of culture per well). Plastic film was used to seal the microtiter wells to prevent drying. After 14 days, all inoculated wells were observed by dark-field microscopy (magnification, ×400) to determine the presence of spiroplasmas.

Serological techniques have been described previously (12, 34, 36); isolates were compared with all previously established species, groups, and subgroups and putative groups. Hyperimmune antisera were obtained from reference collections at the Beltsville Agricultural Research Center and the National Institute of Allergy and Infectious Diseases laboratory in Frederick, Md.

Genomic analysis. Extraction and purification of DNA from mollicutes were performed as described previously (3). Estimates of the guanine-plus-cytosine (G+C) content of purified DNA of strain LD-1^T were obtained by three independent procedures, the buoyant density, melting temperature, and high-performance liquid chromatography procedures (4). The genome size was determined by pulsed-field gel electrophoresis (2).

RESULTS AND DISCUSSION

Cultural, biological, and morphological properties. Strain LD-1^T and other isolates grew rapidly in DCCM broth under aerobic or anaerobic conditions and in M1D broth and SP-4 broth under anaerobic conditions at all pHs tested. They also adapted to grow in M1D broth (pH 6.0 to 7.0) or SP-4 broth (pH 6.25 to 6.5) under aerobic conditions. Strain LD-1^T did not grow in DCCM base broth containing 1% bovine serum fraction, 0.5% glucose, and 0.21% arginine (DSFGA medium). In M1D medium, optimal growth occurred at 30 to 37°C, and multiplication occurred at 20 to 41°C but not at 15 or 43°C (19); the doubling times at 20, 25, 30, 32, 37, and 41°C were 41.9, 15.2, 7.2, 7.2, 7.2, and 21.6 h, respectively. In DCCM medium, optimal growth occurred at 30°C, and multiplication occurred at 20 to 37°C but not at 15 or 41°C (19); the doubling times at 20, 25, 30, 32, and 37°C were 32.6, 12.4, 7.2, 11.4, and 8.5 h, respectively.

Strain LD-1^T grew on solid M1D medium (Fig. 1D) and SP-4 medium incubated at 30°C under aerobic conditions. When the medium contained 0.8% agar, the colonies were diffuse. When the medium contained 2.5% agar, the colonies were discrete, and there were two types, small smooth colonies and larger colonies with numerous small satellites. Dark-field microscopy of logarithmic-phase cultures of strain LD-1^T revealed filamentous cells with no evidence of a cell wall; large medusa-like spiroplasma clumps were frequently observed (Fig. 1C). Electron microscopy confirmed the presence of spiral cells surrounded by a single cytoplasmic membrane in culture (strain LD-1^T) (Fig. 1B) and in the beetle gut (Fig. 1A).

Passage of broth cultures of strain LD-1^T through 300- and 450-nm-pore-size filters did not decrease the titer from the initial level of 10⁸ cells per ml; however, filtration through 220-nm-pore-size membranes reduced the viable cell titer 1,000-fold, and a 100-nm-pore-size membrane filtrate was free of viable cells.

Biochemical properties. Strain LD-1^T hydrolyzed arginine, as shown by a change in pH from 6.4 to 7.4 or more in DBFA medium and DBFGA medium and an increase in pH to 6.8 in DBF medium and DBFG medium. Urea was not utilized. The data suggested that glucose was utilized. However, acidification of the medium was weak; a decrease in pH of only 0.4 pH unit (pH 6.8 to 6.4) was detected in DBF medium and DBFG medium, and no decrease in pH was detected in DBFA medium and DBFGA medium. We suspect that the weak glucose reaction was a consequence of the fastidious nature of the organism. Also, in contrast to the results of previous studies of

TABLE 1. Serological reactions of spiroplasma strains that exhibited positive cross-reactions in deformation and metabolism inhibition tests performed with strain LD-1^T and its specific antiserum^a

Group	Strain	Homologous reaction		Heterologous reaction			
		Deformation test titer ^b	Metabolism inhibition test titer ^c	Deformation test titer		Metabolism inhibition test titer	
				AG direction ^d	AS direction ^e	AG direction ^d	AS direction ^e
I-1	<i>Spiroplasma citri</i> R8A2 ^T	2,560	>117,000	<20	<20	<18	162
I-2	<i>Spiroplasma melliferum</i> BC-3 ^T	10,240	>117,000	320	<20	<18	18
I-4	277F	2,560	39,000	<20	<20	<18	18
I-5	LB-12	5,120	>117,000	<20	<20	<18	18
I-6	<i>Spiroplasma insolitum</i> M55 ^T	640	13,000	<20	<20	<18	54
I-7	N525	1,280	39,000	<20	<20	<18	18
I-8	<i>Spiroplasma phoeniceum</i> P40 ^T	5,120	>117,000	<20	40	<18	18
II	DW-1	2,560	>117,000	<20	<20	54	18
IV	<i>Spiroplasma apis</i> B31 ^T	1,280	13,000	<20	<20	<18	18
V	<i>Spiroplasma mirum</i> SMCA ^T	2,560	13,000	<20	<20	<18	18
VI	<i>Spiroplasma ixodetis</i> Y32 ^T	10,240	39,000	<20	<20	<18	18
VII	<i>Spiroplasma monobiae</i> MQ-1 ^T	5,120	>117,000	<20	<20	<18	18
VIII-1	EA-1	1,280	39,000	<20	<20	<18	54
VIII-2	DF-1	5,120	39,000	<20	<20	<18	54
VIII-3	TAAS-1	2,560	39,000	<20	20	<18	162
IX	<i>Spiroplasma clarkii</i> CN-5 ^T	320	4,374	<20	<20	<18	54
XI	<i>Spiroplasma vellocrescens</i> MQ-4 ^T	640	4,374	40	<20	<18	<18
XII	DU-1	1,280	13,000	<20	<20	<18	18
XIV	EC-1	1,280	13,000	<20	40	<18	<18
XV	I-25	2,560	>117,000	<20	40	<18	486
XVI-1	<i>Spiroplasma cantharicola</i> CC-1 ^T	1,280	39,000	<20	<20	<18	162
XVI-2	CB-1	1,280	39,000	80	<20	<18	486
XVI-3	Ar-1357	1,280	>117,000	320	<20	<18	18
XVIII	TN-1	1,280	>117,000	<20	160	<18	18
XIX	PUP-1	5,120	13,000	40	<20	54	<18
XXI	W115	2,560	4,374	<20	<20	<18	486
XXII	<i>Spiroplasma taiwanense</i> CT-1 ^T	640	13,000	<20	<20	<18	18
XXIII	TG-1	2,560	39,000	<20	<20	<18	486
XXIV	<i>Spiroplasma chinense</i> CCH ^T	2,560	1,458	<20	20	<18	486
XXV	<i>Spiroplasma diminutum</i> CUAS-1 ^T	5,120	13,000	<20	<20	<18	54
XXVI	PLHS-1	2,560	117,000	<20	40	162	18
XXVII	TALS-2	640	1,458	<20	<20	<18	18
XXVIII	PALS-1	1,280	4,374	20	<20	1,458	162
XXX	BIUS-1	2,560	13,000	<20	<20	<18	54
XXXI	HYOS-1	1,280	13,000	<20	<20	<18	486
XXXII	TABS-2	5,120	39,000	<20	<20	<18	54
XXXIII	TAUS-1	1,280	4,374	<20	<20	<18	162

^a Strain CUAS-1^T was tested reciprocally against sera and antigens of the 23 existing groups, including all 11 designated subgroups, and eight strains representing ungrouped spiroplasma clusters.

^b Reciprocal of the highest antiserum dilution that deformed one-half of the spiroplasmas in either homologous or heterologous tests. The homologous deformation test titer of strain LD-1^T was 20,480.

^c Reciprocal of the highest antiserum dilution that resulted in metabolism inhibition in either homologous or heterologous tests. The homologous metabolism inhibition titer of strain LD-1^T was 117,000.

^d Test in which we used the spiroplasma indicated as the antigen and antiserum to strain LD-1^T.

^e Test in which we used strain LD-1^T as the antigen and antiserum to the strain indicated.

arginine and glucose metabolism in serum fraction media, in which the media were first acidified and then turned basic, DCCM medium inoculated with strain LD-1^T turned basic and then acidic. This was probably due to characteristics of the medium rather than due to the spiroplasma, for *Spiroplasma clarkii* CN-5, which was used as a positive control for arginine and glucose metabolism, exhibited similar color-changing characteristics in DCCM medium.

Serological reactions. Strain LD-1^T exhibited no consistent serological reactivity with members of other existing or putative spiroplasma groups or subgroups. In metabolism inhibition tests, the organism was inhibited at low titers by many antisera (Table 1), which presumably reflected its fastidious growth habits. Because reactivity of sera against LD-1^T antigen in the metabolism inhibition test occurred with members of many groups, some of which have been shown to be genotyp-

ically unrelated by DNA-DNA homology or G+C content data (35), it is likely to be nonspecific. In support of this conclusion, antisera prepared against strain LD-1^T gave a positive metabolism inhibition test reaction with only one of the organisms (group XXVII strain PALS-1; reaction titer [T], 1,458 that cross-reacted in the other direction. Since the homologous LD-1^T spiroplasma metabolism inhibition titer was high (117,000), this reaction was not significant. Minor cross-reactions were also observed with anti-LD-1^T serum against heterologous antigens from strains DW-1 (group II) and PUP-1 (group XIX) (T, 54) and ungrouped strain PLHS-1 (T, 162).

Data from deformation tests confirmed that strain LD-1^T was serologically unrelated to members of other groups and subgroups. In this test, the homologous titer was 20,480, and there was little variation (T range, 5,120 to 40,960) among the other CPBS isolates tested. The LD-1^T antigen reacted at low

levels with sera to strains P40 (group I-8; T, 40), TAAS-1 (group VIII-3; T, 20), EC-1 (group XIV; T, 40), I25 (group XV; T, 40), TN-1 (group XVIII; T, 160), CCH (group XXIV; T, 20), and PLHS-1 (ungrouped; T, 40). When serum directed against strain LD-1^T was tested against heterologous antigens, cross-reactions were observed only with strains BC-3 (group I-2; T, 320), MQ-4 (group XI; T, 40), PUP-1 (group XIX; T, 40), and PALS-1 (ungrouped; T, 40). Thus, in deformation tests, as in metabolism inhibition tests, there was essentially no reciprocal cross-reactivity. The almost complete lack of reciprocity in any serological cross-reaction clearly indicates that strain LD-1^T and related isolates are serologically unique among the spiroplasmas.

For differentiation of intragroup CPBS variation, the spiroplasma motility inhibition test (12) was developed. Strain LD-1^T homologous antisera reacted strongly with several CPBS isolates, including North American isolates Can-1 (T, 166,000), Ves-1 (T, 71,000), Ari-1 (T, 69,000), NC-1 (T, 56,000), LD-1^T (T, 55,000), Mex-1 (T, 1,400), Tex-2 (T, 1,300), and Tex-3 (T, 1,000) and Polish isolates JL23 (T, 137,000), JL32 (T, 115,000), JL26 (T, 101,000), JL3 (T, 1,800), JL5 (T, 1,300), JL17 (T, 1,100), JL22 (T, 1,000), JL9 (T, 800), and JL2 (T, 700) (12). Serovars showed evidence of biogeographical influence (10, 12).

Sterol requirement. Strain LD-1^T grew well in media containing 20 to 25 mg of cholesterol per liter, weakly in the presence of 15 mg of cholesterol per liter, and not at all in the presence of 11 to 12 mg of cholesterol per liter.

Genomic properties. The G+C base composition of the DNA of strain LD-1^T was determined to be 25 ± 1 mol% (29), and the genome size was determined to be 1,085 kb (2).

Habitat. The CPBS appears to be host specific for the genus *Leptinotarsa*. Insect predators have been found to harbor CPBS in their guts (9), but it is not known whether the spiroplasma can multiply or persist in these insects.

Evidence suggests that the CPBS is adapted to inhabit only the gut lumen, where it is probably a commensal (5, 6). Clark (6) and Hackett et al. (15a) were unable to produce disease (mortality or loss of egg production) by feeding the CPBS to potato beetles. This spiroplasma does not become established after intrahemocoelic inoculation into potato beetle larvae or adults (5). Whether the CPBS plays a beneficial role in the nutrition of the beetle or a pathogenic role against its natural enemies has not been studied. Efforts are under way to genetically modify this organism to express an insect-lethal toxin for beetle control (15).

The fact that the CPBS has been isolated from both larvae and adults (9), including adults exiting from overwintering sites in May (5, 9), suggests that passage of this organism is vertical, which has been verified in laboratory assays (5, 9). Presumably, the organism is transmitted to feeding beetles after regurgitation and defecation of viable forms onto leaves (9), although isolations from leaves have not been attempted. The relatively high (among spiroplasmas) temperature range for growth of strain LD-1^T (20 to 41°C [19]) may reflect adaptation to mid-summer exposure on leaf surfaces.

The incidence of infection in larvae and adults in the field varied from 0 to 100% in North America (5, 9, 10) and from 0 to 40% in Europe (22). Factors such as host species susceptibility (*L. texana* and *L. decemlineata* may differ in susceptibility), CPBS biovar, and especially Colorado potato beetle population density and seasonality are probably responsible for the variations in incidence of infection observed.

The narrow host range may have arisen because the beetle adults and larvae feed on leaves of the same hosts (9), providing an opportunity for completion of the spiroplasma life cycle

within the potato beetle, with adults serving as the overwintering reservoir (9). The CPBS is probably a commensal in the beetle gut, where it adheres to midgut microvilli (11). The spiroplasma's high level of translational motility (9) may be an adaptation that facilitates attachment to microvilli and maintenance of its position in the midgut against the flow of food. The adaptive value of the spiroplasma's spiral shape (compared with a helical shape) and the formation of two-dimensional coils when the organism is present at high titers in the beetle gut (9) are unknown. Perhaps the coil forms are more stable when the organisms are deposited onto leaves; when the coils are exposed to fresh media, they regain their three-dimensional shape and their translational motility. The two-dimensional coils may therefore be considered a resting stage of the organism. Overall, the unusual morphological properties of the CPBS are remarkable evidence of the ability of cells to adapt to specific niches.

The properties of strain LD-1^T described previously or reported in this paper fulfill proposed criteria (17) for descriptions of species of the class *Mollicutes*. Properties mandating assignment to this class include the absence of a cell wall, filterability, the lack of reversion to walled bacteria when the organism is grown in antibiotic-free media, and penicillin resistance. The sterol requirement of strain LD-1^T, its inability to utilize urea, and its spiral shape and motility place this organism in the family *Spiroplasmataceae* (27). The results of serological comparisons of strain LD-1^T with representatives of other *Spiroplasma* species and groups demonstrate that strain LD-1^T is a distinct spiroplasma species. Other isolates are serologically similar to strain LD-1^T as determined by the spiroplasma deformation test and are similar in other characteristics, including host specificity for potato beetles, spiral shape, rapid translational motility, and fastidious growth requirements. Since strain LD-1^T and related isolates appear to be host specific for the beetle genus *Leptinotarsa*, we propose the name *Spiroplasma leptinotarsae* for this bacterium.

The taxonomic description below summarizes the properties of the organism.

***Spiroplasma leptinotarsae* sp. nov.** *Spiroplasma leptinotarsae* (lep. ti. no. tar' sac. M.L. gen. no. *leptinotarsae*, from *L. decemlineata*, the Colorado potato beetle [Coleoptera: Chrysomelidae]).

Cells are filamentous with helical, spiral, or compressed coils and motile, pass through 220-nm-pore-size filters with a 1,000-fold decrease in titer, and do not pass through 100-nm-pore-size filters. Cells lack true walls. Colonies on solid media containing 2.0% Noble agar are slightly diffuse to discrete and produce numerous satellite colonies.

Chemoorganotrophic. Acid is produced weakly from glucose. Hydrolyzes arginine. Does not utilize urea.

The sterol requirement is fulfilled by 15 to 20 mg of cholesterol per liter in a DCCM-based medium containing serum.

In M1D medium optimal growth occurs at 30 to 37°C (doubling time, 7.2 h), and multiplication occurs at 20 to 41°C but not at 15 or 43°C. In DCCM medium, optimal growth occurs at 30°C (doubling time, 7.2 h), and multiplication occurs at 20 to 37°C.

Serologically distinct from other established and putative *Spiroplasma* species, groups, and subgroups.

Strain LD-1^T was isolated from the gut of a Colorado potato beetle (*L. decemlineata*) larva. Related isolates have been obtained from the guts of *L. decemlineata* and *L. texana* adults and larvae. Believed to be a commensal.

The G+C content of the DNA is 25 ± 1 mol%. The genome size is 1,085 kb.

The type strain is strain LD-1 (= ATCC 43213).

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