Spiroplasma litorale sp. nov., from Tabanid Flies (Tabanidae: Diptera) in the Southeastern United States

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Spiroplasma strain TN-1^T (T = type strain), a strain serologically distinct from other *spiroplasma* species, groups, and subgroups, was isolated from the gut of a horsefly (*Tabanus nigrovittatus*) from a barrier island off the coast of North Carolina. Related strains were isolated from other *Tabanus* spp., *T. atratus*, *T. americanus*, *T. gladiator*, *T. lineola*, *T. sulcifrons*, and *T. zythicolor*, from coastal Georgia. Cells of strain TN-1^T in culture were helical and motile with an average of 5 to 10 helical turns per cell. Electron microscopic studies determined that the cells of strain TN-1^T were surrounded by a single cytoplasmic membrane, and there was no evidence of a cell wall. The *spiroplasma* grew well in M1D and SP-4 liquid media. Serum fraction (1%) medium and conventional horse serum medium also supported growth of strain TN-1^T. Fried-egg colonies were not produced; instead, the strain produced small diffuse colonies that were surrounded by satellite growth. The optimum temperature for growth was 32°C, but multiplication was observed at temperatures from 10 to 41°C. The doubling time at the optimum temperature (32°C) was 1.6 h. No growth was observed at 5 or 43°C. *Spiroplasma* strain TN-1^T passed through 220-nm filter pores but failed to pass through 100-nm filter pores. Strain TN-1^T catabolized glucose but hydrolyzed neither arginine nor urea. The guanine-plus-cytosine content of the DNA was about 25 ± 1 mol%, and the genome size was 1,370 kbp. Based on results from this study and previously published data, strain TN-1^T (= ATCC 43211) (group XVIII) is designated the type strain of a new *spiroplasma* species, *Spiroplasma litorale*.

In 1983, one of us (R.F.W.), while visiting North Carolina's Ocracoke Island, observed that the booth of a campground attendant was besieged, as was the entire island, with innumerable individuals of a green-eyed horsefly, *Tabanus nigrovittatus* (4). The attendant graciously permitted us to collect a sample of flies, from which *spiroplasma* strain TN-1^T (T = type strain) was derived. Isolates which have similar serological profiles or which exhibit partial serological cross-reactivity with strain TN-1^T were subsequently isolated from other *Tabanus* spp., *T. atratus*, *T. americanus*, *T. gladiator*, *T. lineola*, *T. sulcifrons*, and *T. zythicolor* collected from a barrier island off the coast of Georgia or from Bulloch County in Georgia's coastal plain (6, 7, 22, 23). Strain TN-1^T has been designated the representative strain of *spiroplasma* group XVIII (19). In this paper we describe the morphological, physiological, and biochemical properties of strain TN-1^T (= ATCC 43211^T) and designate this strain the representative of a new *spiroplasma* species, the group XVIII species *Spiroplasma litorale*.

MATERIALS AND METHODS

Spiroplasma strains. Strain TN-1^T was isolated by standard techniques (3, 4, 12) by one of us (T.B.C.) in M1D medium from the gut of a female green-eyed horsefly (*T. nigrovittatus*) collected at Ocracoke, N.C. Serologically related isolates were also obtained from the guts of six other tabanid fly species, *T. americanus*, *T. atratus*, *T. gladiator*, *T. lineola*, *T. sulcifrons*, and *T. zythicolor* (6, 7, 23). Some of the genomic and serological features of the organism have been re-

ported previously (2, 19). Representative strains of all previously recognized groups (16, 17, 19, 25, 26) and subgroups, including the type strains of all previously recognized species, were also employed.

Culture medium and cultivation techniques. *Spiroplasma* strain TN-1^T was grown in M1D liquid broth (20) at 30°C. The culture was filtered and cloned (14) in M1D broth containing 500 U of penicillin per ml. A triply cloned strain was designated TN-1^T and was subsequently used in characterization studies. Other culture media used in the study were SP-4, conventional 20% horse serum medium, and a 1% bovine serum fraction broth (20). Solid medium was prepared by adding 2.25% Noble agar (Difco Laboratories, Detroit, Mich.). Agar cultures were incubated at 30°C either aerobically or anaerobically in a hydrogen GasPak system (BBL Microbiology Systems, Cockeysville, Md.).

Temperature studies. The temperature requirements of cultures of strain TN-1^T in the late logarithmic phase in M1D broth were determined as previously described (10, 11). Growth was measured at temperatures of 5, 10, 15, 20, 25, 30, 32, 37, 41, and 43°C.

Filtration. Filtration characteristics were determined in M1D broth by passing logarithmic-phase cultures through filters with 450-, 300-, 220-, and 100-nm pores. Each filtrate, as well as an unfiltered control, was subcultured in a series of 12 tubes by using 10-fold dilutions and was monitored for growth (14).

Morphological studies. Cells of strain TN-1^T in M1D broth cultures in the logarithmic phase were examined by dark-field microscopy by using a magnification of ×1,250. Electron microscopic examination of the membrane structure of strain TN-1^T by standard methods (27) has been described previously (19).

Sterol requirements. Strain TN-1^T was tested for a sterol requirement by two methods; the growth obtained in a serum-free broth formulation containing various concentrations of cholesterol was assessed, and the ability of the strain to show sustained growth in serum-free or serum-containing broth media (13, 15) was determined.

Biochemical and physiological properties. The ability of strain TN-1^T to utilize glucose, arginine, and urea was assessed by previously described methods (1, 30).

Serological tests. Antiserum to strain TN-1^T was raised in rabbits as previously described (29). Hyperimmune antisera to all previously established groups, putative groups, and subgroups (16, 17, 19, 25, 26) of *Spiroplasma* species were taken from reference collections at the Beltsville Agricultural Research Center and the National Institute of Allergy and Infectious Diseases laboratory in Frederick, Md. These antisera and *spiroplasma* TN-1^T were tested reciprocally in both metabolism inhibition (28) and *spiroplasma* deformation tests (28, 29).

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FIG. 1. Colonies of strain TN-1^T on SP-4 medium supplemented with 2.25% Noble agar after 3 days of aerobic incubation at 30°C. Bar = 50 μ m.

Genomic analysis. The guanine-plus-cytosine content of purified DNA of strain TN-1^T and the genome size of the organism have been reported previously (2, 17, 19, 25).

RESULTS AND DISCUSSION

Cultural and morphological properties. Spiroplasma strain TN-1^T grew well in M1D and SP-4 broth media containing 500 U of penicillin per ml. The strain also grew in conventional mycoplasma media containing horse serum or bovine serum fraction. Growth occurred over a temperature range of 10 to 41°C; optimum growth was observed at 32°C. The doubling time at the optimum temperature was 1.6 h. Although the organism did not replicate at 5°C, it was able to retain its helical morphology and remained viable at this temperature. Clark and colleagues (4) reported an optimum temperature of 35°C for strain TN-1^T and noted that this organism grew at 37°C, but a complete range of temperatures was not tested.

Colonies of spiroplasma strain TN-1^T on SP-4 medium supplemented with 2.25% Noble agar grown under aerobic conditions were granular with dense centers, uneven margins, and multiple satellites (Fig. 1). Logarithmic-phase cultures of strain TN-1^T in M1D and SP-4 media examined by dark-field microscopy contained numerous helical motile filaments. Electron microscopy revealed filamentous cells with no evidence of a cell wall (19). Representative cells were about 200 to 300 nm in diameter and were surrounded by a single cytoplasmic membrane.

Passage of broth cultures of strain TN-1^T through 450- and 300-nm-pore-size membrane filters did not reduce the viable cell titer of a nonfiltered control broth (10^9 color-changing units/ml). The titer of a filtrate obtained after passage through a 220-nm-pore-size membrane filter was reduced about 100-fold (to 10^7 color-changing units/ml); a 100-nm-pore-size membrane filtrate was free of viable cells.

Sterol requirements and physiological properties. The response of strain TN-1^T to cholesterol supplementation of serum-free SP-4 medium is shown in Table 1. No growth was apparent in control base broth devoid of any supplement, but growth was enhanced when 2 to 20 μ g of cholesterol per ml was included in the serum-free broth. In experiments to test

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

Medium	Cholesterol concn (μ g/ml)	Amt of protein (mg/100 ml)
M1D	0 (Control)	3.06 ^a
Serum-free base medium with 1% serum fraction	0 (Control)	2.28
Serum-free base medium alone	0	0.06
Serum-free base medium with cholesterol	1	0.12
	2	0.57
	5	0.82
	10	0.76
	20	1.21

^a Amount of total cell protein obtained from a cell pellet cultivated from 100 ml of each serum control medium or serum-free base medium supplemented with cholesterol.

the ability of strain TN-1^T to sustain growth through 23 serial dilutions in serum-free broth, in serum-free broth supplemented with 0.04% Tween 80, or in serum-containing media, the organism showed consistent and sustained growth only in medium containing serum. Growth was apparent only in a single 10-fold dilution in each of the two serum-free formulations.

Spiroplasma strain TN-1^T produced acid from glucose, but no evidence of arginine or urea hydrolysis was observed.

Serological tests. Metabolism inhibition and deformation tests performed with antisera prepared against known and putative *Spiroplasma* groups and subgroups indicated that strain TN-1^T was not related serologically to representatives of previously established groups or species or to any of the eight representatives of putative new groups tested. No reciprocal crosses were observed in either test. One-way crosses were observed with 11 groups or putative groups when antiserum against strain TN-1^T was reacted with heterologous antigen (Table 2). When strain TN-1^T antigen was tested against heterologous sera, a low-level one-way cross-reaction was observed with strain PALS-1 (an ungrouped representative of a putative new group) in deformation tests. No other cross-reactions were observed.

Genome size and DNA base composition. The genome size of strain TN-1^T was found to be 1,370 kbp by pulsed-field gel electrophoresis (2). The base composition (guanine-plus-cytosine content) of the DNA of strain TN-1^T was 25 ± 1 mol% (19).

Habitat. The genus *Spiroplasma* offers a rich opportunity to study the ecology of microorganisms associated with insects (3, 8). Strain TN-1^T described here was isolated from the gut of the horsefly *T. nigrovittatus* from a barrier island, Ocracoke Island, N.C. Strain TN-1^T has not been studied as an insect pathogen. Hundreds of spiroplasma cultures have been obtained from tabanids collected on the coastal plains from Florida to Nova Scotia and in the Rocky Mountains from Montana to New Mexico. Other sites of isolation include Vermont and Texas. The most extensive survey was performed from 1987 to 1994 in Bulloch County, Ga., some 80 km from the Atlantic Ocean. Cultures closely related serologically to strain TN-1^T were isolated from six other *Tabanus* species (4–7, 22, 23). Four isolates were obtained from *T. americanus* from Bulloch County, Ga., and Johnston County, N.C.; eight cultures were obtained from *T. gladiator* from Bulloch County, Ga.; nine isolates were obtained from *T. lineola* from Bulloch and Camden Counties, Ga.; one isolate was obtained from *T. sulcifrons* from Bulloch County, Ga.; and one isolate was obtained from *T. zythicolor* from Camden County, Ga. A closely related

TABLE 2. Serological reactions and cross-reactions of strain TN-1^T in deformation and metabolism inhibition serological tests^a

Group	Strain	Titer in:			
		Spiroplasma deformation test		Metabolism inhibition test	
		Antiserum	Antigen	Antiserum	Antigen
I-8	P40	0 ^b	0 ^b	162 ^c	0 ^c
II	DW-1	40	0	18	0
IV	B31	0	0	18	0
VII	MQ-1	0	0	54	0
VIII-1	EA-1	0	0	486	0
IX	CN-5	40	0	0	0
XVIII	TN-1 ^T	2,560 ^d	2,560	>117,000 ^d	>117,000
XIX	PUP-1	80	0	0	0
XX	LD-1	160	0	18	0
XXI	W115	40	0	18	0
XXX	BIUS-1	0	0	18	0
XXVI	PLHS-1	20	0	162	0
XXVIII	PALS-1	0	40 (1,280) ^e	486	0
All others	All others	<20	<20	<18	<18

^a Strain TN-1^T antigen and antisera were tested in all heterologous combinations against representatives of all known and putative groups and subgroups. All cross-reactions were negative except those shown. All cross-reactions observed were in one direction only, demonstrating the serological uniqueness of strain TN-1^T.

^b Reciprocal of the endpoint in a deformation test in which the antigen was tested against the homologous antiserum and vice versa.

^c Reciprocal of the endpoint in a metabolism inhibition test in which the antigen was tested against the homologous antiserum and vice versa.

^d Homologous titers of strain TN-1^T in the test systems. The reactions, obtained once, are listed in each column for completeness.

^e The value in parentheses is the homologous titer for antisera against which strain TN-1^T cross-reacted when it was used as the antigen in a heterologous test.

strain, TAAS-2, was isolated from *Tabanus atratus* from Cumberland Island, Camden County, Ga. A large number of isolates were obtained from *T. lineola* collected in Florida, Georgia, North Carolina, and Maryland. Overall, the spiroplasma infection rate of these flies was about 50% (7). However, a low prevalence of isolates related to TN-1^T was indicated by the fact that only 3.9% (10 of 256) of the isolates obtained from *T. lineola* reacted to TN-1^T antiserum. Although other spiroplasmas were isolated from *T. lineola* collected during May and June, isolates related to strain TN-1^T were obtained from flies collected from July to November, thus indicating possible seasonality in carriage by tabanids. Many tabanids belonging to the genera *Chrysops* and *Hybomitra* were collected with the *Tabanus* hosts of TN-1^T, but no strains related to TN-1^T have been detected in cultures derived from members of these horsefly genera.

Since mixed infections of different spiroplasmas are common (21), strain TN-1^T may be frequently overgrown by members of other *Spiroplasma* groups in vivo or in vitro. The occurrence of spiroplasmas in narrow habitats in insects (tabanid fly viscera) has suggested that these microorganisms could be studied in biogeographic terms (24). Clark et al. (4) were unable to isolate any group XVIII spiroplasmas from *T. nigrovittatus* collected in Maryland. Group XVIII spiroplasmas have been found on two barrier islands (Ocracoke Island, N.C., and Cumberland Island, Ga.) and 130 km inland from the Atlantic coast (Johnston County, N.C.). The restriction of this spiroplasma to the coastal areas of the southeastern United States makes it one of the best-known examples of a geographically limited spiroplasma.

The properties described here for strain TN-1^T fulfill the proposed criteria of taxonomic nomenclature (9) for species of the class *Mollicutes*, including the absence of a cell wall, filterability, and penicillin resistance. The inability of this strain to utilize urea, its temperature requirements, and its helicity and motility place the organism in the family *Spiroplasmataceae* and the genus *Spiroplasma* (18). Finally, a serological comparison of strain TN-1^T with other *Spiroplasma* species and with other unclassified spiroplasma strains that represent putative

new groups demonstrated the uniqueness of the new insect strain. We therefore propose the name *Spiroplasma litorale* for this organism.

The taxonomic description below summarizes the properties of the organism.

Description of *Spiroplasma litorale* sp. nov. *Spiroplasma litorale* (li.to.ra'le. L. neut. adj. *litorale*, of the shore or coastal area).

Cells are filamentous, helical, and motile, vary from 200 to 300 nm in diameter, and lack true cell walls. Colonies on solid medium containing 2.25% Noble agar are granular with dense centers, uneven margins, and multiple satellites and never have a fried-egg appearance.

Chemorganotroph. Acid is produced from glucose. Hydrolyzes neither arginine nor urea.

Cholesterol or serum is required for growth.

The temperature range for growth is 10 to 41°C, with optimum growth occurring at 32°C. The doubling time in M1D medium at the optimum temperature is 1.6 h.

Serologically distinct from previously established *Spiroplasma* species. Isolated from the gut of *T. nigrovittatus* (Diptera: Tabanidae). Pathogenicity for insects has not been determined.

The guanine-plus-cytosine content of the DNA is 25 ± 1 mol%, as determined by the buoyant density method. The genome size is 1,370 kbp.

The type strain is TN-1 (= ATCC 43211).

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REFERENCES

1. 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.
2. Carle, P., F. Laigret, J. G. Tully, and J. M. Bové. 1995. Heterogeneity of genome sizes within the genus *Spiroplasma*. *Int. J. Syst. Bacteriol.* 45:178-181.

3. Clark, T. B. 1982. Spiroplasmas: diversity of arthropod reservoirs and host-parasite relationships. *Science* **212**:57-59.
4. Clark, T. B., R. V. Peterson, R. F. Whitcomb, R. B. Henegar, K. J. Hackett, and J. G. Tully. 1984. Spiroplasmas in the Tabanidae. *Isr. J. Med. Sci.* **20**:1002-1005.
5. French, F. E., R. F. Whitcomb, D. V. Hagan, J. A. Rafter, M. Konai, and E. A. Clark. 1992. Dynamics of spiroplasma infections in tabanid (Diptera: Tabanidae) flies. *IOM Lett.* **2**:114.
6. French, F. E., R. F. Whitcomb, J. G. Tully, K. J. Hackett, E. A. Clark, R. B. Henegar, and D. L. Rose. 1990. Tabanid spiroplasmas of the southeast USA: new groups and correlation with host life history strategy. *Zentralbl. Bakteriologie. Suppl.* **20**:919-921.
7. French, F. E., R. F. Whitcomb, D. L. Williamson, and R. B. Henegar. 1996. Spiroplasmas of *Tabanus lineola*. *IOM Lett.* **4**:211-212.
8. Hackett, K. J., and T. B. Clark. 1989. Ecology of spiroplasmas, p. 113-200. In R. F. Whitcomb and J. G. Tully (ed.), *The mycoplasmas*, vol. 5. Academic Press, San Diego, Calif.
9. International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Mollicutes*. 1995. Revised minimal standards for descriptions of new species of the class *Mollicutes*. *Int. J. Syst. Bacteriol.* **45**:605-612.
10. Konai, M., E. A. Clark, and R. F. Whitcomb. 1992. Temperature ranges, growth optima, and growth rates of *Spiroplasma* (*Spiroplasmataceae*: class *Mollicutes*) species. *Curr. Microbiol.* **32**:1-7.
11. Konai, M., R. F. Whitcomb, and M. Camp. 1994. Growth of spiroplasmas at the upper temperature range limit. *IOM Lett.* **3**:527-528.
12. Markham, P. G., T. B. Clark, and R. F. Whitcomb. 1983. Culture techniques for spiroplasmas from arthropods. *Methods Mycoplasma*. **2**:217-223.
13. Rose, D. L., J. G. Tully, J. M. Bové, and R. F. Whitcomb. 1993. A test for measuring growth responses of mollicutes to serum and polyoxyethylene sorbitan. *Int. J. Syst. Bacteriol.* **43**:527-532.
14. Tully, J. G. 1983. Cloning and filtration techniques for mycoplasmas. *Methods Mycoplasma*. **1**:173-177.
15. Tully, J. G. 1995. Determination of cholesterol and polyoxyethylene growth requirements of mollicutes, p. 381-389. In S. Razin and J. G. Tully (ed.), *Molecular and diagnostic procedures in mycoplasma*, vol. 1. Academic Press, San Diego, Calif.
16. Tully, J. G., and S. Razin (ed.). 1996. Appendix, p. 460-462. In S. Razin and J. G. Tully (ed.), *Molecular and diagnostic procedures in mycoplasma*, vol. 2. Academic Press, San Diego, Calif.
17. Tully, J. G., and R. F. Whitcomb. 1991. The genus *Spiroplasma*, p. 1960-1980. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), *The prokaryotes*, 2nd ed., vol. 2. Springer-Verlag, New York, N.Y.
18. Tully, J. G., J. M. Bové, F. Laigret, and R. F. Whitcomb. 1993. Revised taxonomy of the class *Mollicutes*: proposed elevation of a monophyletic cluster of arthropod-associated mollicutes to ordinal rank (*Entomoplasmatales* ord. nov.), with provision for familial rank to separate species with nonhelical morphology (*Entomoplasmataceae* fam. nov.) from helical species (*Spiroplasmataceae*), and emended descriptions of the order *Mycoplasmatales*, family *Mycoplasmataceae*. *Int. J. Syst. Bacteriol.* **43**:378-385.
19. Tully, J. G., D. L. Rose, E. Clark, P. Carle, J. M. Bové, R. B. Henegar, R. F. Whitcomb, D. E. Coffesh, and D. L. Williamson. 1987. Revised group classification of the genus *Spiroplasma* (class *Mollicutes*), with proposed new groups XII to XXIII. *Int. J. Syst. Bacteriol.* **37**:357-364.
20. Whitcomb, R. F. 1983. Culture media for spiroplasmas. *Methods Mycoplasma*. **1**:147-158.
21. Whitcomb, R. F., and K. J. Hackett. 1996. Identification of spiroplasmas from insects, p. 313-322. In J. G. Tully and S. Razin (ed.), *Molecular and diagnostic procedures in mycoplasma*, vol. 2. Academic Press, San Diego, Calif.
22. Whitcomb, R. F., F. E. French, and R. B. Henegar. Unpublished data.
23. Whitcomb, R. F., K. J. Hackett, J. G. Tully, E. A. Clark, F. E. French, R. B. Henegar, and D. L. Rose. 1990. Tabanid spiroplasmas of the southeastern USA: new groups, and correlation with host life history strategy. *Zentralbl. Bakteriologie. Suppl.* **20**:441-444.
24. Whitcomb, R. F., K. J. Hackett, J. G. Tully, E. A. Clark, F. E. French, R. B. Henegar, D. L. Rose, and A. G. Wagner. 1990. Tabanid spiroplasmas as a model for mollicute biogeography. *Zentralbl. Bakteriologie. Suppl.* **20**:931-933.
25. Whitcomb, R. F., D. L. Williamson, J. M. Bové, F. E. French, M. Konai, G. Gasparich, M. Abalain-Colloc, C. Saillard, C. Chastel, E. A. Clark, P. Carle, D. L. Rose, R. B. Henegar, and K. J. Hackett. 1992. Revised classification of spiroplasmas. *IOM Lett.* **2**:134.
26. Williamson, D. L., J. G. Tully, and R. F. Whitcomb. 1989. The genus *Spiroplasma*, p. 71-111. In R. F. Whitcomb and J. G. Tully (ed.), *The mycoplasmas*, vol. 5. Academic Press, San Diego, Calif.
27. Williamson, D. L. 1983. Specialized electron microscopic techniques for spiroplasmas in plant and insect tissues. *Methods Mycoplasma*. **1**:71-76.
28. Williamson, D. L., J. G. Tully, and R. F. Whitcomb. 1979. Serological relationships of spiroplasmas as shown by combined deformation and metabolism inhibition tests. *Int. J. Syst. Bacteriol.* **29**:345-351.
29. Williamson, D. L., R. F. Whitcomb, and J. G. Tully. 1978. The spiroplasma deformation test, a new serological method. *Curr. Microbiol.* **1**:203-207.
30. Williamson, D. L., J. G. Tully, L. Rosen, D. L. Rose, R. F. Whitcomb, M.-L. Abalain-Colloc, P. Carle, J. M. Bové, and J. Smyth. 1996. *Spiroplasma diminutum* sp. nov., from *Culex annulus* mosquitoes collected in Taiwan. *Int. J. Syst. Bacteriol.* **46**:229-233.