Acholeplasma florum, a New Species Isolated from Plants†

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Three acholeplasmas isolated from floral surfaces of healthy plants in Florida were found to be similar in their biochemical and serological properties. These organisms did not require serum or cholesterol for growth, although addition of some supplementary fatty acids (as represented by Tween 80) was necessary for growth to occur in serum-free medium. The three strains possessed biochemical properties typical of the *Acholeplasmataceae* and were distinguished from the nine previously recognized *Acholeplasma* species by serological and deoxyribonucleic acid-deoxyribonucleic acid hybridization techniques. The genome molecular weight of the three *Acholeplasma* strains was 10^9 , and the guanine-plus-cytosine content of the deoxyribonucleic acid was 27 to 28 mol%. On the basis of these results and other morphological, biological, and serological properties, we propose that these organisms represent a new species, *Acholeplasma florum*. Strain L1 (= ATCC 33453) is the type strain.

Plant surfaces, particularly flowers, have recently been proven to be fertile sites for isolation of members of the *Mycoplasmatales* (5, 11-13, 26). Members of the genus *Acholeplasma* have been recovered from flower surfaces in Florida and the central United States (26; McCoy, Basham, Tully, and Rose, Proc. 3rd Conf. Int. Org. Mycoplasma., Custer, S.D., 1980, abstr. no. 88), from decaying coconut palm (*Cocos nucifera* L.) tissue in Jamaica (8), and from commercial vegetables intended for human consumption (19). Most of these isolations yielded strains of *Acholeplasma laidlawii*, *Acholeplasma oculi*, and *Acholeplasma axanthum* (8, 19), species that have been previously identified as having animal origins (22). However, although three isolates from citrus and ornamental flowers in Florida were related to each other, they were not related to any previously described *Acholeplasma* species. In this paper we define the basic characteristics of these flower surface acholeplasmas and describe their unique biological properties. We propose that these organisms be given taxonomic status as a new species of *Acholeplasma*.

MATERIALS AND METHODS

***Acholeplasma* strains.** Details of the primary isolation technique for flower surface-inhabiting strains have been described previously (13). Strain PP2 was obtained from blossoms of *Calliandra haematocephalus*, whereas strains L1^T (T = type strain) and GF1 were obtained from flowers of lemon (*Citrus limon*) and grapefruit (*Citrus paradisi*), respectively. Primary isolation was in MC (14) or SP-4 (24) broth medium without antibiotics. Each *Acholeplasma* strain was purified by a 3× filtration-cloning technique (21) before characterization studies were performed. The reference *Acholeplasma* species and other members of the *Mycoplasmatales* used in this study for comparative tests were stock cultures maintained in the Mycoplasma Section, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Frederick, Md. For other serological and biological comparisons we used stock cultures maintained at the University of Florida Agricultural Research and Education Center, Fort Lauderdale, Fla.

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Media and cultivation procedures. Isolates were routinely grown in MC broth or in the serum fraction medium described previously (17). We developed a serum-free medium that contained mycoplasma broth base (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10% fresh 25% yeast extract (Microbiological Associates, Bethesda, Md.), 0.5% glucose, 500 U of penicillin G per ml, 0.002% phenol red, 0.5% bovine serum albumin, 0.04% Tween 80, and 10 µg of palmitic acid per ml. Solid medium was prepared by adding 0.8% Noble agar (Difco Laboratories, Detroit, Mich.) or 1.0% agarose to the broth base before autoclaving. Cultures were grown aerobically at 25 to 30°C.

Reversion studies. Primary isolation of strains L1^T, PP2, and GF1 was in antibiotic-free media, and more than 30 passages were made in the absence of penicillin. In addition, transfer of these strains to penicillin G-containing serum fraction medium followed by serial passage in serum-free broth with transfer to blood agar plates at each passage was done to screen for bacterial revertants.

Filtration studies. Eighteen-hour cultures of strains L1^T, PP2, and GF1 were passed through cellulose acetate membrane filters having pore diameters of 0.65, 0.45, 0.3, 0.22, and 0.1 µm, and the numbers of color-changing units in the filtrates were determined from 10-fold-serial dilutions in broth.

Morphology. Broth cultures of all three acholeplasmas were examined by phase-contrast microscopy and dark-field microscopy. Ammonium molybdate contrast preparations of whole organisms from cultures and ultrathin sections of pelleted organisms were examined by transmission electron microscopy as described previously (13).

Tests for biological and biochemical properties. Sterol requirements were determined by a broth culture method (16). A 2% inoculum was used, and the cells were harvested after 24 h. Susceptibility to digitonin was assayed by a plate method, using MC agar medium and a paper disk (diameter, 6 mm) soaked in 1.5% digitonin (10). The procedures used to determine carbohydrate fermentation and arginine and urea hydrolysis have been described previously (1). Production of β-D-glucosidase was tested by using the methods of Williams and Wittler (27) and Ernø and Stipkovits (9). Carotenoid production was tested by the method of Tully and Razin (23).

Serological tests. Antisera to strains L1^T, GF1, and PP2 were raised in rabbits as described previously (7). Hyperim-

mune antisera were raised from strains maintained at the Mycoplasma Section, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases (MS), and the other antisera used represented the collection of antisera in the National Institutes of Health Mycoplasma Reference Reagent Program (4) (NIH). We used antisera to *Acholeplasma axanthum* H86NT (MS), *Acholeplasma equifetale* N93 (MS), *Acholeplasma granularum* BTS 39^T (NIH), *Acholeplasma hippikon* Cl^T (MS), *Acholeplasma laidlawii* PG8^T (NIH), *Acholeplasma modicum* PG49^T (MS), *Acholeplasma morum* 72-043^T (MS), *Acholeplasma oculi* 19L^T (MS), *Acholeplasma parvum* H23M^T (MS), *Mycoplasma alvi* Ilsley^T (MS), *Mycoplasma anatis* 1340^T (NIH), *Mycoplasma bovigenitalium* PG11^T (NIH), *Mycoplasma bovirhinis* PG43^T (NIH), *Mycoplasma bovoculi* M165/69^T (MS), *Mycoplasma californicum* ST6^T (MS), *Mycoplasma canis* PG14^T (NIH), *Mycoplasma capricolum* California Kid^T (MS), *Mycoplasma caviae* G122^T (MS), *Mycoplasma citelli* RG2C^T (MS), *Mycoplasma columborale* MMP4^T (MS), *Mycoplasma conjunctivae* HRC581^T (MS), *Mycoplasma cynos* H831^T (MS), *Mycoplasma dispar* 462/2^T (MS), *Mycoplasma edwardii* PG24^T (MS), *Mycoplasma equigenitalium* T37^T (MS), *Mycoplasma fastidiosum* H822^T (MS), *Mycoplasma feliminutum* Ben^T (MS), *Mycoplasma felis* Cat27 (MS), *Mycoplasma fermentans* PG18^T (NIH), *Mycoplasma flocculare* Ms42^T (MS), *Mycoplasma gallinaceium* SA-J (MS), *Mycoplasma gallisepticum* PG31^T (NIH), *Mycoplasma gallopavonis* WR1^T (MS), *Mycoplasma genitalium* G37^T (MS), *Mycoplasma hyopneumoniae* J^T (MS), *Mycoplasma hyorhinis* BTS7^T (NIH), *Mycoplasma iowae* 695^T (MS), *Mycoplasma moatsii* MK405^T (MS), *Mycoplasma molare* H542^T (MS), *Mycoplasma mycoides* subsp. *capri* PG3^T (MS), *Mycoplasma mycoides* subsp. *mycoides* B3 (MS), *Mycoplasma neurolyticum* Type A^T (NIH), *Mycoplasma ovipneumoniae* Y98^T (MS), *Mycoplasma pneumoniae* FH^T (NIH), *Mycoplasma pullorum* CKK^T (MS), *Mycoplasma pulmonis* PG34^T (NIH), *Mycoplasma putrefaciens* KS1^T (MS), *Mycoplasma sualvi* Mayfield B^T (MS), *Mycoplasma verecundum* 107^T (MS), *Mycoplasma* sp. strain B5P (MS), *Mycoplasma* sp. strain California calf (MS), *Mycoplasma* sp. strain 70-159 (MS), *Mycoplasma* sp. strain M1 (MS), *Mycoplasma* sp. strain 831-C4 (MS), and *Mycoplasma* sp. strain Gough (MS). Disk growth inhibition tests (3) were performed on serum fraction or MC agar medium, and direct plate immunofluorescence antibody tests were performed by using fluorescein-conjugated antisera to the organisms listed above (6). A direct enzyme-linked immunosorbent assay was performed as previously described (7).

Polyacrylamide gel electrophoresis. One-dimensional slab polyacrylamide gel electrophoresis was performed as described by Mouches and Bové (14). Whole cell proteins were solubilized in 16% glycerol–3% sodium dodecyl sulfate–0.17% dithiothreitol, diluted in gel buffer, and electrophoresed on 10% acrylamide gels at 100 V (constant voltage; ca. 160 mA). The gels were stained with Coomassie brilliant blue or silver stains (BioRad Laboratories, Richmond, Calif.).

DNA characteristics. The guanine-plus-cytosine contents of the deoxyribonucleic acids (DNAs) of the three *Acholeplasma* strains were determined from the buoyant density profiles established by equilibrium density gradient centrifugation in CsCl₂ in Spinco model E and MSE Centriscan 10 analytical ultracentrifuges, using the method of Schildkraut et al. (18). The DNAs of *Spiroplasma citri* and *Escherichia coli* were used as reference standards. Genome sizes were determined from kinetic complexity by the method of Wetmur and Davidson (25).

FIG. 1. Electron micrograph of an ultrathin section of *A. florum* strain L1^T. Bar = 0.1 μ m.

RESULTS

Morphological and cultural properties. Broth cultures of strains PP2, L1^T, and GF1 grew rapidly, producing heavy turbidity in 18 h in MC broth, serum fraction broth, or serum-free Tween 80 broth. Light microscopy revealed round forms and heavy clumping of cells to form the suspended microcolonies responsible for the turbidity. Electron microscopy of ammonium molybdate-contrasted cells also revealed some clumping (13), and ultrathin sections showed a trilaminar unit membrane and no evidence of cell wall material outside the cytoplasmic membrane (Fig. 1). The organisms grew at 18 to 37°C, but not at 40°C. No growth occurred in serum-free media without the fatty acids provided by the Tween 80–palmitic acid–bovine serum albumin supplement. The colonies on agar media exhibited typical fried egg morphology and ranged in size from 200 to 300 μ m in diameter (Fig. 2).

Reversion studies. No reversion to bacterial forms was observed after 30 passages in antibiotic-free media, nor were bacterial forms observed after transfer to penicillin-G containing media and subsequent passage in serum-free medium.

Filtration studies. Cloned broth cultures of all three *Acholeplasma* strains contained 10⁷ to 10⁸ color-changing units per ml of medium (Table 1). The number of color-changing units decreased logarithmically with decreasing pore size after filtration through filters having pore sizes of 0.65, 0.45, 0.3, 0.22, and 0.1 μ m. No organisms were detected in the filtrate that passed through 0.1 μ m pores.

Biochemical and biological properties. All three strains fermented glucose but not mannose, did not hydrolyze esculin or arbutin, did not produce carotenoid pigments, did not hydrolyze arginine or urea, and did not liquefy coagulated serum or hemadsorb guinea pig erythrocytes. Although all three strains developed 2- to 4-mm radial inhibition zones to digitonin on 20% horse serum agar, no response to added cholesterol was detected when the broth culture method was used (Table 2).

FIG. 2. Colonies of *A. floridum* strain L1^T on 20% horse serum agar (48 h, 37°C).

Serological tests. Growth inhibition and plate immunofluorescence tests indicated that the three *Acholeplasma* strains which we studied are distinct from all other *Acholeplasma* species and the glucose-fermenting *Mycoplasma* species. Reciprocal growth inhibition tests and enzyme-linked immunosorbent assays performed on the three strains confirmed that they were related to each other but not to *A. laidlawii* (Table 3).

Polyacrylamide gel patterns. The one-dimensional polyacrylamide gel electrophoresis patterns of the three flower *Acholeplasma* strains were similar and were readily distinguished from those of *A. laidlawii* (Fig. 3). The silver stain produced more and sharper bands than the usual Coomassie brilliant blue gel stain.

DNA characteristics. The guanine-plus-cytosine contents of the DNAs of the three *acholeplasma* strains averaged 27.3 ± 1 mol%, as determined independently in two laboratories (Institut National de la Recherche Agronomique, Bordeaux, France, and John Innes Institute, Norwich, England). The genome molecular weight was $1.05 \times 10^9 \pm 0.3 \times 10^9$.

DISCUSSION

A unique feature of the three strains described here is the fact that they were isolated from the surfaces of subtropical flowers. This is the first documented evidence of the isola-

TABLE 2. Effect of cholesterol on the growth of strain L1^T in serum-free medium

Cholesterol concn (μg/ml)	Amt of cell protein (mg) ^a
0 ^b	<0.02
0 ^c	<0.02
0 ^d	2.45
1.0 ^e	2.55
5.0	2.25
10.0	2.75
20.0	2.55
Control ^f	4.30

^a Amount of protein in the cell pellet obtained from 100 ml of medium.

^b Serum-free base medium alone.

^c Serum-free base medium supplemented with 0.5% albumin and 10 μg of palmitic acid per ml.

^d Serum-free base medium supplemented with 0.5% albumin, 10 μg of palmitic acid per ml, and 0.04% Tween 80.

^e Serum-free base medium supplemented with 0.5% albumin, 10 μg of palmitic acid per ml, 0.04% Tween 80, and cholesterol.

^f Serum-free base medium supplemented with 1% bovine serum fraction.

tion of new, non-sterol-requiring mycoplasmas from sources other than animals. Subsequent isolations of related organisms have been made from flowers in temperate regions (Colorado, Nebraska, and Illinois) (26). The relationship of the temperate region isolates to the Florida strains was demonstrated by nucleic acid hybridization and fluorescent antibody serological tests. It is yet to be determined whether flower surfaces represent primary habitats for these organisms or whether the plant surfaces are just resting sites. We suspect that insects carry the flower mycoplasmas from site to site, and indeed several flower spiroplasmas have been demonstrated to be pathogenic to insects (2, 7, 15).

The morphology, the filterability, the guanine-plus-cytosine content of the DNA, and the absence of reversion to bacteria of these organisms indicate that they belong to the

FIG. 3. Polyacrylamide gel electrophoresis patterns of whole cell proteins of *A. floridum* strains L1^T, PP2, GF1 and *A. laidlawii* PG8.

TABLE 1. Passage of *A. floridum* strains L1^T, GF1, and PP2 through cellulose acetate filters of varying pore sizes

Filter pore size (μm)	No. of color-changing units/ml		
	L1 ^T	GF1	PP2
Unfiltered	10 ⁸	10 ⁷	10 ⁷
0.65	10 ⁶	10 ⁸	10 ⁷
0.45	10 ⁶	10 ⁶	10 ⁷
0.30	10 ⁴	10 ³	10 ⁶
0.22	<10	<10	10 ²
0.10	<10	<10	<10

TABLE 3. Comparison of *A. florum* L1^T, GF1, and PP2 and *A. laidlawii* PG8 by the growth inhibition test and the enzyme-linked immunosorbent assay

Anti-gen	Antiserum							
	PG8		L1 ^T		GF1		PP2	
	Zone of growth inhibition (mm)	% Of homologous reaction ^a	Zone of growth inhibition (mm)	% Of homologous reaction	Zone of growth inhibition (mm)	% Of homologous reaction	Zone of growth inhibition (mm)	% Of homologous reaction
PG8	3	100	0	3	0	7	0	9
L1 ^T	0	0	1.5	100	1.5	45	3.5	29
GF1	0	0	2	15	2	100	4	35
PP2	0	0	1	25	1	42	5	100

^a Percentage of homologous reaction in the enzyme-linked immunosorbent assay.

class *Mollicutes*. Their lack of a sterol requirement for growth, their ability to grow aerobically, and their genome molecular weight place them in the family *Acholeplasmataceae* and the genus *Acholeplasma*. The results of extensive serological tests indicate the lack of relationship of these strains to any of the nine previously established *Acholeplasma* species or to all known fermenting *Mycoplasma* species. Additional evidence that these isolates represent a new *Acholeplasma* species comes from the DNA-DNA hybridization data published previously (20). We propose that these organisms be named *Acholeplasma florum* (*flo*'rum, sp. nov. L.gen. pl.n. *florum*, of flowers, indicating the recovery site of the organism). The specific epithet refers to the site of isolation of the strains. Strain L1 is designated the type strain; a cloned line of this strain has been deposited in the American Type Culture Collection as strain ATCC 33453.

Acholeplasma florum sp. nov. Ultrathin sections reveal ovoid particles with a single trilaminar membrane and no cell wall.

Cells pass through filters having 0.45-μm pores, but most are retained on filters having 0.22-μm pores.

Colonies on agar are umbonate.

Cultures are resistant to penicillin, and no reversion to walled forms occurs in the absence of penicillin.

No growth response occurs when supplementary cholesterol is added, although digitonin is slightly inhibitory to growth on horse serum-containing agar.

Films and spots are produced on serum-containing media.

Glucose is utilized, but mannose, arginine, and urea are not.

Carotenes are not produced, nor is β-D-glucosidase.

The species is serologically distinct from other *Acholeplasma* species.

The known strains were isolated from flower surfaces; other hosts are unknown.

The guanine-plus-cytosine content of the DNA is 27.3 ± 1 mol%.

The genome molecular weight is 1.05 × 10⁹ ± 0.3 × 10⁹.

The type strain is strain L1 (= ATCC 33453). The type strain has all of the characteristics given above for the species.

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LITERATURE CITED

1. Aluotto, B. B., R. G. Wittler, C. O. Williams, and J. E. Faber. 1970. Standardized bacteriologic techniques for the characterization of *Mycoplasma* species. *Int. J. Syst. Bacteriol.* **20**:35-58.
2. Clark, T. B. 1977. *Spiroplasma* sp., a new pathogen in honey bees. *J. Invertebr. Pathol.* **29**:112-113.
3. Clyde, W. A., Jr. 1964. *Mycoplasma* species identification based upon growth inhibition by specific antisera. *J. Immunol.* **92**:958-963.
4. Cunningham, S. (ed.). 1978. NIAID catalog of research reagents. Department of Health, Education and Welfare publication (NIH) 78-899. National Institutes of Health, Bethesda, Md.
5. Davis, R. E. 1978. *Spiroplasmas* associated with flowers of the tulip tree. *Can. J. Microbiol.* **24**:954-959.
6. Del Giudice, R. A., N. F. Robillard, and T. R. Carski. 1967. Immunofluorescence identification of *Mycoplasma* on agar by use of incident illumination. *J. Bacteriol.* **93**:1205-1209.
7. Dowell, R. F., H. G. Basham, and R. E. McCoy. 1981. Influence of five *spiroplasma* strains on growth rate and survival of *Galleria mellonella* larvae. *J. Invertebr. Pathol.* **37**:231-235.
8. Eden-Green, S. J., and J. G. Tully. 1979. Isolation of *Acholeplasma* spp. from coconut palms affected by lethal yellowing disease in Jamaica. *Curr. Microbiol.* **2**:311-316.
9. Ernø, H., and L. Stipkovits. 1973. Bovine mycoplasmas: cultural and biochemical studies. II. *Acta Vet. Scand.* **14**:450-463.
10. Freundt, E. A., B. E. Andrews, H. Ernø, M. Kunze, and F. T. Black. 1973. The sensitivity of *Mycoplasma* to sodium polyanethanol-sulfonate and digitonin. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig.* **225**:104-112.
11. Junca, P., C. Saillard, J. Tully, O. Garcia-Jurado, J.-R. Degorce-Dumas, C. Mouches, J.-C. Vignault, R. Vogel, R. McCoy, R. Whitcomb, D. Williamson, J. Latrille, and J. M. Bové. 1980. Caractérisation de spiroplasmes isolés d'insectes et de fleurs de France continentale, de Corse et du Maroc. Proposition pour une classification des spiroplasmes. *C. R. Acad. Sci.* **290**:1209-1212.
12. McCoy, R. E., H. G. Basham, and R. E. Davis. 1982. Powder puff *spiroplasma*, a new epiphytic mycoplasma. *Microbial Ecol.* **8**:169-180.
13. McCoy, R. E., D. S. Williams, and D. L. Thomas. 1979. Isolation of mycoplasmas from flowers, p. 75-81. In H. J. Su and R. E. McCoy (ed.), *Proceedings of the Republic of China-United States Cooperative Science Seminar on Mycoplasma Diseases of Plants*. National Science Council Symposium Series, no. 1. National Science Council, Taipei, Taiwan.
14. Mouches, C., and J. M. Bové. 1983. Electrophoretic characterization of mycoplasma proteins, p. 241-255. In S. Razin and J. G. Tully (ed.), *Methods in mycoplasmaology*, vol. 1. Academic Press, Inc., New York.
15. 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. *Ann. Microbiol. (Paris)* **134A**:383-397.
16. Razin, S., and J. G. Tully. 1970. Cholesterol requirement of mycoplasmas. *J. Bacteriol.* **102**:306-310.
17. Rose, D. L., J. G. Tully, and R. A. Del Giudice. 1980. *Acholeplasma morum*, a new non-sterol-requiring species. *Int. J. Syst. Bacteriol.* **30**:647-654.

18. Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J. Mol. Biol.* **4**:430-433.
19. Somerson, N. L., J. P. Kocka, D. Rose, and R. A. Del Giudice. 1982. Isolation of acholeplasmas and a mycoplasma from vegetables. *Appl. Environ. Microbiol.* **43**:412-417.
20. Stephens, E. B., G. S. Aulakh, R. E. McCoy, D. L. Rose, J. G. Tully, and M. F. Barile. 1981. Lack of genetic relatedness among animal and plant acholeplasmas by nucleic acid hybridization. *Curr. Microbiol.* **5**:367-370.
21. 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.
22. Tully, J. G. 1979. Special feature of the acholeplasmas, p. 431-449. In M. F. Barile and S. Razin (ed.), *The mycoplasmas*, vol. 1. Cell biology. Academic Press, Inc., New York.
23. Tully, J. G., and S. Razin. 1968. Physiological and serological comparisons among strains of *Mycoplasma granularum* and *Mycoplasma laidlawii*. *J. Bacteriol.* **95**:1504-1512.
24. Tully, J. G., R. F. Whitcomb, H. F. Clark, and D. L. Williamson. 1977. Pathogenic mycoplasmas; cultivation and vertebrate pathogenicity of a new spiroplasma. *Science* **195**:892-894.
25. Wetmur, J. G., and M. Davidson. 1968. Kinetics of renaturation of DNA. *J. Mol. Biol.* **31**:349-370.
26. Whitcomb, R. F., J. G. Tully, D. L. Rose, E. B. Stephens, A. Smith, R. E. McCoy, and M. F. Barile. 1982. Wall-less prokaryotes from fall flowers in central United States and Maryland. *Curr. Microbiol.* **7**:285-290.
27. Williams, C. O., and R. G. Wittler. 1971. Hydrolysis of aesculin and phosphatase production by members of the order *Mycoplasmatales* which do not require sterol. *Int. J. Syst. Bacteriol.* **21**:73-77.