Isolation of the Corn Stunt Spiroplasma from Maize in Florida

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ABSTRACT

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An anaerobic atmosphere with 5% CO_2, when compared with an aerobic atmosphere, improved growth of the corn stunt spiroplasma in broth cultures. Upon primary isolation from corn, the maximum titer of spiroplasmas was greater in all media when the cultures were anaerobically incubated than when aerobically incubated. The frequency of successful primary isolations of spiroplasmas from field maize in south Florida was also improved by anaerobic incubation, especially when the C-3G medium was used. Spiroplasma strains representing field collections from 1978 through 1982 were readily obtained from plants showing corn stunt symptoms and inconsistently obtained from plants with maize bushy stunt symptoms. All strains were identified as the corn stunt spiroplasma on the bases of serology, electrophoresis patterns of cellular proteins in polyacrylamide gels, and pathogenicity to sweet corn.

Corn stunt disease is considered one of the most economically important diseases of maize in the United States, Mexico, and Central and South America (2,22). Several types of corn stunt were once thought to be caused by strains of what was regarded as the same "virus" on the bases of symptomatology and vector transmission (11,19,22). Several viruses, a mycoplasmalike organism, and a spiroplasma are presently believed to be responsible for symptoms once attributed to "strains" of corn stunt (22). The disease originally described by Kunkel (15) as corn stunt and later described by Maramorosch (18) as Rio Grande corn stunt is now designated corn stunt (CS) and is caused by the corn stunt spiroplasma (CSS) (5,10,26). Mesa Central corn stunt as described by Maramorosch (18) is now believed to be caused by the maize bushy stunt mycoplasmalike organism (1,21). The presence of spiroplasmas in plants with symptoms of Mesa Central corn stunt was reported (7); however, a double infection with CSS and an unknown agent now suspected to be the maize bushy stunt mycoplasmalike organism was postulated (22).

Gordon and Nault (12) found that maize chlorotic dwarf virus, and not the CSS, was the most prevalent corn-stunting pathogen in many parts of the United States during 1973–1975. When 168 samples from 15 states were examined, the CSS was found in only two samples (0.01%) from Texas. However, CSS was consistently found in maize with corn stunt in Mexico in 1972 (7) and Florida in 1979 and 1980 (2). In Florida, samples were taken during a maize disease epidemic in which 68.4 and 98.5% of the diseased field samples in 1979 and 1980, respectively, contained CSS. Maize chlorotic dwarf was not observed in Florida, but mixed infections of CSS and viruses, including the maize rayado fino, maize mosaic, and maize stripe viruses, were detected. Spiroplasmas were observed in plants with a variety of symptoms but were most frequently observed in plants with corn stunt symptoms (2).

Identification of CSS in field maize from Mexico and Florida has been based on dark-field microscope observations of spiroplasmas in expressed sap. Further characterization was not conducted to determine if the spiroplasmas were the same, except in one brief report (6). In that report, a spiroplasma strain isolated from diseased maize in Florida was described as differing from established CSS strain I-747 in serological and cultural properties; however, specific details were not given. More recently, isolation of spiroplasmas from vectors and diseased maize with reddened leaves was reported in California (14). The California spiroplasmas were described as differing from CSS in a number of properties, and their nonidentity with CSS was suggested. Spiroplasmas subsequently isolated from maize in California have been identified as strains of CSS (8).

In view of the suggestions that different pathogenic spiroplasmas might be found in maize, we endeavored to determine the identity of maize spiroplasmas in Florida. Our initial attempts to isolate spiroplasmas from diseased maize repeatedly failed, even when motile spiroplasmas could be observed in the inocula. These preliminary setbacks led us to also investigate culture conditions and media for spiroplasma isolation. The results of these experiments are reported in this paper.

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MATERIALS AND METHODS

Plant material. In fall 1978 through 1981, leaf samples of field-grown maize (*Zea mays* L.) with corn stunt symptoms were collected in Dade County, FL. They were used in attempts to culture CSS and in tests for transmission of CSS by laboratory-reared corn leafhopper, *Dalbulus maidis* (Delong & Wolcott). Presence of spiroplasmas in sap expressed from leaf samples of plants experimentally inoculated by *D. maidis* was confirmed by dark-field microscopy. In 1978, two isolates were obtained from field-grown plants and designated red-leaf and R-type isolates. From 1979 through 1981, only one isolate was kept for each year and designated as 1979 CS, 1980 CS, and 1981 CS. All isolates were routinely transmitted (using *D. maidis*) to a series of healthy corn plants. *D. maidis* was reared on *Z. mays* var. *saccharata* 'Guardian' with 12 hr of light per day at 25 ± 1 C. Guardian sweet corn was used for source and test plants. On 8 December 1982, 20 healthy and 20 diseased plants from one field were sampled to test the efficiency of procedures for isolation of CSS in culture. Diseased plants showed typical symptoms of corn stunt, except they had extensive leaf reddening. A single mature leaf was detached from each plant, placed in a plastic bag, and transported to the laboratory at ambient temperatures. Samples were kept at 4 C overnight before isolations were attempted the following day.

Culture media. The following media recommended for cultivation of spiroplasmas were used: C-3G (17), C-3GH (C-3G supplemented with 0.06 M HEPES [N-2-hydroxyethyl-piperazine-*N*-2-ethanesulfonic acid] buffer) (17), LD8A (4), SP4 (25), and M1A (13). A modified M1A medium, the M1AS medium, was prepared by increasing the concentration of Schneider's drosophila medium (Grand Island Biological Co., Grand Island, NY) to 66.6% (v/v).

In addition, a new medium (L20 medium) was prepared as follows: 2.0 g Lactalysate Peptone (Baltimore Biological Laboratories, Cockeysville, MD), 5.0 g sorbitol, 0.1 g sucrose, 0.1 g fructose, 1.0 ml hemin stock (0.1% bovine hemin chloride [Sigma Chemical Co., St. Louis, MO] in 0.05 N NaOH), 0.02 g

$MgSO_4 \cdot 7H_2O$, and 0.5 ml phenol red stock (0.1%) were added to 74.5 ml deionized H_2O and dissolved. The pH was adjusted to 7.3 and the solution was autoclaved at 120 C for 25 min. After autoclaving, the medium was cooled and the following ingredients were added: 1.0 ml salts stock (8% KCl, 2% KH_2PO_4, 10% NaCl, 3.5% Na_2HPO_4, and 10% Na_2SO_3; pH 6.8), 1.0 ml bicarbonate stock (7% $NaHCO_3$; pH 8.9), 1.0 ml KS stock (1% α-ketoglutarate, 1% sodium succinate; pH 7.3), 1.0 ml cysteine stock (1%, free base; without pH adjustment; freshly made), 20 ml agamma horse serum (Grand Island Biological Co.; inactivated at 56 C for 60 min), 10.0 ml Yeastolate stock (2% Yeastolate, Difco) and 50.0 ml Schneider's drosophila medium. All stock solutions were filter-sterilized (0.2-μm pore size membrane filter) except the bicarbonate stock, which was autoclaved. The final pH of the L20 medium was 7.4. Potassium-penicillin-G solution (100,000 units per milliliter, Sigma) was added to all media at 1% (v/v).

Isolation and cultivation. Leaf tissue was used in all isolation attempts from plants. Two 2-cm^2 samples were cut from each leaf, one near the leaf base and one about midway between the leaf base and leaf tip. The samples included the leaf midrib. Samples were surface-sterilized for 1 min in 70% ethanol, then rinsed twice in sterile deionized water. The samples, after excess water was removed with sterile paper towels, were placed in sterile petri dishes with 1 ml of C-3G medium and chopped into small pieces (about 1–2 mm^2) with a sterile razor blade while submerged in the medium. The medium was then transferred to a 3-ml syringe and passed through a 0.45-μm pore size Acrodisc membrane filter unit (Gelman, Ann Arbor, MI) into a sterile culture tube. Additional culture tubes containing 2 ml of fresh media were inoculated with 50 μl of the filtrate per tube. Filtrates from several samples taken from the same plant were combined when larger volumes of inoculum were needed in some experiments.

Attempts to reisolate CSS strains from injected *D. maidis* were made after incubation and inoculation access periods on sweet corn. Groups of 10 *D. maidis* were used. They were frozen at −40 C, suspended in 2 ml of C-3G medium, ground in a glass homogenizer, and the brei clarified by centrifugation at 5,000 *g* for 10 min before filtration and inoculation of media as described before. All cultures in tubes were incubated at 30 C in GasPak 100 jars (Baltimore Biological Laboratories) containing either air, 5% CO_2 in air, or an anaerobic atmosphere with 5% CO_2 (GasPak disposable generator envelopes were used to obtain the modified atmospheres). For larger culture volumes, 450-ml volumes of C-3GH medium in 500-ml screw-cap bottles were inoculated with 2 ml of culture in late log phase that had been grown under anaerobic conditions. The large-volume cultures were incubated with tightly sealed caps and without agitation at 30 C.

Growth measurements. Growth of spiroplasmas in culture was measured by direct cell counts. Five microliters of culture was deposited on a microscope slide, covered with a coverslip (18 × 18 mm), and the cells in 10 microscope fields counted using dark-field microscopy (×1600). The depth of each field was determined using the micrometer on the microscope; with the field diameter, this value was used to calculate the field volume. The cells-per-unit volume was then calculated. In preliminary experiments to determine which media and incubation atmospheres should be examined further, duplicate cultures of CSS strain E275 (ATCC 29320), which had been grown in M1A medium, were prepared for each treatment and examined after 7 and 14 days of incubation. Subsequent tests were performed using inoculum from sweet corn infected with the 1980 CS isolate. Each treatment was replicated five times, and cell counts were taken at intervals of 3–5 days.

Serology. Antisera were produced by immunization of rabbits as described (27). CSS strain E275 (26), *Spiroplasma citri* strain SC-27 (provided by E. C. Calavan), and group IV spiroplasma strain PPS1 (20) were used for comparison. Antisera to strains E275, PPS1, and SC-27 from bleedings having homologous metabolic inhibition titers (24) of 13×10^3, 32×10^3, and 4×10^3, respectively, were used. Spiroplasma deformation tests (27) and enzyme-linked immunosorbent assay (ELISA) (20) were performed as described.

Polyacrylamide gel electrophoresis. Spiroplasmas grown in 450 ml of C-3GH medium were harvested in late log phase and washed three times by centrifugation at 25,000 *g* at 4 C for 30 min in phosphate buffer (0.02 M, pH 7.4) containing 10% (w/v) sucrose. Washed cells were resuspended in 5 ml of phosphate-buffered sucrose and stored frozen at −40 C. For solubilization, 0.5 ml of the cell suspension was mixed with 0.5 ml of equilibration buffer (10 ml of glycerol, 0.1 g of dithiothreitol, 0.07 g of bromphenol blue, and 2 g of sodium dodecyl sulfate in 60 ml of deionized water) in a 1.5-ml polypropylene centrifuge tube, capped, and placed in a boiling water bath for 5 min. Precipitates were than removed by centrifugation at 8,000 *g* for 3 min. Electrophoresis was performed after the method of Laemmli (16) in 10% polyacrylamide resolving gels with 3% polyacrylamide stacking gels in a vertical electrophoresis unit (model 2001-001, LKB Instruments, Inc., Durham, NC) at 15 C and a constant 100V. Low-molecular-weight protein standards (Pharmacia Fine Chemicals, Uppsala, Sweden) and soluble proteins from CSS strain I-747 (5) and flower spiroplasma strain SR 3 (9) were used for comparison.

Pathogenicity tests. Spiroplasma cultures stored frozen in C-3G or L20 medium at −40 C were transferred to fresh C-3G medium and incubated for 7–10 days under anaerobic conditions. For each strain, two 2-ml cultures were combined, centrifuged at 28,000 *g* for 30 min at 4 C, and the pellet was washed once with phosphate-buffered sucrose. The cells were resuspended in 2 ml of phosphate-buffered sucrose and the suspension was used as inoculum for leafhopper injections. Healthy *D. maidis* adults were immobilized with CO_2, placed under stretched Parafilm, and injected through the Parafilm into the abdominal cavity (3). Injection needles capable of delivering about 0.25–0.50 μl per injection were made from glass capillary tubes. About 60–70 insects were injected per treatment; normally, at least 30 of these insects survived after 30 days. Injected insects were then caged singly on corn seedlings and transferred serially to a new series of plants at intervals of 2–3 days for 4–6 wk. Control plants exposed to insects randomly collected from the colonies and to insects injected with deionized water remained healthy. Test plants were kept in a separate screenhouse for 3 mo for symptom development.

RESULTS

The E275 strain of the CSS grew in the M1A, L20, and SP4 media but not in the LD8A medium. An initial titer of 1×10^5 cells per milliliter was used. Anaerobic conditions improved growth in the M1A, L20, and SP4 media. Cell populations of 2×10^8/ml in the M1A medium, 1×10^8/ml in the L20 medium, and 2×10^7/ml in the SP4 medium were observed after 7 days of anaerobic incubation. In comparison, cell populations of 1×10^6/ml in the M1A medium, 5×10^6/ml in the L20 medium, and 1×10^6/ml in the SP4 medium were observed in aerobically grown cultures, and populations were slightly higher in the M1A medium (6×10^6/ml) and the L20 medium (2×10^7/ml) when an atmosphere of 5% CO_2 in air was used.

In preliminary tests with an established culture of the CSS, anaerobic conditions in combination with the appropriate culture medium seemed to provide improved conditions for primary isolation and subculture of the CSS. Primary isolations from experimentally maintained corn stunt-infected sweet corn were attempted under anaerobic conditions using the M1A, M1AS, L20, and C-3G media. Five tubes of each medium were uniformly inoculated, and spiroplasmas grew consistently in all four media. The cultures had an initial titer of 1×10^5 cells per milliliter. Average cell populations between 7×10^7 and 3×10^8/ml were obtained in the media after 10–17 days of incubation. The highest population was

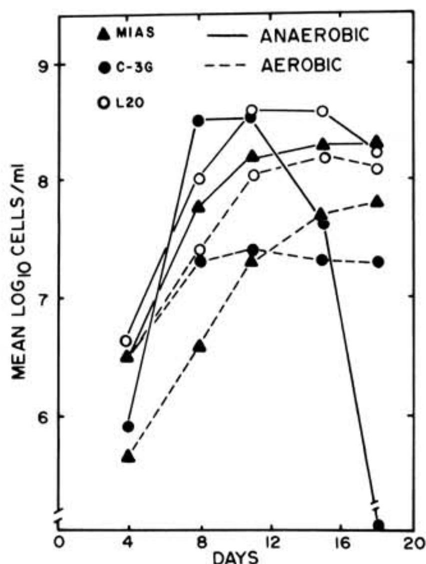

Fig. 1. Growth of primary cultures of spiroplasmas from maize when incubated aerobically or anaerobically in the M1AS, C-3G, or L20 media. Each point represents the average of populations from five replicate cultures for each medium and atmosphere combination. All cultures were equally inoculated with portions of the same extract from sweet corn infected with the 1980 corn stunt isolate. The initial titer of the cultures was about 2.5×10^5 cells per milliliter.

observed in the L20 medium and the lowest in the M1A medium. The L20, C-3G, and M1AS media were tested further under both aerobic and anaerobic conditions; the results are shown in Figure 1. Although spiroplasmas were isolated consistently in all three media regardless of the atmosphere used, higher populations were observed consistently in cultures incubated under anaerobic conditions. During log-phase growth under anaerobic conditions, the growth rate (cells/milliliter/hour) was significantly ($P = 0.01$) faster in the C-3G medium than in the L20 and M1AS media.

Anaerobic incubation, compared with aerobic incubation, considerably improved the frequency of successful primary isolation attempts (Table 1) from field-collected maize leaf samples with corn stunt symptoms when the C-3G medium was used. The isolation frequency was about the same under anaerobic conditions as under aerobic conditions when the L20 medium was used. Spiroplasmas were isolated in both media from the same 18 of 20 symptomatic plants but not from any of the 20 asymptomatic plants. In addition, the filtrate used as inoculum was incubated under anaerobic conditions and also produced positive isolations from the same 18 of 20 symptomatic plants.

In a subsequent test when only experimentally infected maize with corn stunt (1980 CS isolate) was available for sampling, the C-3G, C-3GH, M1AS, L20, and LD8A media were compared for primary isolation of spiroplasmas under anaerobic conditions. The LD8A medium (obtained for this experiment from R. E. Davis, USDA, Beltsville, MD), was used instead of C-3G medium for preparing filtrate for inoculum. Growth in the C-3G, M1AS, and L20 media was similar to that previously observed as shown in Figure 1. Growth was not observed in the LD8A medium, except in a remaining portion of the filtrate that had not been used as inoculum. Growth in the C-3GH medium was similar to that in the C-3G medium, except that the C-3GH medium supported significantly ($P = 0.01$) higher maximum average titers of spiroplasmas (1.0×10^9 cells per milliliter after 12 days of incubation) than the C-3G medium (3.0×10^8 cells per milliliter after 12 days of incubation).

The L20 and C-3G media in combination with anaerobic incubation were used to obtain a collection of spiroplasma strains from maize. These strains were readily obtained from experimentally infected sweet corn representing the 1978

Table 1. Frequency of primary isolation of spiroplasmas from maize under aerobic and anaerobic conditions and in the C-3G and L20 media[a]

Symptoms	No. plants sampled	Culture atmosphere[b]	No. positive isolations in: C-3G medium	L20 medium
Corn stunt	20	Air	7(6)[c]	17(1)
		H_2 + 5% CO_2	18	18
None	20	Air	0	0
		H_2 + 5% CO_2	0	0

[a] Isolations were attempted from leaf samples taken from asymptomatic plants and plants with corn stunt symptoms in one field in south Florida.
[b] BBL anaerobic generator envelopes were used to obtain anaerobic conditions with oxygen replaced by hydrogen and containing about 5% CO_2.
[c] Number of cultures with spiroplasmas averaging less than one per microscope field (×1600) is given in parentheses. Data were taken after 10 days of incubation. No spiroplasmas were observed in the cultures immediately after inoculation.

Table 2. Serological relationship among strains of the Florida corn stunt spiroplasma (FCSS), corn stunt spiroplasma strain E275, *Spiroplasma citri* strain SC-27, and spiroplasma strain PPS1

	Serological assay					
	ELISA[b]			Deformation[c]		
Antigen[a]	E275	SC-27	PPS1	E275	SC-27	PPS1
SC-27	0.10	2.34	0.02	54	13,122	0
PPS1	0.10	0.02	2.95	0	0	4,374
E275	1.58	0.03	0.02	1,458	0	0
FCSS-D1	1.84	0.04	0.03	ND	ND	ND
FCSS-D13	ND[d]	ND	ND	486	0	0
FCSS-T79	1.44	0.04	0.02	972	0	0
FCSS-T80	1.46	0.04	0.01	486	0	0
FCSS-T81	1.34	0.03	0.03	162	18	0
FCSS-TR	1.40	0.04	0.03	486	0	0
FCSS-TRT	1.44	0.04	0.01	1,458	18	0
FCSS-M1	1.64	0.08	0.01	162	0	0
FCSS-M2	1.59	0.06	0.02	162	0	0

[a] Designation is the same as for corresponding spiroplasma strains listed in the text.
[b] ELISA (enzyme-linked immunosorbent assay) reaction is given as the average absorbance (405 nm) of two-well composite samples using immunoglobulin G to the indicated strain.
[c] Deformation reaction is given as the dilution titer at which 50% of the cells were deformed by antisera to the indicated strain.
[d] ND = not done.

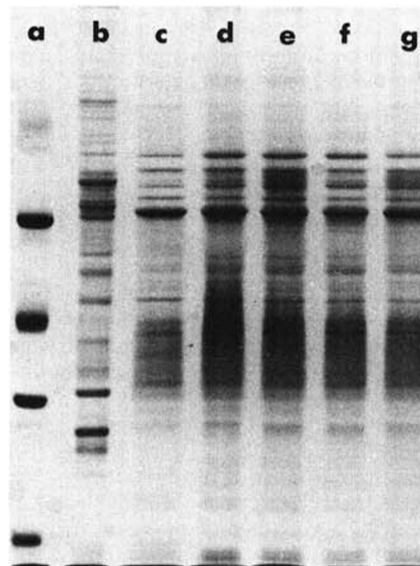

Fig. 2. Cellular protein patterns of spiroplasmas from diseased maize in Florida. Cells from culture were solubilized and subjected to polyacrylamide gel electrophoresis. A sample containing standard proteins, which were (top to bottom) phosphorylase b (mol wt 94,000), bovine serum albumin (mol wt 67,000), ovalbumin (mol wt 43,000), carbonic anhydrase (mol wt 30,000), and soybean trypsin inhibitor (mol wt 20,100), was contained in lane a. Lane b contained extracts from flower spiroplasma strain SR3. Lane c contained extract from corn stunt strain I-747. Lanes d through g contained extracts from Florida maize strains FCSS-TR, FCSS-T79, FCSS-D1, and FCSS-M1, respectively.

R-type isolate (strain FCSS-TRT) and red-leaf isolate (strain FCSS-TR) and the 1979, 1980, and 1981 CSS isolates (strains FCSS-T79, FCSS-T80, and FCSS-T81, respectively). Strains FCSS-D1, FCSS-D3, FCSS-D6, and FCSS-D13 were isolated in 1982 from naturally infected maize with corn stunt symptoms. In addition, two strains were obtained from maize with bushy stunt symptoms. One of these strains (FCSS-M1) was isolated from one of the two sweet corn plants that represented two separate collections of experimentally maintained maize bushy stunt. A second spiroplasma strain (FCSS-M2) was obtained from a field-collected sample with maize bushy stunt symptoms. Primary spiroplasma cultures obtained under anaerobic conditions were readily subcultured after growth was first observed.

All maize spiroplasma strains from Florida reacted with antisera (or purified immunoglobulin G) to the E275 strain of the CSS in both deformation tests and ELISA (Table 2). Comparable reactions did not occur when antisera to the SC-27 strain of *S. citri* and to spiroplasma strain PPS1.

Polyacrylamide gel electrophoresis patterns (Fig. 2) of soluble proteins from the maize spiroplasma strains from Florida, including patterns for three additional strains (FCSS-D6, FCSS-TR, and FCSS-M2) not shown in Figure 2, were indistinguishable from the Rio Grande CSS strain I-747 but easily distinguishable from the SR3 flower strain.

All nine maize spiroplasma strains from Florida that were tested for pathogenicity incited typical corn stunt symptoms in sweet corn. These strains included one obtained from plants with maize bushy stunt symptoms. The frequency of transmission of the strains by injected *D. maidis* adults ranged from 13.3 to 66.7% (Table 3). Each spiroplasma strain was reisolated in one attempt from *D. maidis* after the transmission attempts were completed.

Table 3. Transmission of Florida corn stunt spiroplasma strains to Guardian sweet corn by *Dalbulus maidis* in 1983

Spiroplasma strain injected[a]	Date injected	No. insects	No. infectious insects[b]
FCSS-D6	1 January	30	14 (46.7%)
FCSS-D14		30	15 (50.0%)
FCSS-D20		31	21 (67.7%)
FCSS-M1		30	13 (43.3%)
FCSS-T79	1 February	30	10 (33.3%)
FCSS-T80		30	15 (50.0%)
FCSS-T81		30	11 (36.7%)
FCSS-TR		30	4 (13.3%)
FCSS-TRT		30	11 (36.7%)

[a] After injection with a spiroplasma strain, each insect was transferred serially at 2- to 3-day intervals for 4–6 wk to new corn seedlings.

[b] An insect was considered infectious if any corn seedlings exposed to its feeding developed corn stunt symptoms.

DISCUSSION

Our initial inability to isolate the CSS from maize with corn stunt in Florida was overcome by incubating primary cultures under anaerobic conditions instead of aerobic conditions and by using appropriate culture media. Other researchers have reported improved growth of established cultures of the CSS and other spiroplasmas on solid media when the spiroplasmas were grown under anaerobic or microaerophilic conditions (5,25,26). The reason for better growth in modified culture atmospheres is not known. Depending on the terminal electron acceptor in spiroplasma respiration, which is not known, growth might be favored by microaerophilic or anaerobic conditions (23). The chemical composition in media might also be affected by different culture atmospheres. Liao and Chen (17) attributed difficulties in obtaining primary cultures of the CSS from maize to the release of one or more toxic compounds during isolation procedures. If the putative toxic compounds are oxidation products, then anaerobic conditions might prevent their formation. However, this does not explain why we also observed improved growth under anaerobic conditions in established cultures of the CSS.

Anaerobic growth of CSS was faster in the C-3G medium than in the M1A or L20 media, but populations of detectable organisms declined rapidly once maximum growth was obtained. Liao and Chen (17) demonstrated that the rapid decline in CSS cell numbers was related to increased acidity and that addition of HEPES buffer prolonged the exponential growth phase under aerobic conditions. We found that the addition of HEPES buffer also improved growth under anaerobic conditions. The C-3G and C-3GH media have few components and are easy to prepare, whereas the other media used were more complicated and expensive to prepare. Under aerobic conditions, the L20 medium supported the development of higher maximum titers in primary culture than the other media tested. Also, the frequency of aerobic isolation of the CSS from field-collected material was greater in the L20 medium than in the C-3G medium. The availability of oxygen to organisms growing in broth medium can be limited by such factors as chemical composition, depth, and viscosity of the medium. It is probable that growth of the CSS can be improved without incubating cultures in a modified atmosphere by modification of some media, especially if the factors limiting growth under aerobic conditions can be elucidated.

All spiroplasmas isolated from maize in this study appear to be the CSS. Pathogenicity tests, serology, and cellular protein patterns in polyacrylamide electrophoresis gels all supported identification of the strains tested as CSS. However, physiological differences in strains of the CSS from different geographical locations might exist. This might explain why Florida strains do not grow readily in the LD8A medium as reported previously for other CSS strains (4,10).

Although some variation in symptomatology was noted in the original diseased plants, plants experimentally inoculated with cultured CSS had similar symptom profiles. Some of the original diseased plants showed typical corn stunt symptoms, with extensive chlorosis but not leaf reddening (1979 CS, 1980 CS, and 1981 CS isolates), whereas others had extensive leaf reddening (red-leaf isolate and all 1982 corn stunt field samples). Chlorosis, but not leaf reddening, was observed in experimentally inoculated plants, including both the plants in which field isolates were maintained using plant-to-plant transmission by *D. maidis* and plants inoculated with spiroplasma strains from culture. Therefore, factors such as genotypes of the host and environmental conditions, rather than differences in CSS strains, must be responsible for the variations in corn stunt symptomatology observed in the field. In some situations, mixed infections with other maize pathogens might be responsible for variations in corn stunt symptomatology (2,8). Dual infection of maize with the maize bushy stunt mycoplasmalike organism and the CSS probably accounts for our isolation of the CSS from some, but not all, plants with maize bushy stunt symptoms.

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