New Media for Rapid Growth of *Spiroplasma citri* and Corn Stunt Spiroplasma

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ABSTRACT

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Several media (LD series) for cultivation of *Spiroplasma citri* and corn stunt (CS) spiroplasma were developed by study of growth-promoting factors and eventual reformulation of a complex medium (L) originally developed for cultivation of *S. citri*. The maximum titer of *S. citri* grown in one of the new media, LD8, reached over 6×10^9 colony-forming units (CFU) per milliliter, with an estimated doubling time of about 4 hr at 31 C. In another medium, LD8A, CS spiroplasma (strain I-747) grew to a titer of about 2×10^9 CFU/ml, with a doubling time of 11–12 hr at 31 C. Media LD8 and LD8A were highly suitable for primary isolation of *S. citri* and CS spiroplasma, respectively, from diseased plants.

Additional key words: Catharanthus roseus, plant mycoplasmas, Zea mays.

Isolation and cultivation in vitro of yellows disease agents have been the goal of many researchers since the discovery of mycoplasmalike organisms (MLO) in infected plants (10). Thus far, only two mycoplasmalike agents of plant disease, one from citrus stubborn diseased and other plants and one from corn stunt (CS) diseased plants, have been cultivated in vitro and proved to be plant pathogens (4,12,19–21,24). Unlike other microbes in the order Mycoplasmatales, these two organisms possess helical morphology (9,22). The term "spiroplasma" was proposed (9) and later adopted as the genus name for this unusual type of microorganism (22). *Spiroplasma citri*, causal agent of citrus stubborn disease, is the only phytopathogenic species that has been named thus far (5,22).

Subsequently, studies have been devoted to determining factors in media which contribute to successful cultivation of spiroplasmas and to improving growth of spiroplasmas in culture. Thus, several media for these organisms have been developed (1,2,13–17). Among these media, medium L (15), which included 20 amino acids, sorbitol, succinic acid, and mineral salts, permitted good in vitro growth of *S. citri*, but not that of corn stunt spiroplasma. In the present study, our goal was to reformulate medium L so that it would support good growth of both *S. citri* and CS spiroplasma. Our working hypotheses were that: the complex medium L contained materials deleterious to spiroplasma growth, and growth of spiroplasmas could be improved by simplification of this medium and (based on a recent report [14]) by incorporation of α-ketoglutaric acid. We report here improved media for growth of both *S. citri* and CS spiroplasma. A preliminary report on part of this study has been presented (16).

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MATERIALS AND METHODS

Spiroplasmas. *S. citri* strain Maroc R8A2 (ATCC 27556) and corn stunt (CS) spiroplasma I-747 (ATCC 29051) were used in this study.

Media and growth conditions. Medium L and its modifications in the LD media series were prepared according to the formulations listed in Table 1. All components except fresh yeast extract (Flow Laboratories, Rockville, MD 20852) and fetal bovine serum (Flow

Laboratories) were dissolved in deionized water, and the pH was adjusted to 7.5 with 2 N NaOH. After being autoclaved at 121 C for 15 min and cooled, this basal medium was supplemented with sterile fresh yeast extract and serum. For solid medium, 0.65 or 1.0 gm of Noble agar (Difco) was added per 100 ml of final volume of complete medium before autoclaving. Calavan's medium and four other media (DSM4 with yeast extract, C-3G, M1, and SMC) were prepared according to procedures described by Fudl-Allah et al (12), Davis (6), and Liao and Chen (17), Williamson and Whitcomb (24), and Saglio et al (21), respectively. In the present study, 0.06 M HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethane-sulfonic acid) buffer (Calbiochem-Boehring Corp., La Jolla, CA 92037) was incorporated into all media, and the pH of each was adjusted to 7.5. No phenol red or antibiotic was added to any of the media. All cultures were incubated at 30–32 C (15,18).

Growth assessment. Two parameters were employed to estimate the titer of spiroplasma cells in broth media: turbidity and colony-forming units (CFU). Turbidity was estimated by measuring absorbance at 450 or 660 nm with a Beckman DB spectrophotometer. Standard curves were prepared relating turbidity and CFU per milliliter. For CS spiroplasma, the CFU data were obtained by duplicate dilution plating of culture on 0.65% agar medium. For *S. citri*, dilutions of culture were plated on 1% agar medium. The seeded agar plates were incubated in closed moist containers at 30 ± 1 C (for CS spiroplasma) or 31 ± 1 C for *S. citri*, and colonies were counted after 7–10 days of incubation for *S. citri*, or 14 days for CS spiroplasma. When several broth media were compared, titer was estimated by measuring turbidity and CFU per milliliter in all media when titer in the best medium approached maximum turbidity based on preliminary tests.

Source plants for spiroplasma isolations. Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don) plants infected with *S. citri* were kindly provided by G. Oldfield (USDA, Riverside, CA). Maize (*Zea mays* L.) plants infected with corn stunt spiroplasma were supplied by J. Kloepper (Department of Plant Pathology, University of California, Berkeley). These plants were greenhouse-grown seedlings that had been inoculated experimentally by means of leafhoppers (*Circulifer tenellus* for periwinkle and *Dalbulus maidis* for maize plants).

Primary isolations of spiroplasmas from plants. Midribs of periwinkle leaves or pieces of young stem tissue (1 cm^3) of maize plants were surface sterilized by immersion in 20% (v/v) Chlorox (5 min) and rinsed in three changes of sterile distilled water. The tissues were then chopped aseptically in 10 ml of sterile liquid medium (LD8 for *S. citri* and LD8A for CS spiroplasma) and rinsed in the medium. The brei was incubated for 10 min to enhance release of spiroplasmas from broken sieve elements and was then passed through a sterile 0.45-μm pore diameter Acrodisc (Gelman, Ann Arbor, MI 48106) filter. The filtrate was diluted immediately with nine volumes of sterile medium to give a total of 8 ml in a 15-mm-diameter culture tube. One-tenth milliliter of this primary broth culture was immediately plated on solid agar medium. These constituted the primary agar cultures. Both broth and agar cultures were incubated at 30 ± 1 C. In the case of corn stunt spiroplasma,

TABLE 1. Composition of medium L and some LD media[a]

Components	L	LD2	LD4	LD5	LD6	LD8	LD8A	LD10	LD10A
Inorganic salts									
KCl	0.4	0.4	0.4	0.4	0.4	0.4	...	0.4	...
KH_2PO_4	0.3	0.3	0.3	0.3	0.3	0.3	...	0.3	...
$MgSO_4\ 7H_2O$	0.2	0.2	0.2	0.2	0.2	0.2	...	0.2	...
NaCl	1.4	1.4	1.4	1.4	1.4	1.4	...	1.4	...
Na_2HPO_4	0.2	0.2	0.2	0.2	0.2	0.2	...	0.2	...
Na_2SO_3	0.5	0.5	0.5	0.5	0.5	0.5	...	0.5	...
Amino acids									
Eagle's BM-amino acid solution (ml) (×100)[b]	10.0	...	...	...	...	...	...	...	...
L-Arginine	0.6	0.6	0.6	0.6	0.6	0.6	0.6	...	...
L-Asparagine	0.6	0.6	0.6	0.6	0.6	0.6	0.6	...	...
L-Cysteine HCl	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.6	0.6
L-Glutamine	0.6	0.6	0.6	0.6	0.6	0.6	0.6	...	...
Glycine	0.4	...	...	...	...	...	...	...	...
L-Lysine HCl	0.6	...	...	...	...	...	...	...	...
Methionine	0.4	0.4	0.4	0.4	0.4	0.4	0.4	...	...
L-Proline	0.4	...	...	...	...	...	...	...	...
Organic acids									
α-Ketoglutaric acid	...	...	...	...	...	0.4	0.4	0.4	0.4
Pyruvic acid	...	...	...	...	...	0.4	0.4	0.4	0.4
Succinic acid	0.4	0.4	0.4	...	...	...	...	...	...
Carbohydrates									
Fructose	2.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Glucose	2.0	...	...	...	...	...	...	...	...
Sorbitol	25.0	...	...	...	...	...	...	...	...
Sucrose	10.0	60.0	60.0	60.0	60.0	60.0	90.0	80.0	90.0
Other components									
Tryptone	5.0	5.0	5.0	...	...	...	...	...	...
PPLO broth	5.0	5.0	5.0	12.0	12.0	12.0	12.0	12.0	12.0
Fresh yeast extract (ml)	50	50	50	50	...	...	...	...	...
Yeastolate	...	...	...	...	2.0	2.0	1.5	2.0	1.5
HEPES (buffer) (gm)	15.0	15.0	15.0	15.0	15.0	15.0	20.0	15.0	20.0
Fetal bovine serum (ml)	150	150	100	100	100	100	100	100	100

[a] Amounts are given in grams per liter unless specified otherwise. Final pHs of media were 7.4–7.5. See text for details.

[b] Contains 12 amino acids: L-arginine (2.1 g/L), L-cysteine (1.2 g/L), L-histidine (0.8 g/L), L-isoleucine (2.6 g/L), L-leucine (2.6 g/L), L-lysine (3.65 g/L), L-methionine (0.75 g/L), L-phenylalanine (1.65 g/L), L-threonine (2.4 g/L), L-tryptophane (0.4 g/L), L-tyrosine (1.8 g/L), and L-valine (2.35 g/L).

broth primary cultures were subcultured by passing 0.5 ml of primary culture into 5 ml of fresh sterile broth LD8A after 3 days of incubation.

RESULTS

Turbidity estimates of *Spiroplasma* titer. A linear or near-linear relationship between absorbance at 660 or 450 nm and titer of either *S. citri* or CS spiroplasma was observed at titers ranging from 1.0×10^8 to over 6×10^9 CFU/ml (Figs. 1 and 2). The relationship between absorbance and cell titer was characteristic for a given strain in the medium L variations that were examined. Thus, we concluded that with media like the L series in which precipitation is not significant, turbidity can be used for estimation of titers, provided that a standard curve (absorbance versus cell titer) is prepared for a spiroplasma in the culture medium. In our work, we were able to use turbidity measurements as an approximation of titer to assess the effects of modifications in the L medium. Results from determinations of CFU per milliliter verified the relative assessments of media based on turbidity. Similar agreement between CFU and turbidity measurements has been reported for mycoplasmas by Snell (23).

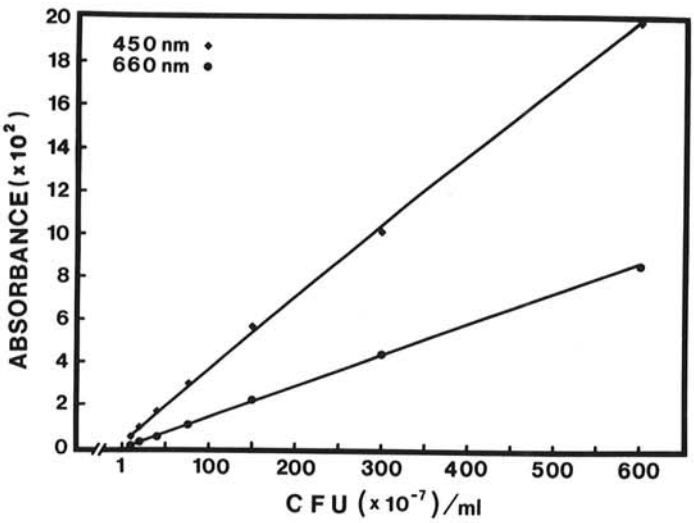

Fig. 1. Relationship between colony-forming units (CFU) per milliliter and absorbance at 450 or 660 nm in cultures of *Spiroplasma citri* Maroc R8A2 in medium LD8. Each data point is the average of two determinations.

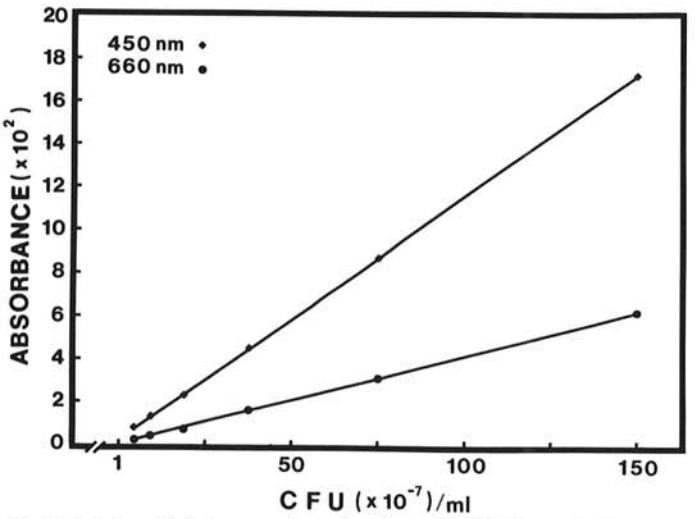

Fig. 2. Relationship between colony-forming units (CFU) per milliliter and absorbance at 450 or 660 nm in cultures of corn stunt spiroplasma (strain I-747) in medium LD8A. Each data point is the average of two determinations.

Effect of deletion of several supplemental amino acids from medium L. Deletion of supplemental Eagle's BM-Amino acids, glycine, lysine, and proline from medium L, and replacement of glucose and sorbitol with fructose and sucrose, did not impede growth of *S. citri*. Growth in the resulting simplified medium (LD2) was at least as good as that in medium L. Among the remaining five supplemental amino acids (arginine, asparagine, cysteine, glutamine, and methionine) tested separately in medium LD2, cysteine was associated with greatest yield (Table 2).

Modification of medium LD2. Modification of medium LD2 resulted in further improvement of growth of *S. citri*. Reduction of fetal bovine serum content from the original 15% (in medium LD2) to 10% resulted in a formulation (termed LD4, see Table 1) that permitted better growth. When succinate and tryptone or when succinate, tryptone, and yeast extract were deleted from medium LD4 and replaced with 0.7% (w/v) PPLO broth base (Difco) or with 0.7% PPLO broth base and 0.2% yeastolate, the resulting media, LD5 and LD6 (see Table 1), respectively, were at least as good as media LD2 and LD4 for growth of *S. citri*. For example, growth of *S. citri* in media LD2, LD4, LD5, and LD6 gave titers estimated at 4.4×10^9, 4.8×10^9, 6.8×10^9, and 6.5×10^9 CFU/ml (average of two experiments), respectively.

Growth of *S. citri* and CS spiroplasma in LD6 medium with various organic acids. Several intermediates in the glycolytic and tricarboxylic acid cycles were added individually or in combination to medium LD6. Among these organic acids, non-ketoacids (citrate, succinate, fumarate, and malate) failed to promote growth, whereas the ketoacids α-ketoglutarate, oxalacetate, and pyruvate) stimulated growth of both CS spiroplasma and *S. citri* (Table 3). Medium LD6 supplemented with α-ketoglutarate and pyruvate was designated medium LD8.

Inorganic salts and growth of *S. citri* and CS spiroplasma. Deletion of Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4, NaCl, and

TABLE 2. Effect of supplemental amino acids in medium LD2 on growth of *Spiroplasma citri* (Maroc R8A2)

LD2 medium no.	Presence or absence of indicated amino acid[a]					CFU/ml[b] (×10⁻⁹)
	Arg	Asn	Cys	Gln	Met	
1	+	+	+	+	+	7.8
2	+	−	−	−	−	5.1
3	−	+	−	−	−	5.3
4	−	−	+	−	−	7.1
5	−	−	−	+	−	4.8
6	−	−	−	−	+	4.5
7	−	−	−	−	−	4.5

[a] +, Present in; −, deleted from; supplemental amino acids: arginine, asparagine, and glutamine were added at 600 μg/ml; cysteine and methionine, were added at 400 μg/ml. Abbreviations are the same as in Table 1.

[b] Estimated by turbidity measurement. Average value from two experiments.

TABLE 3. Growth of *Spiroplasma citri* (Maroc R8A2) and corn stunt spiroplasma in LD6 medium supplemented with selected organic acids[a]

Medium	CFU/ml[b]	
	S. citri (×10⁻⁹)	CS spiroplasma (×10⁻⁸)
LD6	6.1	3.4
LD6 + citrate	5.4	2.2
LD6 + α-ketoglutarate	7.4	9.0
LD6 + succinate	6.1	2.2
LD6 + fumarate	6.1	2.2
LD6 + malate	6.1	2.9
LD6 + oxalacetate	8.4	8.7
LD6 + pyruvate	7.4	8.2
LD6 + α-ketoglutarate + pyruvate (= LD8)	7.9	9.2

[a] Each individual organic acid was added at 400 μg/ml.

[b] Estimated by turbidity measurement. Average value from two experiments.

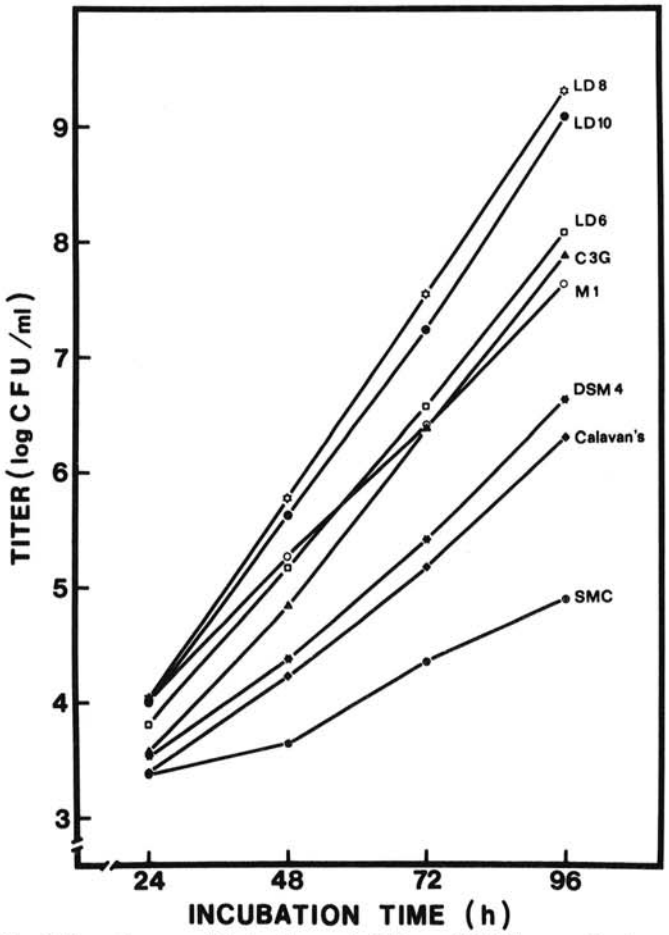

Fig. 3. Growth rates of *Spiroplasma citri* Maroc R8A2 in several culture media as measured by estimation of colony-forming units (CFU) per milliliter. Data are presented as $\log_{10}$ CFU per milliliter. Each point represents the average from two culture tubes, each titered in quadruplicate on solid agar medium.

Mg_2SO_4 from media LD6, LD8, and LD10 slowed growth of *S. citri*, but tended to promote growth of CS spiroplasma (Table 4). Na_2SO_3, which promoted growth of *S. citri*, inhibited CS spiroplasma.

Growth of *S. citri* and CS spiroplasma in LD and other media. Comparisons were made of growth of *S. citri* in several LD media and in five other media that have been used for cultivation of this spiroplasma. These media were each seeded with a filtrate (Millipore; 0.45-μm pore diameter of a log-phase culture of *S. citri* grown in medium C-3G and diluted with sterile C-3G to give a starting titer of 1.4×10^3 CFU/ml in each medium. The cultures were incubated at 31 ± 1 C until any one of the cultures reached near-stationary growth phase. Based on the number of CFU assessed after 96 hr of incubation, *S. citri* grew most rapidly in media LD8 and LD10 followed, in order, by growth in LD6, C-3G, M1, DSM4 with yeast extract, Calavan's medium, and SMC. The average doubling time for the period between 24 and 96 hr of incubation in these media was 4.0, 4.3, 5.1, 5.2, 6.0, 7.0, 7.5, and 14.7 hr, respectively (Fig. 3). Growth quantitation based on turbidity at 660 nm measured after 104 hr of incubation agreed well with the results based on assessment of CFU. For example, cultures of *S. citri* grown in LD6, LD8, and LD10 had higher absorbances at 660 nm (0.039, 0.153, and 0.109, respectively) than cultures grown in any of the other media. Starting from a titer of 1.4×10^3 CFU/ml, *S. citri* reached 2.0×10^9 and 1.2×10^9 CFU/ml in 96 hr in media LD8 and LD10, respectively, in this experiment. In several separate experiments, *S. citri* reached a titer of over 6×10^9 CFU/ml when grown in medium LD8.

Log phase cultures of *S. citri* grown in medium LD8 consisted predominantly of short helical cells (1.5–3.0 gyres in length) and lacked clumps. Colonies of *S. citri* on solid (1% agar) medium LD8 were diffuse and lacked the "fried egg" appearance previously reported for colonies of this spiroplasma on other media (12,21).

Among the LD media, LD8A and LD10A modified from LD8 and LD10 by deleting supplemental inorganic salts and increasing sucrose content from 60 to 90 g/L in LD8A and from 80 to 90 g/L in LD10A, were most favorable for growth of CS spiroplasma. Colonies formed on medium LD8A containing 0.65% Noble agar and were visible to the unaided eye after 10 days of incubation at 30

TABLE 4. Effect of deleting inorganic salts on growth of *Spiroplasma citri* and corn stunt spiroplasma in LD media

Medium from which salts were deleted[a]	Inorganic salt(s)[b] deleted	CFU/ml[c]	
		S. citri ($\times 10^{-9}$)	CS spiroplasma ($\times 10^{-8}$)
LD6	None	3.0	0.7
	Na_2SO_3	0.9	4.4
	Na_2SO_3, KCl	0.8	1.2
	Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4	0.7	1.9
	Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4, NaCl	0.6	5.7
	Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4, NaCl, Mg_2SO_4	0.9	9.7
LD8	None	3.5	1.0
	Na_2SO_3	1.7	12.0
	Na_2SO_3, KCl	1.4	13.0
	Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4	1.2	13.0
	Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4, NaCl	1.2	16.0
	Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4, NaCl, Mg_2SO_4	1.3	14.0
LD10	None	2.7	1.2
	Na_2SO_3	1.0	10.0
	Na_2SO_3, KCl	1.4	9.7
	Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4	1.0	12.8
	Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4, NaCl	1.0	12.8
	Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4, NaCl, Mg_2SO_4	1.1	13.3

[a] Supplemental inorganic salts in media LD6, LD8, and LD10 were Na_2SO_3, KCl, Na_2HPO_4, KH_2PO_4, NaCl, Mg_2SO_4 (see Table 1). Change of osmolarity due to deletion of inorganic salts was compensated by increase of sucrose concentration.
[b] Media LD6, LD8, and LD10 from which the five supplemental inorganic salts were deleted are referred to as media LD6A, LD8A, LD10A, respectively.
[c] Estimated by turbidity measurement. Average value from two experiments.

C. For quantitation of CFU per milliliter, colonies were counted after 14 days. The colonies were diffuse and granular, lacking a clearly defined edge; satellite colonies sometimes formed around the central region (Fig. 4). In broth medium LD8A, CS spiroplasma had an average doubling time of 11–12 hr during exponential growth at 31 C. Predominant morphotypes during this stage were helical cells 2–5 gyres in length (Fig. 5). A few small clumps of cells were seen in cultures during late log phase of growth. Titers in several experiments reached at least 1.5×10^9 CFU/ml.

Primary isolations of spiroplasmas from plants. *S. citri* was successfully isolated from infected periwinkle plants in two out of two attempts on medium LD8. Cell titers in broth primary cultures reached over 10^9 CFU/ml after <6 days of incubation. On solid medium LD8, colonies of *S. citri* were visible with the unaided eye after 7 days of incubation. CS spiroplasma was isolated from infected maize plants in four of four attempts; cell titers in broth primary cultures were $>10^9$ CFU/ml after 10–12 days of incubation. Colonies appeared on solid medium LD8A after 12–15 days of incubation. In a second experiment, five out of five attempts to isolate CS spiroplasma from infected plants were successful, both in LD8A broth and on LD8A agar.

DISCUSSION

Following the development of a medium (C-G) for maintenance of corn stunt (CS) spiroplasma in vitro (3) and of media for cultivation of *S. citri* (12,22), several new and sometimes improved media were formulated for cultivation of these plant pathogens and other spiroplasmas (1,2,4,5,13–17,24). Several investigators have studied factors stimulating spiroplasma growth in vitro. Jones et al (14) concluded, for example, that supplemental free amino acids in their M1A or M1 medium were important factors promoting growth of *S. citri* and CS spiroplasma and that organic acids, especially α-ketoglutaric acid, were indispensible for optimal growth of CS spiroplasma. In other work, one of us (I.-M. Lee) developed for cultivation of *S. citri* a medium (L) similar in complexity to M1 or M1A (15). Like M1A and M1, medium L contained a wide variety of supplemental free amino acids, and the benefit of these amino acids for growth of *S. citri* in medium L was in general agreement with that in medium M1A or M1. However, results of our current work have demonstrated that simplified and improved versions of medium L yield high spiroplasma titers. We found, for example, that medium L containing the supplemental amino acid cysteine either alone or in combination with four other amino acids (asparagine, arginine, glutamine, and methionine), yielded higher spiroplasma titers than did the medium L with a total of 20 supplemental amino acids. The present study confirmed that α-ketoglutaric acid promotes growth of CS spiroplasma and also showed that two other ketoacids, pyruvic acid, and oxaloacetic acid, had a similar effect on growth of CS spiroplasma. Further studies on the roles of these amino acids and ketoacids in the physiology of the plant pathogenic spiroplasmas would be worthwhile when a chemically defined medium for their culture in vitro is eventually available.

Through several steps, the complex medium L has now been reformulated into several simpler, but improved, media for cultivation of *S. citri* and also CS spiroplasma. Superior growth of *S. citri* was obtained in several of the new formulations (ie, media LD6, LD8, and LD10). Media LD8A and LD10A permitted rapid growth to high titers of both CS spiroplasma and *S. citri*. For example, the maximum titer of *S. citri* cultivated in medium LD8 was $>6 \times 10^9$ CFU/ml, with an estimated doubling time of ~4 hr, and in LD8A, CS spiroplasma grew to a titer approaching 2×10^9 CFU/ml with a doubling time of 11–12 hr. These doubling times compare favorably with values of 6 and 20 hr previously reported for *S. citri* (1) and CS spiroplasma (17), respectively, during log phase increase in other media. The relatively high cell titers reached in the improved media made it feasible to estimate growth of both *S. citri* and CS spiroplasma by measuring turbidity. Moreover, CS spiroplasma formed colonies readily on solid agar of LD8A, and it was possible to assess titer of viable CS spiroplasma on the basis of CFU.

Media LD8 and LD8A proved to be highly suitable for cultivation in vitro of *S. citri* and CS spiroplasma as well as for primary isolation of these pathogens from infected plants. These media are already being used by several laboratories for isolations of *S. citri* and corn stunt spiroplasma. In Illinois and Maryland, for example, medium LD8 has been used successfully for isolating *S. citri* from horseradish with brittle root disease (7,11). Medium LD8A has been used in separate experiments by us and others for the isolation of CS spiroplasma from Peru and California (R. E. Davis and I.-M. Lee, *unpublished*, and 8).These media have also proved to be particularly well suited for plaque assays of spiroplasmaviruses (R. E. Davis et al, *unpublished*). New media such as these should aid other studies on spiroplasmas and may also prove useful in developing procedures for culturing other fastidious organisms. In this latter regard, a flagellated protozoan (*Phytomonas* sp.) has been found to multiply to a relatively high titer in medium LD8 (R. E. McCoy, *personal communication*).

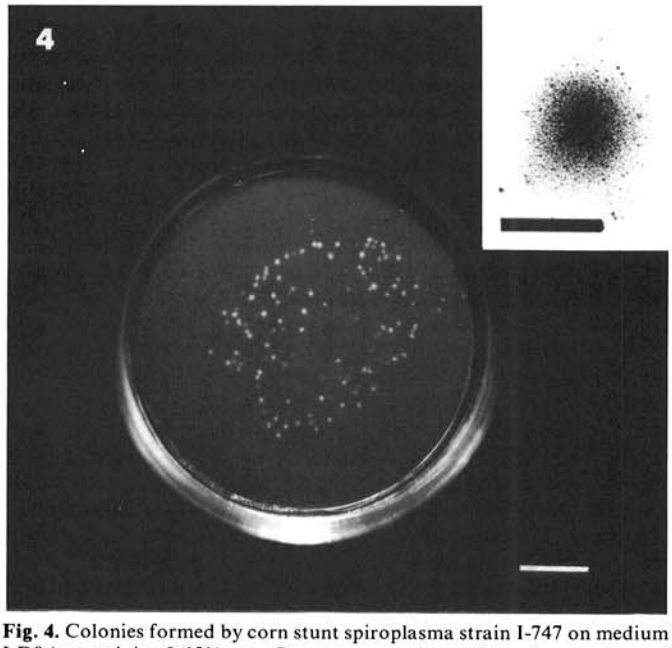

Fig. 4. Colonies formed by corn stunt spiroplasma strain I-747 on medium LD8A containing 0.65% agar. Bar represents about 1.0 cm. Inset: colony of corn stunt spiroplasma stained with Dienes' stain. Note tiny satellite colonies surrounding the central region. Bar represents 0.3 mm.

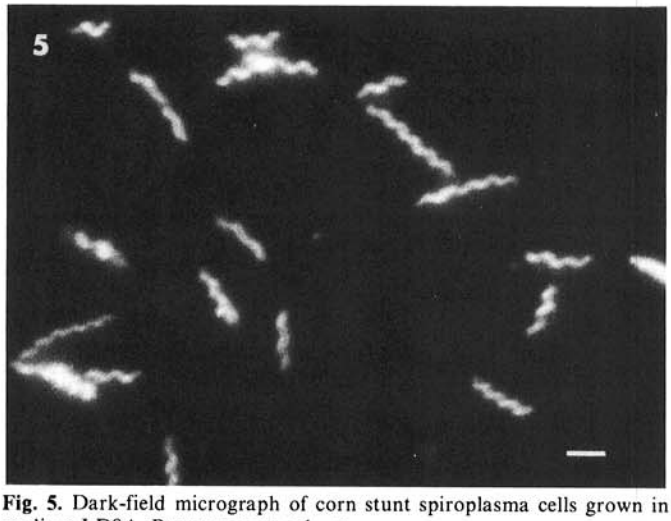

Fig. 5. Dark-field micrograph of corn stunt spiroplasma cells grown in medium LD8A. Bar represents ~1 μm.

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