

Culturing Liberibacter

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Some Direct and Indirect Advantages of Culturing *Liberibacter* spp.

- Facilitate development methodologies for detection and identification of the bacteria and greening disease
- Facilitate studies on greening disease epidemiology
- Facilitate studies on host/pathogen/insect vector interactions.
- Facilitate studies on genomics and molecular biology of *Liberibacter*.
- Facilitate development of procedures to manage greening disease.

Fastidious Prokaryotic Plant Pathogens

Xylem-Limited

Xylella fastidiosa

Clavibacter xyli subsp. *xyli*

Clavibacter xyli subsp. *cynodontis*

Phloem-Limited

Spiroplasma citri

Spiroplasma kunkelii

Spiroplasma phoeniceum

Candidatus Phytoplasma spp.

Candidatus Liberibacter spp.

Candidatus Phlomobacter fragariae

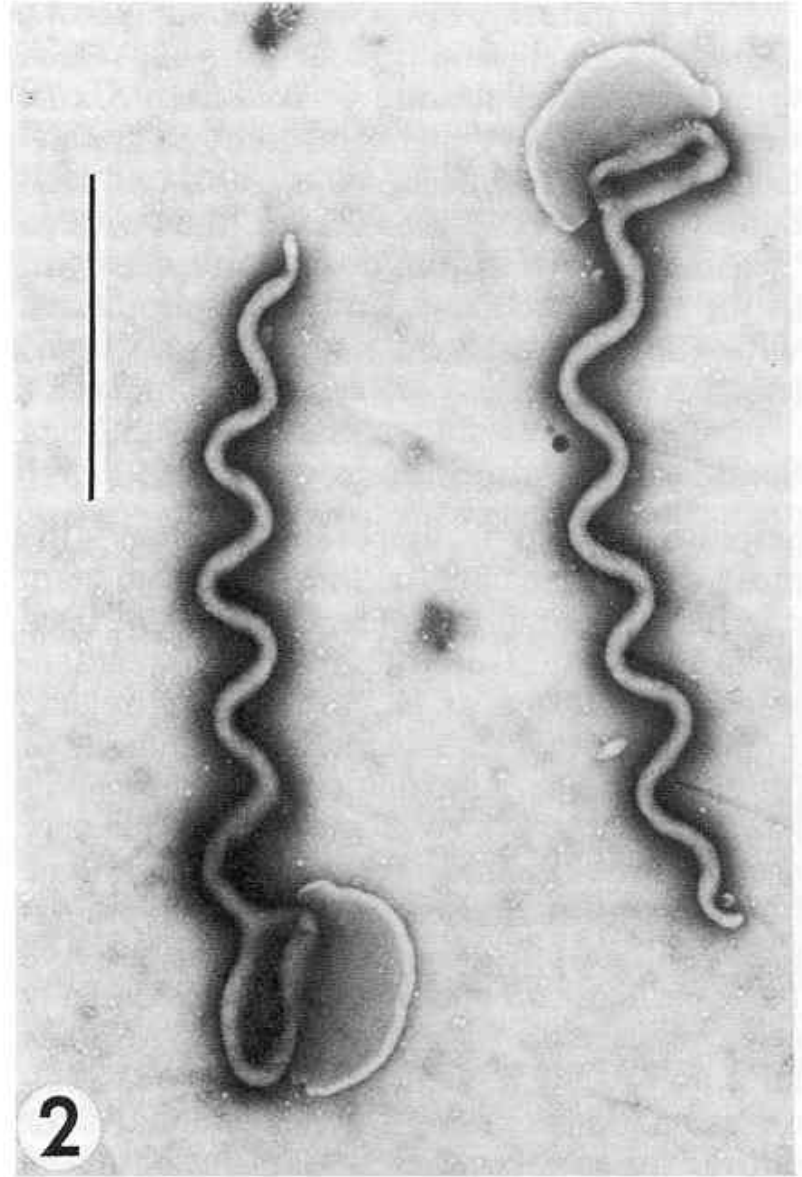
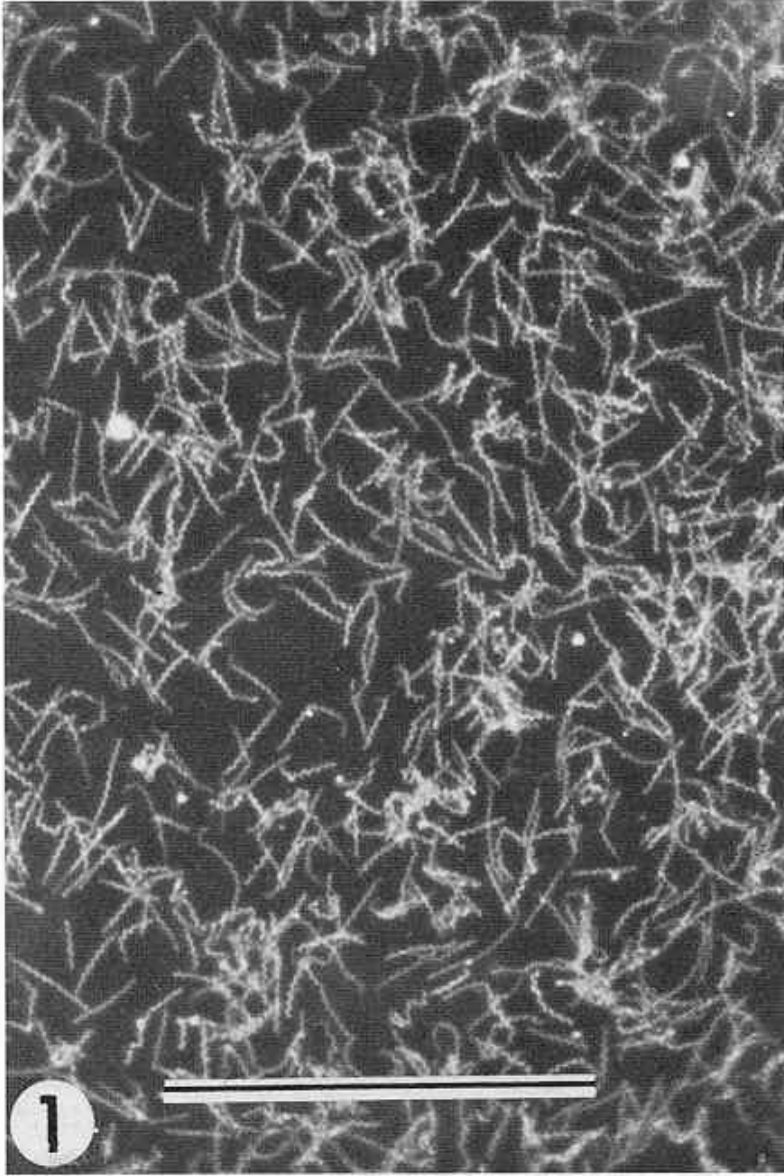
Candidatus Phlomobacter betae

Serratia marcescens

Unnamed Bacteria

Laticifer-Inhabiting

Papaya Bunchy Top Rickettsia



The Genus *Spiroplasma* JOSEPH G. TULLY and ROBERT F. WHITCOMB

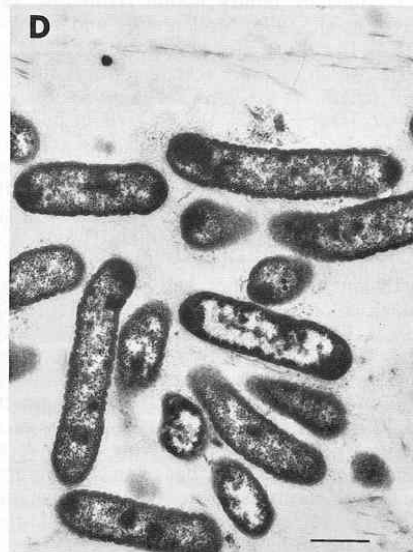
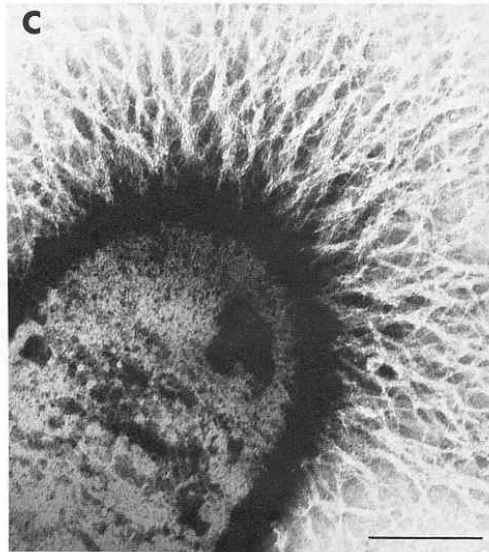
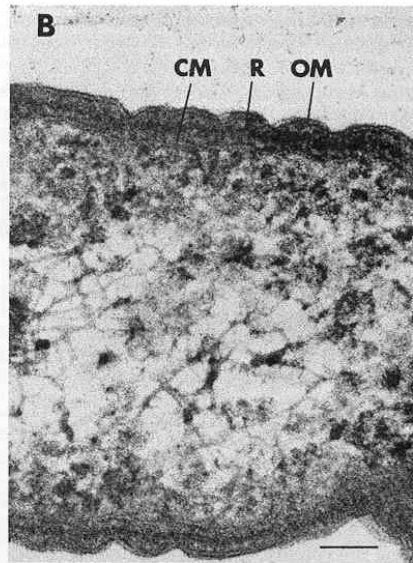
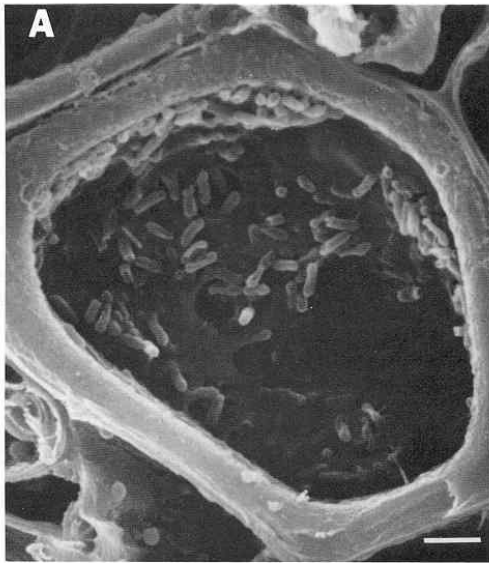
The Prokaryotes

Media for Spiroplasmas

BSR Medium (Bové et al., 1978), per liter

Beef heart infusion	25 g
Glucose	1 g
Fructose	1 g
Sucrose	10 g
Sorbitol	70 g
Phenol red	20 mg
Deionized water	900 ml
Horse serum	100 ml

Fastidious Xylem-limited Bacteria



**Fastidious Bacteria of Plant Vascular Tissues and Their
Invertebrate Vectors** M. J. DAVIS

Clavibacter xyli* subsp. *xyli
R. H. LAWSON

PW Medium for *Xylella fastidiosa*

Pancreatic digest of casein	1 g
Papaic digest of soy meal	4 g
Hemin chloride	10 mg
MgSO ₄ ·7H ₂ O	0.4 g
K ₂ HPO ₄	1 g
KH ₂ PO ₄	1.2 g
Agar	12 g
Phenol red	20 mg
Distilled Water	1 liter
Bovine serum albumin fraction five	6 g
Glutamine	4 g

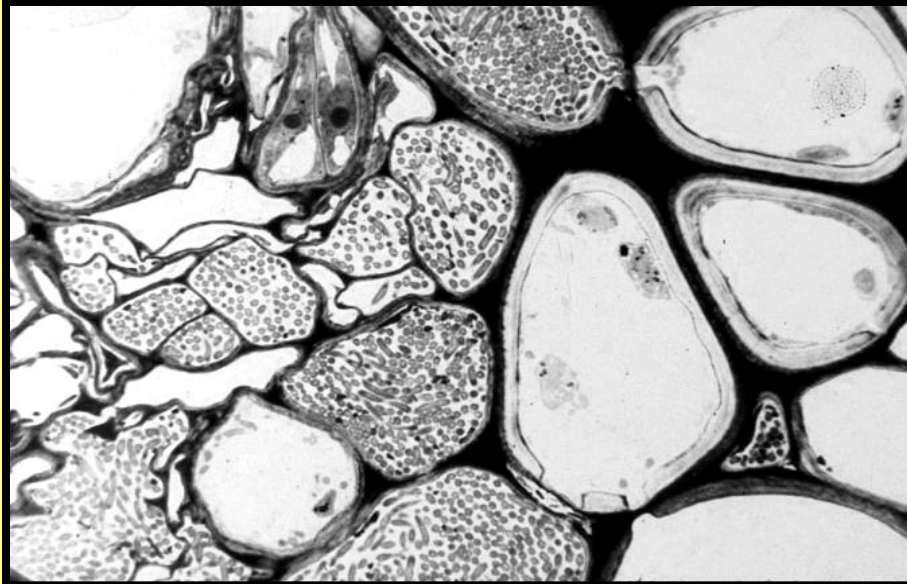
Davis M. J. ; B. C. Raju; R. H. Brlansky; R. F. Lee; L. W. Timmer; R. C. Norris; R. E. McCoy; 1983. *Periwinkle wilt bacterium: Axenic culture, pathogenicity, and relationships to other Gram-negative, xylem-inhabiting bacteria.* *Phytopathology*, vol. 73, pp. 1510–1515

SC Medium for Isolating *Clavibacter xyli*

Papaic digest of soy meal	8 g
Hemin chloride	10 mg
MgSO ₄ ·7H ₂ O	0.2 g
K ₂ HPO ₄	0.5 g
KH ₂ PO ₄	1.5 g
Corn meal agar	17 g
Distilled Water	1 liter
Glucose	0.5 g
Cysteine	1 g
Bovine serum albumin fraction five	2 g

Davis M. J. ; A. G. Gillaspie, Jr.; R. W. Harris; R. H. Lawson;
1980a. *Ratoon stunting disease of sugarcane: Isolation of the causal bacterium.* Science, vol. 210, pp. 1365–1367

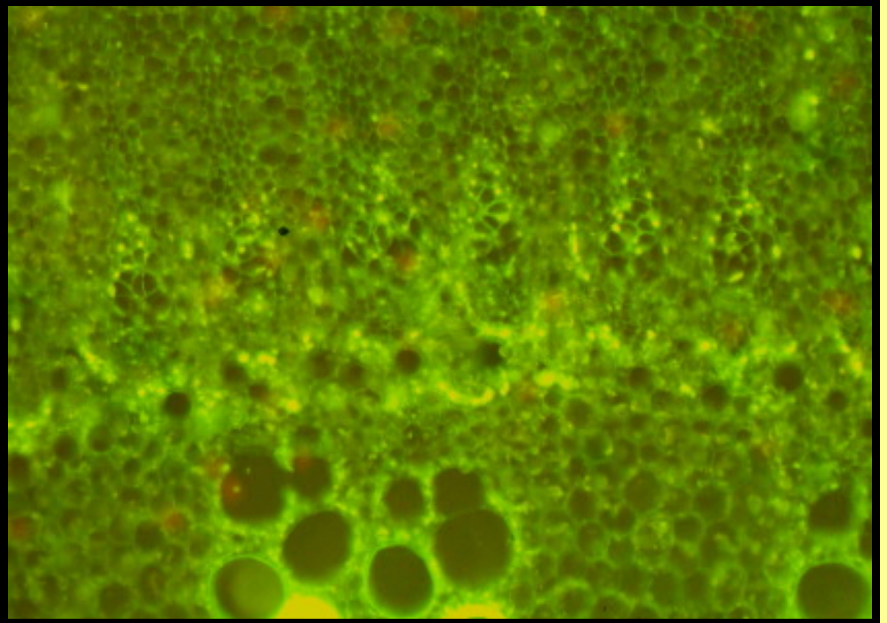
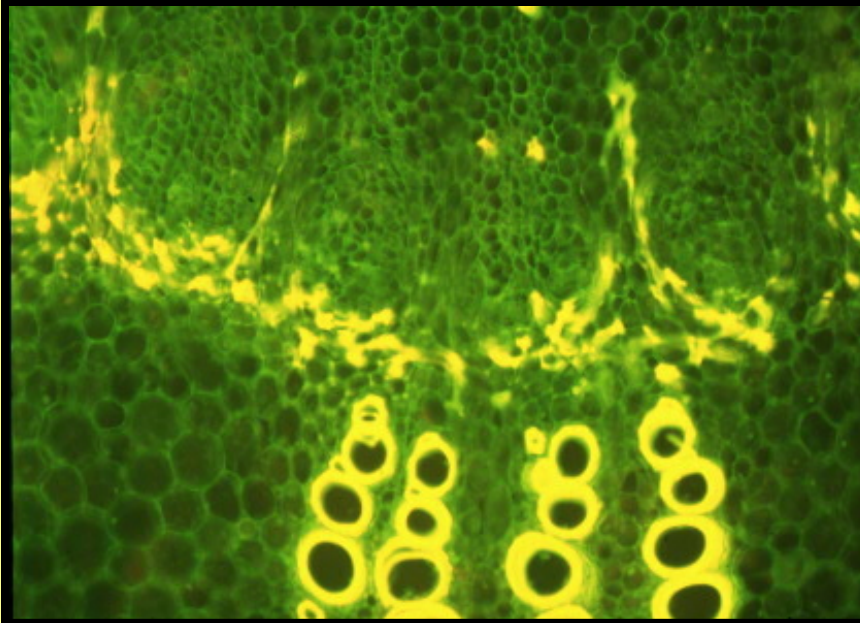
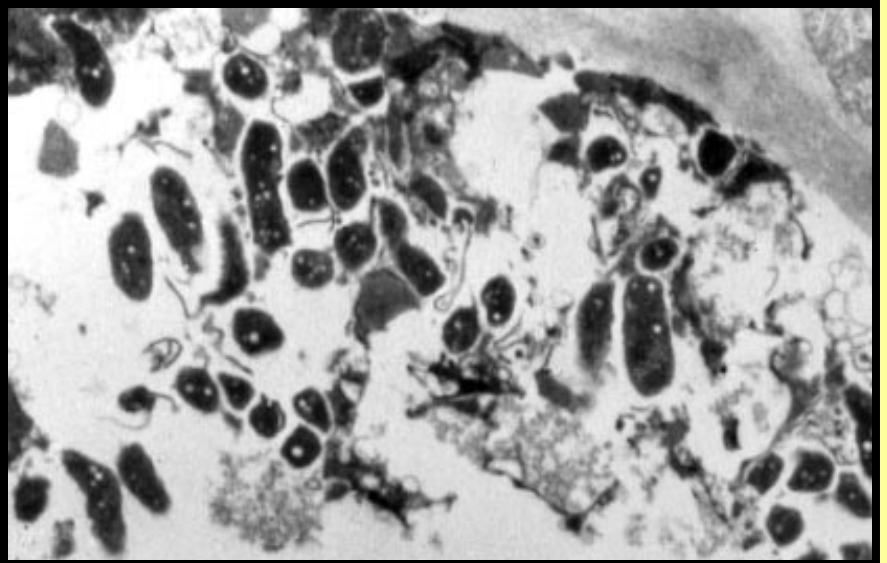
California Buckeye Leaf Scorch



BLAST Search Results for Buckeye Bacterium 16S rRNA Gene Sequence

Sequences producing significant alignments:				Score	E
				(Bits)	Value
gi 20378103 gb AF373185.1			Pectobacterium carotovorum subsp. ...	2557	0.0
gi 20378107 gb AF373189.1			Pectobacterium carotovorum subsp. ...	2553	0.0
gi 20378105 gb AF373187.1			Pectobacterium carotovorum subsp. ...	2549	0.0
gi 20378121 gb AF373203.1			Pectobacterium chrysanthemi strain...	2533	0.0
gi 20378101 gb AF373183.1			Pectobacterium carotovorum subsp. ...	2524	0.0
gi 20378106 gb AF373188.1			Pectobacterium carotovorum subsp. ...	2520	0.0
gi 38373979 gb AY363386.2			Klebsiella sp. rennanqilfy18 16S r...	2494	0.0
gi 71480490 gb DQ068854.1			Uncultured bacterium clone 6s27 16...	2486	0.0
gi 4582000 emb AJ233411.1	ECA233411		Erwinia carotovora 16S rRNA	2482	0.0
gi 20378104 gb AF373186.1			Pectobacterium carotovorum subsp. ...	2478	0.0
gi 2584742 emb Z96089.1	ECZ96089		Erwinia carotovora LMG 2404-T 1	2476	0.0
gi 56159736 gb AY825036.1			Enterobacter aerogenes strain NTG-...	2470	0.0
gi 71480420 gb DQ068784.1			Uncultured bacterium clone f1s5 16...	2464	0.0
gi 51243827 gb AY690721.1			Erwinia sp. MK14 16S ribosomal RNA ge	2462	0.0
gi 71480418 gb DQ068782.1			Uncultured bacterium clone f1s3 16...	2462	0.0
gi 6562390 emb AJ251468.1	EAE251468		Enterobacter aerogenes parti	2462	0.0

Papaya Bunchy Top Disease



BLAST Search Results for the Papaya Bunchy Top Rickettsia 16S rRNA Gene Sequence

Sequences producing significant alignments:	Score (Bits)	E Value
gi 506778 gb U11014.1 RBU11014 Rickettsia bellii 16S rRNA gene,	2789	0.0
gi 535750 gb L36103.1 RIRRGDF Rickettsia bellii 16S ribosomal RN	2763	0.0
gi 1147763 gb U42084.1 RPU42084 Rickettsia PAR 16S ribosomal RNA	2759	0.0
gi 58978283 gb AY753175.1 Rickettsia endosymbiont of Tetrany...	2728	0.0
gi 67003925 gb CP000053.1 Rickettsia felis URRWXCal2, complete	2718	0.0
gi 15620042 gb AE008647.1 Rickettsia conorii str. Malish 7, ...	2686	0.0
gi 506787 gb U11021.1 RRU11021 Rickettsia rickettsii 16S rRNA ge	2686	0.0
gi 535749 gb L36102.1 RIRRGDE Rickettsia sp. 16S ribosomal RNA (2680	0.0
gi 70568364 dbj AB196668.1 Rickettsia sp. PAR-OY gene for 16...	2678	0.0
gi 600169 gb U17645.1 U17645 Rickettsia honei 16S ribosomal RNA	2678	0.0
gi 535753 gb L36106.1 RIRRGDI Rickettsia massiliae 16S ribosomal	2676	0.0
gi 538433 gb L36214.1 RIRRGDM Rickettsia massiliae (strain Mt...	2676	0.0
gi 33438751 gb AF178037.2 Rickettsia heilongjiangii 16S ribo...	2674	0.0
gi 6979193 gb AF120026.1 Rickettsia sp. RpA4 strain RpA4 16S...	2672	0.0
gi 31323700 gb AF510102.1 Rickettsia sp. Chad 16S ribosomal RNA	2670	0.0
gi 600168 gb U17644.1 RAU17644 Rickettsia australis 16S ribosoma	2670	0.0
gi 538435 gb L36216.1 RIRRGDO Rickettsia rhipicephali (strain...	2668	0.0
gi 529688 gb U12460.1 RCU12460 Rickettsia conorii ITT-597 16S rR	2666	0.0

Babaco Bacterium



- Unidentified bacterium isolated from babaco or mountain papaya (hybrid of *Carica stipulata* and *C. pubescens*).
- Bacterium is very fastidious, and grows on media developed for the buckeye bacterium but not those developed for other fastidious bacteria.
- Is an alpha proteobacterium based on its 16S rRNA gene sequence.
- Not the papaya bunchy top rickettsia
 - 16S rRNA sequences differ
 - PBT bacterium did not grow on media developed for the Babaco bacterium

BLAST Search Results for Babaco Bacterium 16S rRNA Gene Sequence

Sequences producing significant alignments:	Score (Bits)	E Value
gi 57918592 gb AY742824.1 Candidatus Liberibacter americanus...	1961	0.0
gi 60267995 gb AY919312.1 Candidatus Liberibacter sp. LSg2 1...	1503	0.0
gi 38426928 gb AY444978.1 Uncultured bacterium clone M16 16S...	1427	0.0
gi 2290246 gb U87772.1 AGU87772 Afipia genosp. 6 16S ribosomal R	1417	0.0
gi 15430883 gb AF407383.1 Uncultured bacterium clone RB7C4 1...	1409	0.0
gi 15072624 emb AJ278726.1 EST278726 Methylocapsa acidiphila ...	1368	0.0
gi 73698030 gb DQ157274.1 Candidatus Liberibacter asiaticus ...	1348	0.0
gi 73698029 gb DQ157273.1 Candidatus Liberibacter asiaticus ...	1348	0.0
gi 2570146 dbj AB008366.1 Liberibacter sp. gene for 16S rRNA, p	1348	0.0
gi 73698031 gb DQ157275.1 Candidatus Liberibacter asiaticus ...	1340	0.0
gi 28397343 gb AY192576.1 Candidatus Liberibacter asiaticus ...	1332	0.0
gi 438690 gb L22533.1 LIE16SRRNB Liberibacter africanus 16S ribo	1330	0.0
gi 60267994 gb AY919311.1 Candidatus Liberibacter asiaticus ...	1324	0.0
gi 11125697 dbj AB038369.1 Liberibacter endosymbiont of Diap...	1312	0.0
gi 438689 gb L22532.1 LIE16SRRNA Liberibacter asiaticus 16S ribo	1300	0.0
gi 46361651 gb AY500255.1 Sinorhizobium sp. ORS 1400 16S rib...	1259	0.0
gi 58294523 gb AY875975.1 Sinorhizobium sp. ORS3178 16S ribo...	1251	0.0
gi 21952355 gb AF523952.1 Uncultured bacterium clone FW8 16S...	1223	0.0

Significance of the Babaco Bacterium

- The Babaco bacterium appears to be the most closely related species to the *Liberibacter* spp. that can be grown in axenic culture at the present time.
- It follows that the Babaco bacterium might be used in a “model system” for the development of media and procedures for culturing *Liberibacter* spp.

Isolation in Culture of Liberibacter

- Numerous media were developed that support growth of the Babaco bacterium and the corn stunt spiroplasma
- Media were tested for supporting growth of Liberibacter
- Fastidious bacteria isolated from two of 64 extracts from HLB infected citrus
- Cultures were PCR-positive for Liberibacter

Liberibacter Cultures

- Mixed culture: The Liberibacter is growing in association with other bacteria.
- Single colony isolations retain mixed culture at a high frequency.
 - Suggests that both bacteria benefit from the presence of the other.
- Second bacterium overshadows Liberibacter.
 - PCR detection of Liberibacter is often weak and inconsistent

Present Efforts

- Attempting to modify medium and culture conditions to support better growth of *Liberibacter*
- Will attempt to use second bacterium to establish new cultures of *Liberibacter*
- Will determine if cell-free supernatants from cultures of the second bacterium support growth of *Liberibacter*