LIBERIBACTERS ASSOCIATED WITH CITRUS HUANGLONGBING AND POTATO ZEBRA CHIP

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‘*Candidatus* Liberibacter’ Species – Associated Diseases

- **Citrus - Huanglongbing**
- **Potato – Zebra Chip**
- **Tomato/Potato yellows**
- **Haywire**

- **Other solanaceous crops**
- **Carrot – carrot yellows ?**
- **Other ?** **[wide host range]**

‘*Candidatus* Liberibacter’ Species-Associated Diseases

DISTRIBUTION

ZC

- Widespread
- Occurs in:
  - North America
    U.S. – (AZ* CA, CO, ID*, KS, MT*, ND, NE, NM, NV, TX, UT*, WY)
    Canada?
  - Mexico & Central America
  - Oceania
    (New Zealand)
  - Europe ?
    (CLso detected in carrots in Finland)
  - Other?

HLB

- Widespread
- Occurs in:
  - Most citrus-producing countries in Asia
  - South America
    (Brazil)
  - North America
    (U.S. - Florida)
  - Mexico & Central America
  - Caribbbean countries
  - Other?

‘*Candidatus* Liberibacter’ Species

- ‘*Ca.* Liberibacter asiaticus’ – Asian
- ‘*Ca.* Liberibacter americanus’ – (So.) American
- ‘*Ca.* Liberibacter africanus’ – African

- ‘*Ca.* Liberibacter africanus subsp. capensis’ – African (South Africa)

[associated with HLB-like leaf mottling in Cape chestnut (*Calodendrum capense*)]

‘*Candidatus* Liberibacter’ Species

- **‘*Ca.* Liberibacter solanacearum’** (Liefting et al., 2009)

  (syn. ‘*Ca.* Liberibacter psyllaurous’ (Hansen et al., 2009))

‘*Candidatus* Liberibacter’ Species

psyllaurous & solanacearum

- associated with solanaceous crops/plants
- probably synonyms based on 16S rRNA gene sequence analyses

❖ suggested preferred phytopathological name:

“*Candidatus* Liberibacter solanacearum”

Alternative:

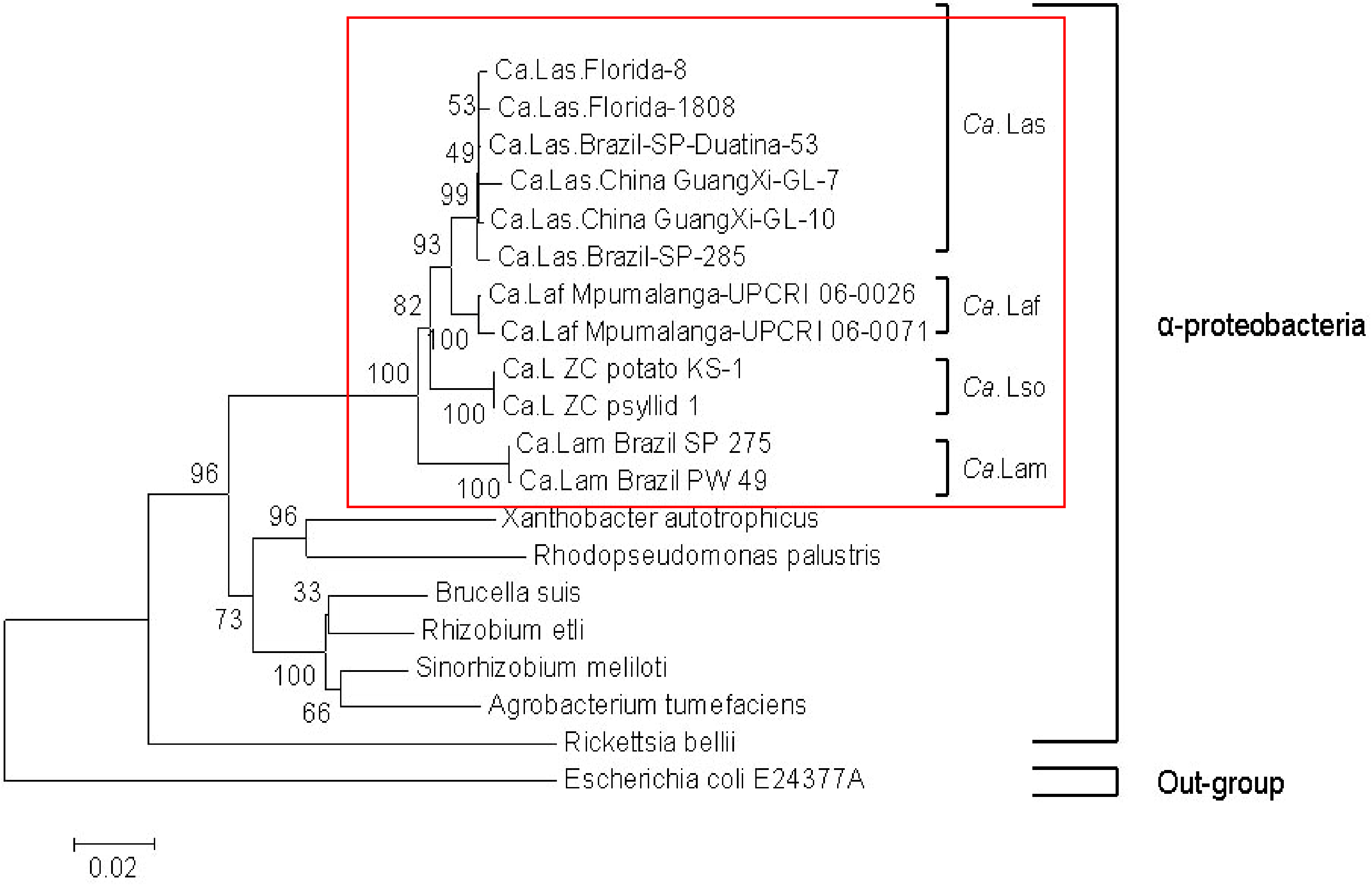
“*Candidatus* Liberibacter solanacearum”
(syn. “*Candidatus* Liberibacter psyllaurous”)

Proposed name published in IJSEM
Description of the bacterium provided
Reference material of the described strain is available

Hansen et al. Appl. Environ. Microbiol. 73:5862-5865, 2008
Liefting et al. Int. J. System. Evol. Microbiol. 59:2274-2276, 2009

‘*Candidatus* Liberibacter’ Species

Phylogenetic Relationships

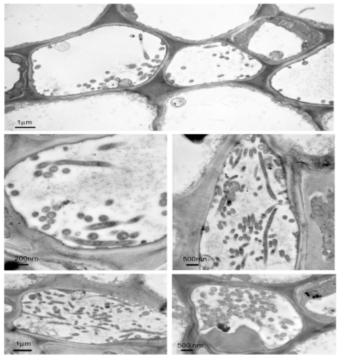


Lin *et al* 2009

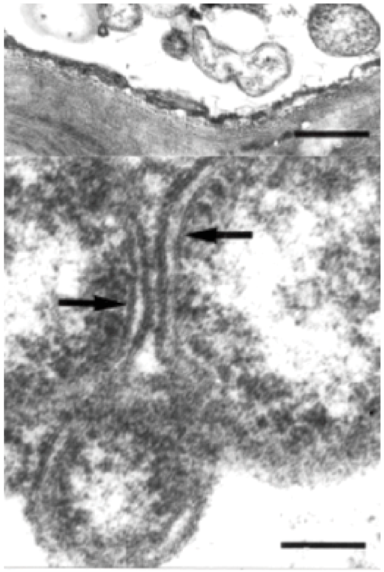
‘*Candidatus* Liberibacter’ Species

CHARACTERISTICS

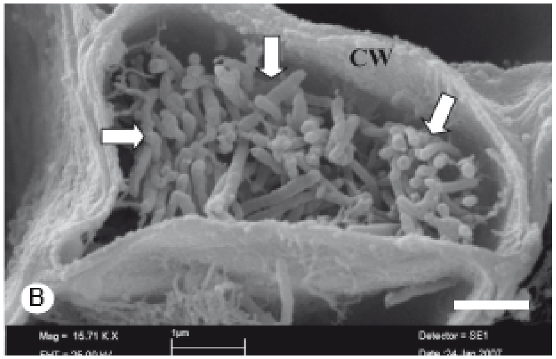
CLso



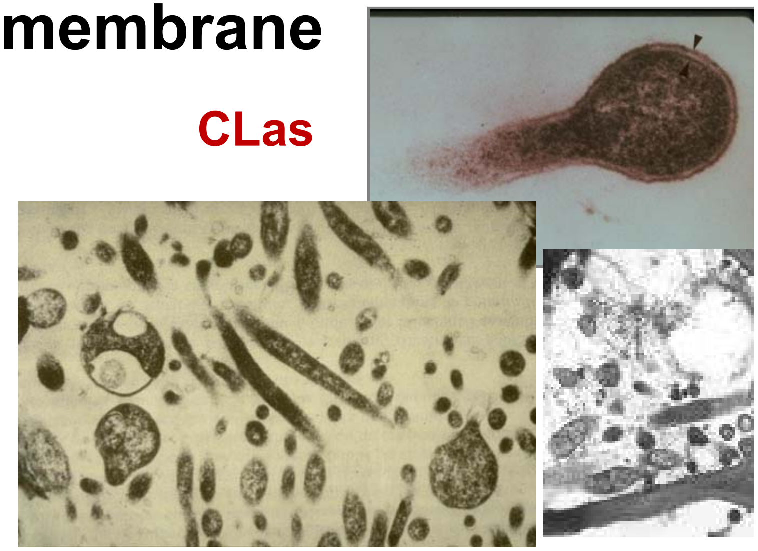
- Pleomorphic
- Triple-layered cell envelope
  - Outer cell wall
  - Peptidoglycan layer
  - Inner cytoplasmic membrane



CLam



CLas

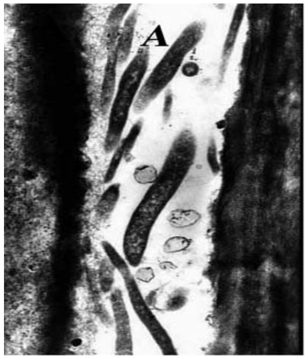


‘*Candidatus* Liberibacter’ Species

- **(Nutritionally) fastidious**
- **Non-culturable *in vitro* (to date)**
- **Phloem-limited and transmitted by psyllids**
- **Koch’s postulates have not been conclusively fulfilled**
- **Etiologies of the diseases not conclusively determined**
- **HLB - long latent period; complex, variable syndrome; nutrient deficiency-like symptoms**
- **ZC – short latent period; complex syndrome; symptoms similar to other potato diseases**

ZC

HLB



Bactericera cockerelli

Diaphorina citri

Trioza erytreae

ZC

HLB

LIBERIBACTER-ASSOCIATED DISEASE DETECTION / DIAGNOSIS

- **Visual symptoms – preliminary**
- **Confirmation by PCR detection/identification of '*Ca*. Liberibacter species' in symptomatic tissue based primarily on ribosomal 16S RNA and β operons**
- **Other methods available:**
  - **ELISA [HLB]**
  - **DNA probes – dot-blot hybridization [HLB]**
  - **Iodine reaction [HLB; ZC?]**
  - **Biological indexing [HLB]**
  - **TEM / SEM [HLB; ZC]**

Need improved, robust, reliable methods & technology to detect clinically asymptomatic plants

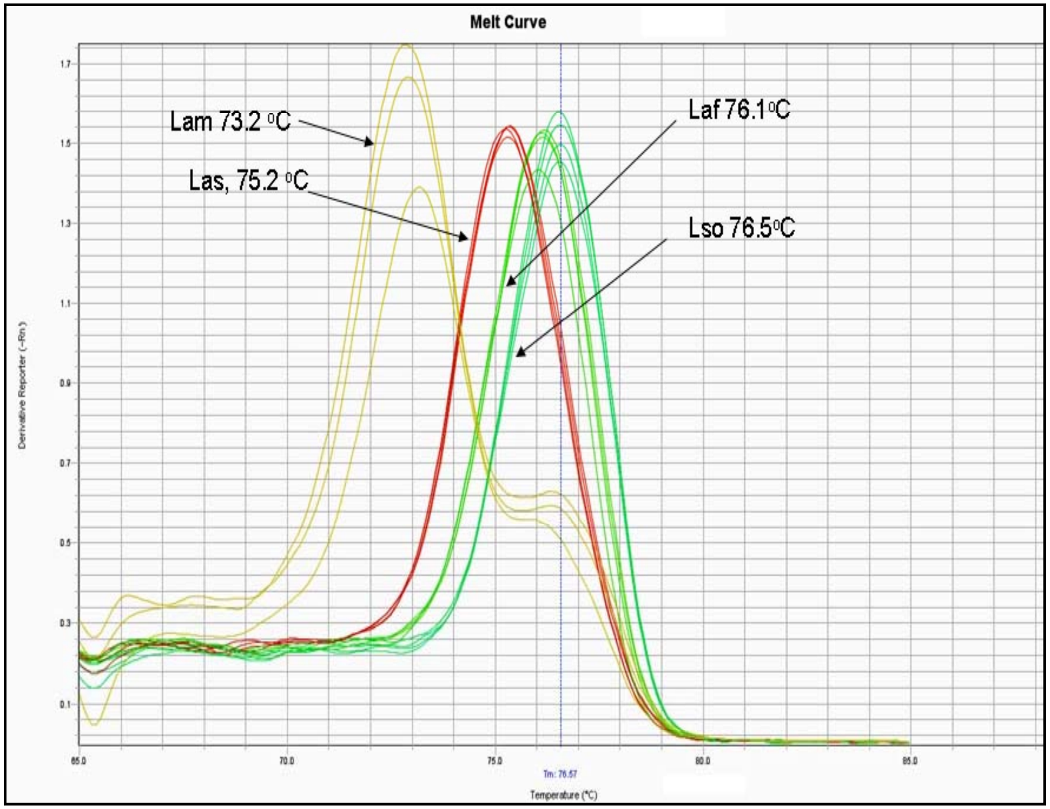
LIBERIBACTER DIAGNOSTICS

- Limitations of PCR-based detection of Liberibacters
  - Relies on detection of the bacterium
  - Detection of sub-clinical (pre-syptomatic/asymptomatic) plants may be problematic?

- Could be enhanced by:
  - Detection of Liberibacter-specific host response(s)?
  - Detection of disease-specific biomarker(s)?

Fluorescent Dye-based qPCR and HRMCA for Detection and Genotyping Liberibacter Species

- 1 pair of universal primers
- High resoluition melting curve analyses
- Simplified detection/identification system – single reaction
- Robust
- Cost-effective



Amplicon melting temperatures:

Lam 73.2 °C, Las 75.2 °C, Laf 76.1 °C, and Lso 76.5 °C.

Lin et al, unpublished data

DISEASE ETIOLOGY

- Single etiological / causal agent
- Multiple etiological / causal agents?
- Different etiologies in different regions?
- Different diseases, same etiological agent(s)?

(Bové, 2006; Wen et al, 2009)

- Complex pathosystems
- Various & variable symptoms associated with a variety of biotic (pathogenic & non-pathogenic) & abiotic factors

DISEASE ETIOLOGY

- **Modified Koch's postulates support etiological role of '*Candidatus* Liberibacter' species**
  - **Transmission**
    - **Psyllid**
    - **Grafting**
    - **Dodder**
  - **PCR detection**
  - **TEM / SEM observation of pleomorphic bacteria-like cells characteristic of '*Ca*. Liberibacter spp.' in phloem tissue**
  - **Genomics – draft genome sequences**
    - **Potential virulence genes/pathogenicity factors identified**
    - **Expression of virulence and CL-specific genes in diseased plants?**

DISEASE ETIOLOGY

Not conclusively determined / established

Other Potential Pathogens Associated with HLB & ZC

HLB Diseases

- Phytoplasmas
  - aster yellows (China)
  - pigeon pea witches' broom (Brazil)
- Other bacteria?
- Virus(es)
- Other?

ZC and Similar Potato Diseases

- Phytoplasmas
  - aster yellows
  - clover proliferation
  - Stolbur?
  - '*Ca*. Phytoplasma americanum' (NE)
  - '*Ca*. Phytoplasma australiense' (NZ)
- Other bacteria?
  - atypical strains of *Serratia marcescens*
- Virus(es)?
- Other?

Polymerase Chain Reaction (PCR) Detection of ‘*Candidatus* Liberibacter asiaticus & ‘*Candidatus* Phytoplasma asteris’ in Citrus with HLB / HLB-like Symptoms in China

Parameter	PCR Result					
	CLas +	CPa +	CLas + CPa +	CLas - CPa -	CLas + CPa -	CLas - CPa +
Number of Samples	89	110	69	11	20	41
%	26.2	32.4	20.3	3.2	5.9	12.0

Two surveys – 2006, 2007
11 cities in Guangdong Province, PRC
340 samples
C. sinensis (sweet orange), *C. maxima* (pummelo), *C. reticulata* (mandarin)
CLas = ‘*Ca*. L. asiaticus’; CPa = ‘*Ca*. P. asteris’
Nested PCR – CLas: fD1/D2 (1st round); OI1/OI2c (2nd round)
CPa: P1/P7 (1st round); fU5/fU3 (2nd round)

Chen et al, Phytopathology 99:236-242, 2009

‘*Candidatus* Liberibacter’ Species

EPIDEMIOLOGY

- Wide Host Range

CLso

Crops

Potato
Tomato
Capsicum species
Tamarillo
Cape gooseberry
Eggplant ?

Carrot

Perennial weeds

Black nightshade
Silverleaf nightshade
Wolfberry

HLB Liberibacters

Citrus species

Other Rutaceae
Clausena sp.
Murraya sp.
Severenia sp.

Experimental Hosts

Dodder
Tobacco

Unknown if alternate & perennial weed hosts are epidemiologically significant as reservoirs / sources of Liberibacter inoculum to initiate infection and disease development in citrus and potato

‘*Candidatus* Liberibacter’ Species

EPIDEMIOLOGY

➢ **Transmitted by:**

- **Psyllids**
- **Grafting**
- **Dodder**
- **Potato tuber seed pieces**
- **(True seed – not conclusive)**

❖ **May be insect endosymbionts that can be plant pathogens**

‘*Candidatus* Liberibacter’ Species

EPIDEMIOLOGY

➢ ***in planta* distribution:**

- **extensive, but uneven / non-uniform, colonization**
- **occur in various tissues from different part of HLB- & ZC-affected citrus and potato, respectively (based on PCR analyses)**

CLas

- root
- leaf midrib
- petal
- pistil
- stamen
- peduncle
- columella
- seed coat
- young, whole fruit

CLso

- stem
- stem epidermis
- stem cortices
- aerial tuber
- root tissues (epidermis, cortices)
- tubers

‘*Candidatus* Liberibacter’

3 species & 1 sub-species (to date)

- **asiaticus (Clas)**
- **africanus (CLaf)**
- **africanus subsp. capensis (CLafc)**
- **solanacearum (CLso) (syn. psyllaurous)**
- **other?**

No conclusive link between:

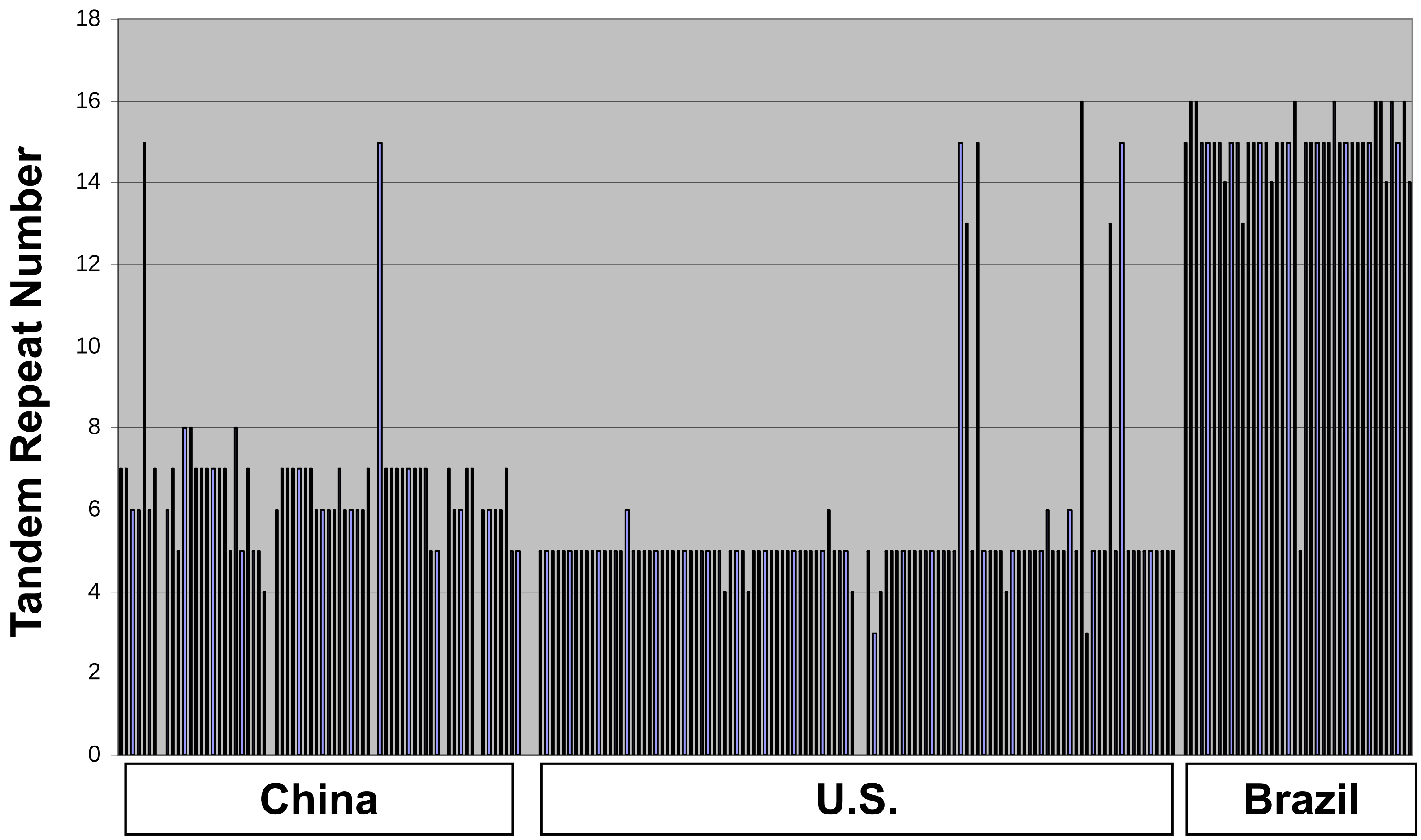
CLso and HLB

or

CLas, CLaf or CLam and ZC

(at this time)

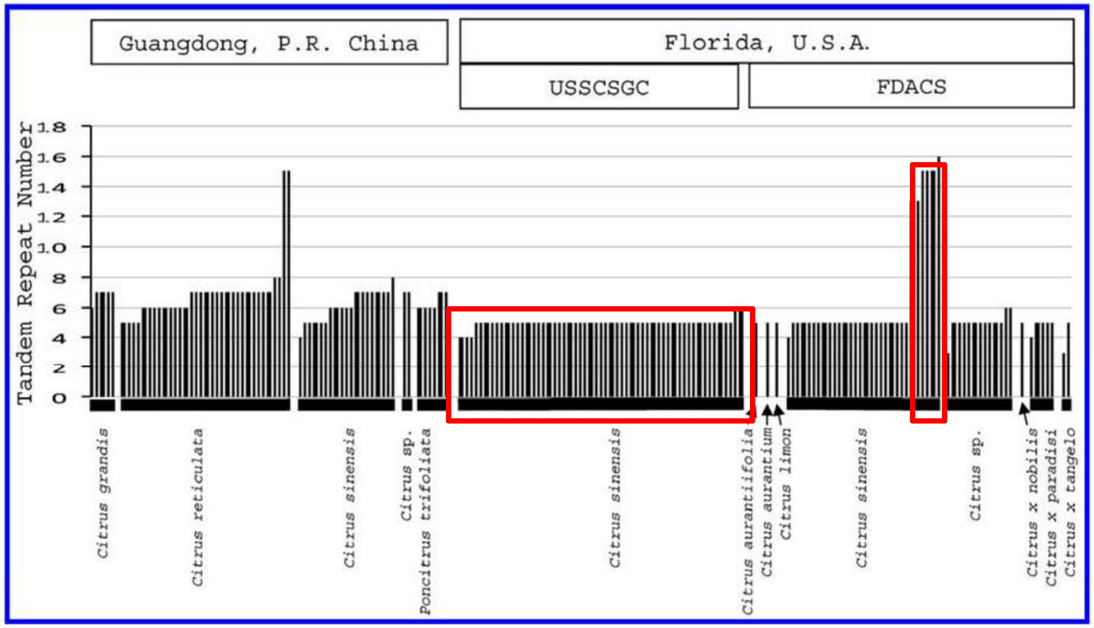
Tandem Repeat Number Analyses
Locus: CLIBASIA_01645 = bacteriophage repressor protein C1



Guangdong ≠ Florida ≠ Sao Paulo

Chen et al, unpublished data

Distribution of Tandem Repeat Numbers in '*Candidatus* Liberibacter asiaticus' Strains from Different Citrus Hosts Guangdong (China) and Florida (U.S.)



Locus: CLIBASIA_01645 = bacteriophage repressor protein C1

All Strains

TRN_{3-16}

No TRN = 9, 10, 11, 12

TRN ≤5, TRN = 6, TRN ≥7

Florida Strains

TRN_5 = 84.4%

$TRN_{>10}$ = 11.1% - all in central FL

Guangdong Strains

TRN_7 = 47.6%

$TRN_{>10}$ = 97.0%

Chen et al, Phytopathology 100:567-572, 2010

Genetic Variation among '*Candidatus* Liberibacter solanacearum' in Commercial Field Potato Samples

Single Nucleotide Polymorphisms

39 strains

- 2 SNPs – 16S rDNA
  1,171 bp sequence
  primer set ZCf/OI2c
- Two clades (Clades C1 & C2)

C1 – 99.8% similarity with C2;
99.8-100% similarity with ZC CLso
94.8-97.1% similarity with HLB
CLas, CLaf, CLam

40 strains

- 6 SNPs – partial 16S-ISR-23S rDNA
  946 or 948 bp sequence
  primers Lp Frag4-1161 & 480R
- Two clades (Clades C1& C2)

C1 – 99.3% similarity with C2
99.8-100% similarity with ZC CLso
85.6-90.5% similarity with HLB
CLas, CLaf, CLam

- CLso-PY and CLso-HW strains identical to CLso-ZC strains
- CLso-PY and CLso-HW strains distributed in both sub-clades in each group based on 16S rDNA and partial 16S-ISR-23S rDNA
- Distinct diseases (e.g., ZC & PY) associated by the same Liberibacter?

Wen et al, Plant Disease 93:1102-1115, 2009

‘*Candidatus* Liberibacter’ Species
Genome Research

- **Provide insights of structures & organization and of Liberibacter genomes**
- **Identify genes and associated metabolic pathways that help predict the lifestyle and nutritional requirements to facilitate culture *in vitro***
- **Identify candidate genes to target sites for disease mitigation**
- **Develop biomarkers for Liberibacter detection**
- **Reveal genetic features of Liberibacters acquired, modified, or lost through evolutionary adaptation, host selection and/or alteration of evolutionary potential**

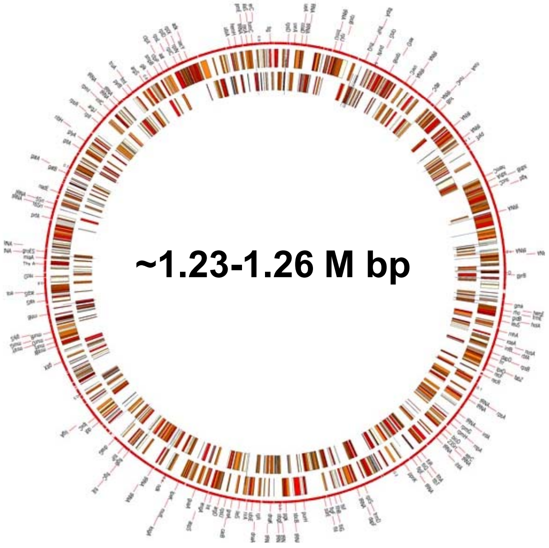
‘*Candidatus* Liberibacter’ Species

Genome-wide Bioinformatics & Functional Analyses

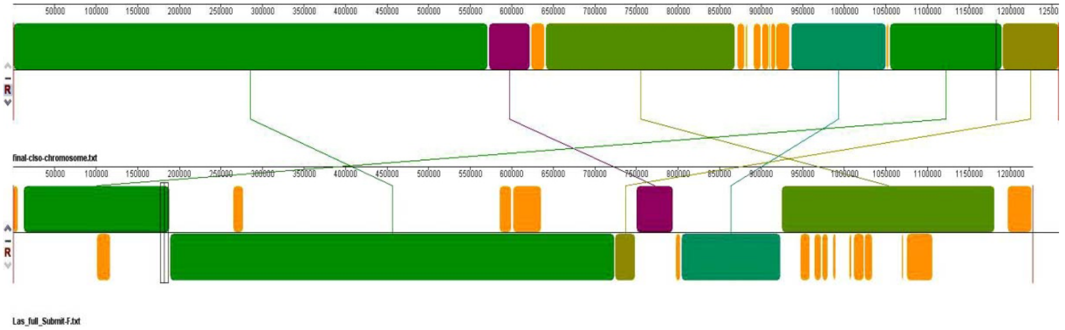
- May not have all of the genes encoding all of the proteins necessary for ‘housekeeping’ activities and metabolic pathways
- Genes encoding proteins that might be virulence or pathogenicty identified
- Functional characterization of proteins potentially involved in Liberibacter-host & Liberibacter-psyllid interactions
- Differentiation of putative biotypes / pathotypes

‘*Candidatus* Liberibacter’ Species

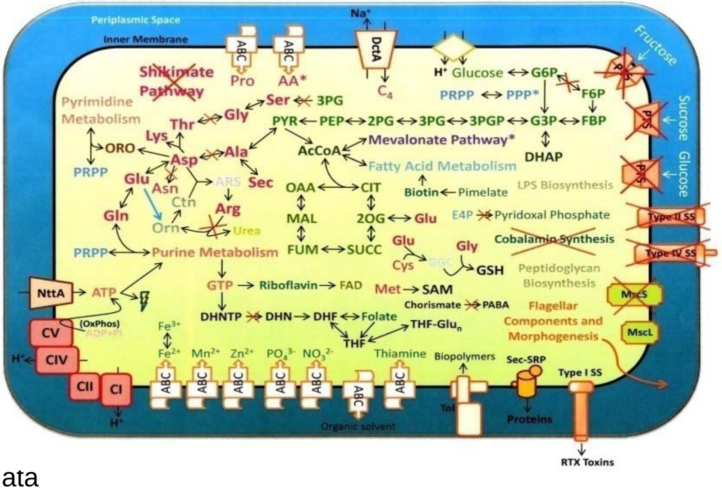
Genomics



Rearrangement of Genomes and Genomic Islands



Reconstruction of Metabolic Pathways of CLso



- **Draft sequences of CLas-FL & CLso-US genomes assembled & annotated**
- **Main differences are genome rearrangements & genomic islands**
- **CLas-China, CLam & CLso-NZ genomes being sequenced**
- **CLaf (& CLafc?) expected to be sequenced**

Lin et al, unpublished data

‘*Candidatus*. Liberibacter’ Genome Sequence and Analysis

- ~1.23-1.26 Mbp
- GC content ~35%
- 1,126 annotated proteins
  - 815 shared between Las & Lso
  - toxin proteins
  - secretion systems
  - transport & motility factors

MANAGEMENT OF LIBERIBACTER-ASSOCIATED DISEASES

- Avoidance, removal of inoculum
  - prevent introduction, establishment & spread
  - phytosanitary measures
    - ✓ quarantines
    - ✓ eradication
    - ✓ certification programs
      - propagation & use of Liberibacter-free planting stock
- Reduce Liberibacter transmission
  - psyllid vsctor control
    - ✓ chemical
    - ✓ biological control (if possible)
- Cultural practices?

MANAGEMENT OF LIBERIBACTER-ASSOCIATED DISEASES

- **Long-term, sustainable management may be based on host resistance – pathogen(s); psyllid**
  - ❖ **genetic**
  - ❖ **transgenic**
  - ❖ **induced**

➢ **Effective, economical management likely to be based on integrated strategy**

❖ **Other novel approaches needed**

SUMMARY

- Widespread
- Broad host ranges
- Associated with some economically-important diseases
- Role in disease is not conclusively known
- Likely involved in disease etiology, but may not be only / sole etiological agent in all cases
- Genetically & pathogenically distinct within & among species
- Biology & ecology poorly understood
- Genomics / bioinformatics research likely to lead to identification of biomarkers for Liberibacter and/or disease detection and candidate genes to target for disease mitigation