Functional and comparative genomics of Liberibacter.

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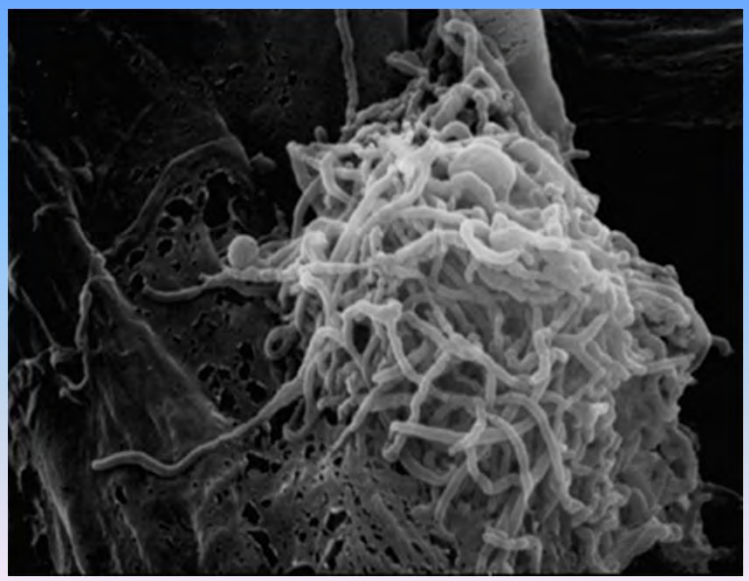
What does the Las bacterium look like?



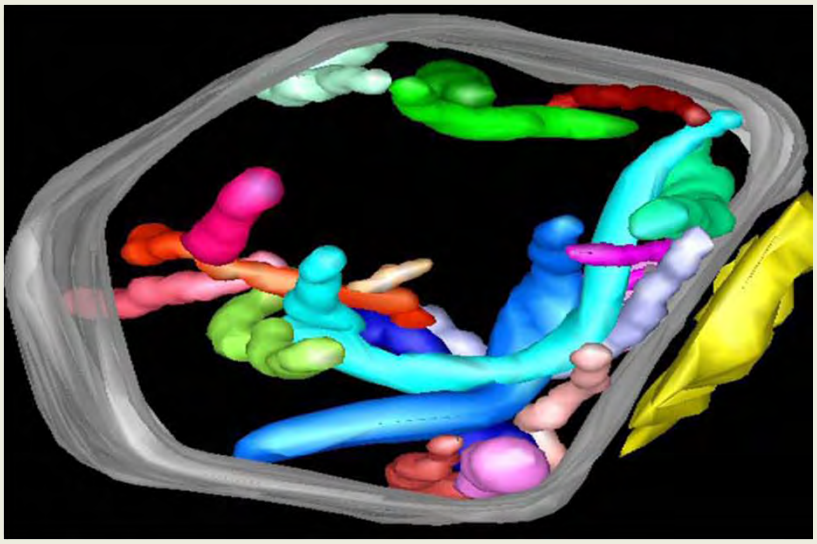
Las in biofilm in midgut of a psyllid. Photo courtesy **Dr. Micheal Davis, UF**

How much of the cell volume is occupied by Liberibacter?

With Las, up to 40% in citrus, and up to 90% in periwinkle.

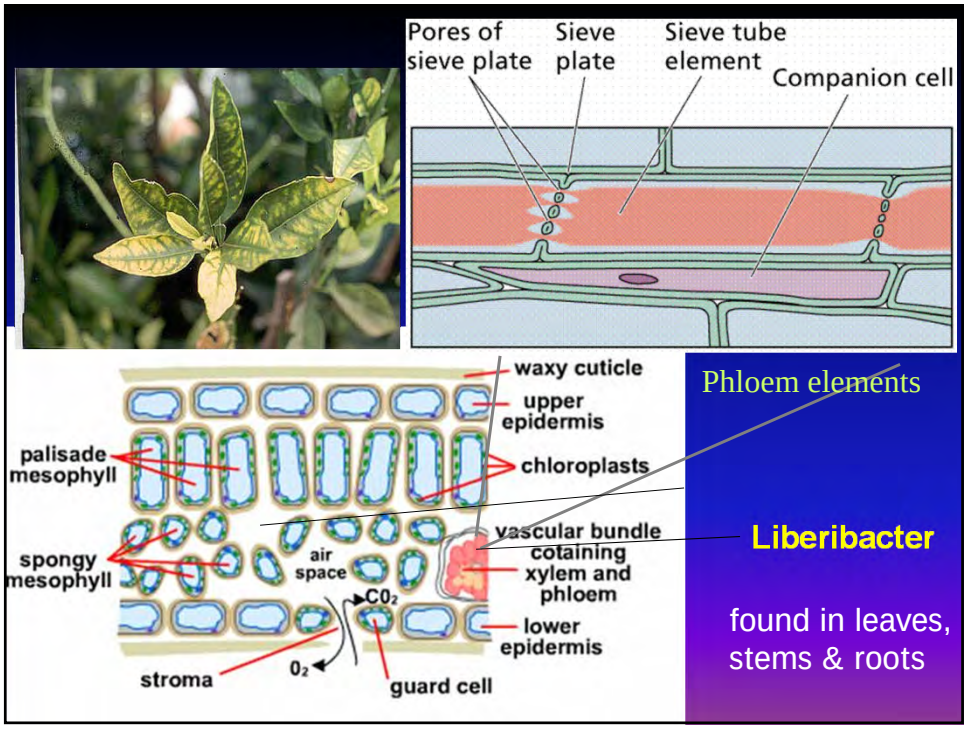
Photos courtesy **Dr. Byung-Ho Kang, UF**

Liberibacter is a biotroph's biotroph



2 microns

Each Liberibacter cell colorized;
courtesy Dr. Byung-Ho Kang, UF



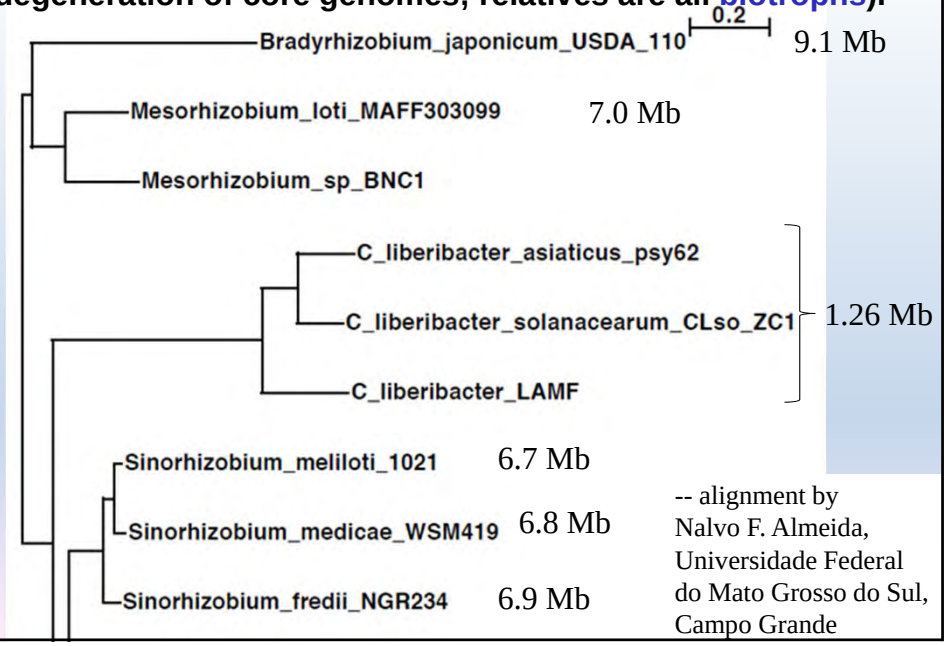
Liberibacters sequenced to date.

- Duan et al. 2009. Ca. L. asiaticus (Florida). MPMI.
- Lin et al. 2011. Ca. L. solanacearum. (Texas) PLoS One.
- Wulff et al. 2012. Ca. L. americanus (São Paulo) In preparation; refer poster this meeting for details.

Liberibacter sequencing projects in process (Hong Lin, USDA-ARS, Parlier, CA).

- '*Ca*. L. asiaticus ' strain from China (Guangxi; 2 contigs)
- '*Ca*. L. africanus' strain from South Africa (Pretoria; 23 contigs)
- '*Ca*. L. americanus' strain from Brazil (São Paulo,; 41 contigs)

What have we learned? (Liberibacters show large scale degeneration of core genomes; relatives are all biotrophs).



-- alignment by Nalvo F. Almeida, Universidade Federal do Mato Grosso do Sul, Campo Grande

Proteins **shared** by Liberibacter asiaticus and (intracellular) *Bartonella henselae*

DNA transactions (Low G+C Genomes)
- Integrase/recombinase
- Carboxynorspermidine decarboxylase
- Hypothetical and phage related proteins

Homeostasis
- Na+/H+ antiporter (homeostasis)

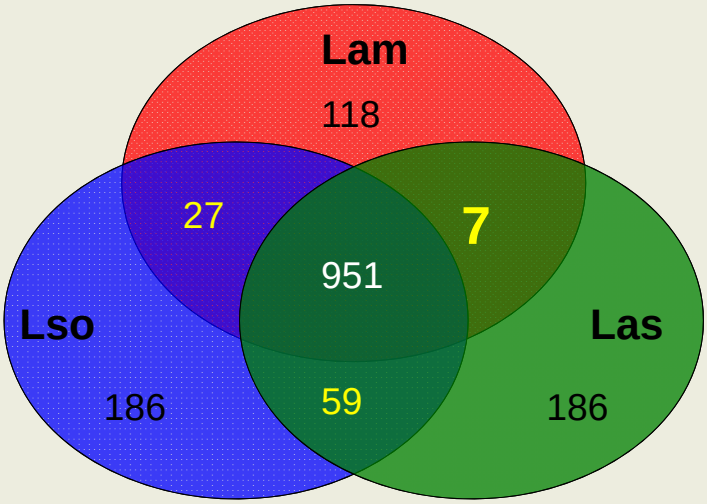
Proteins **Unique** to 'Ca. Liberibacter asiaticus'

3 proteins **for surface polysaccharide synthesis**
- Glysosyl transferase
- dTDP-4-dehydrorhamnose epimerase
- dTDP-4-dehydrorhamnose reductase

Metabolism
- 7 proteins of mevalonic acid pathway (also in Lam)
- ATP/ADP translocase
- Ascorbate phosphotransporter System IIC

--slide courtesy **John Hartung, USDA-ARS**

No obvious citrus host range (offensive) effectors found shared by Lam and Las.



--Figure courtesy João Carlos Setubal, Universidade de São Paulo

What have we learned? Liberibacters preserve disproportionate # of defensive genes...

Functional Class	Functional Categories		% of total	% in Wolbachia	% in alpha proteo	% in Bacteria
Information	J	Translation, ribosomal structure & biogen	10.94	13.91	4.31	4.34
	K	Transcription	2.49	2.07	5.69	5.96
	L	DNA replication, recombination, repair	6.85	6.21	4.36	4.94
Cellular processes	D	Cell division and chromosome partitioning	1.16	1.03	0.71	0.76
	V	Defense mechanisms	0.44	0.23	0.97	1.25
	O	Postranslational modification	4.18	5.86	3.24	2.95
	M	Cell envelope biogenesis, outer membrane	4.98	3.79	4.34	4.35
	P	Inorganic ion transport & metabolism	2.22	4.02	4.79	4.56
	U	Intracellular trafficking & secretion	0.62	3.33	1.78	1.76
	N	Cell motility	4.63	0.11	1.18	1.41
	T	Signal transduction	1.60	1.15	3.70	3.95
Metabolism	F	Nucleotide transport and metabolism	3.56	4.25	1.61	1.78
	G	Carbohydrate transport and metabolism	2.05	2.76	4.90	4.86
	E	Amino acid transport and metabolism	4.27	4.37	8.18	7.26
	H	Coenzyme metabolism	4.72	3.79	2.97	2.99
	I	Lipid metabolism	2.94	2.99	3.54	2.93
	C	Energy production and conversion	5.52	7.82	5.15	4.80
	Q	Secondary metabolite transport & metabo	0.62	1.26	2.80	2.33
Poorly characterize	R	General function prediction only	6.05	7.24	10.62	10.43
	S	Function unknown	4.27	3.56	6.06	6.14
Not in COGs			25.89	20.23	19.03	20.16
			100.00	100.00	99.95	99.93

but few offensive weapons; NO endoglucanase, galacturonase, pectinase, xylanase, pectate lyase, or cellulase

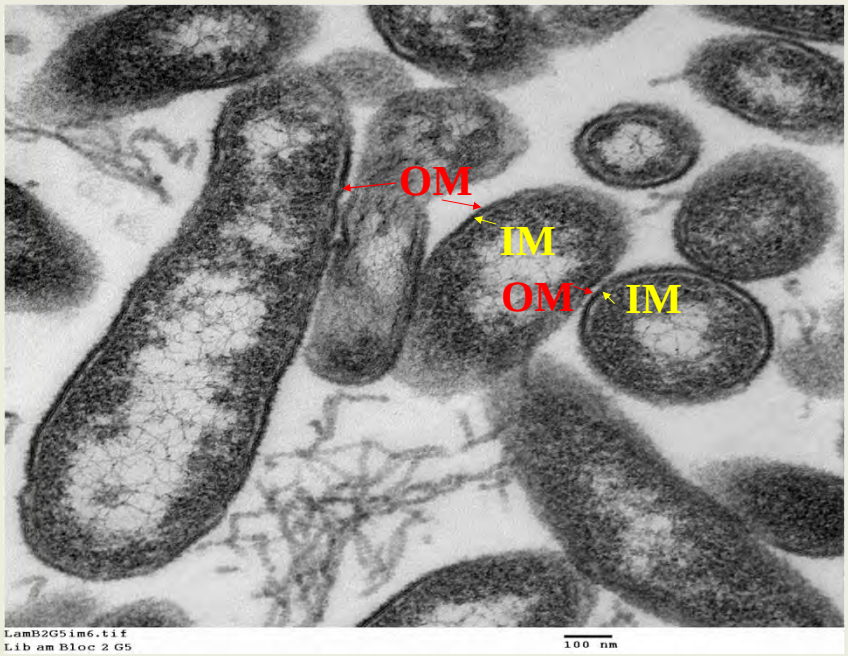
Liberibacters try to shed elicitor PAMPs

Pathogen Associated Molecular Patterns are recognized as part of innate "immune" response, that includes reactive oxygen production and can lead to programmed cell death.

Two classic examples:

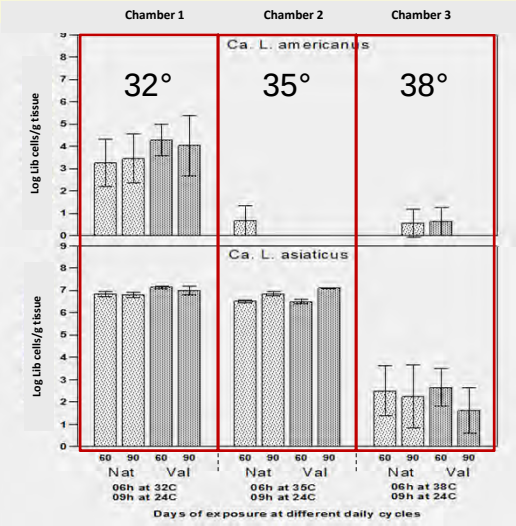
1. Flagellin. No flagella seen with Liberibacter. However, **Dr. Yong Ping Duan, USDA-ARS, Ft. Pierce**, has demonstrated that Las flagellin structural gene functionally complements *S. meliloti* mutation AND when over-expressed in plants, elicits callose deposition.

2. Lipopolysaccharide (LPS). Lam is missing nearly all LPS genes, including core lipidA. These LPS genes are not contiguous (implying selection for multiple events). This likely makes Lam OM more fragile, heat-sensitive, and sensitive to reactive O2. Annotation results of **Dr. Ricardo Harakava, Instituto Biológico, São Paulo**.



Ca. L. americanus has OM --Photo courtesy J. Bove

Lam is less heat tolerant than Las.



***Ca.* L. americanus multiplication *in planta* was significantly affected when incubated at 32°C, 35°C or 38°C, 6 h/day for 60 days.**

***Ca.* L. asiaticus multiplication *in planta* was significantly affected only when when incubated at 38°C, 6 h/day for 60 days.**

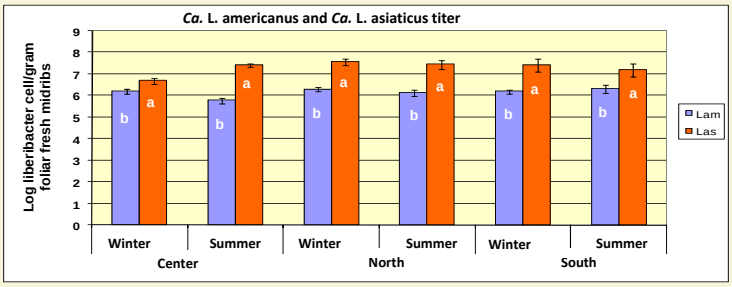
Lopes et al., 2009.

FUNDECITRUS

Las reaches higher titers in citrus than Lam.

Better Las defenses?

Bacteria	Log liberibacter cell/gram fresh midribs	
	Field	Graft transmited
Las	6.67	6.42
Lam	5.74	4.87



Lopes et al., 2009a

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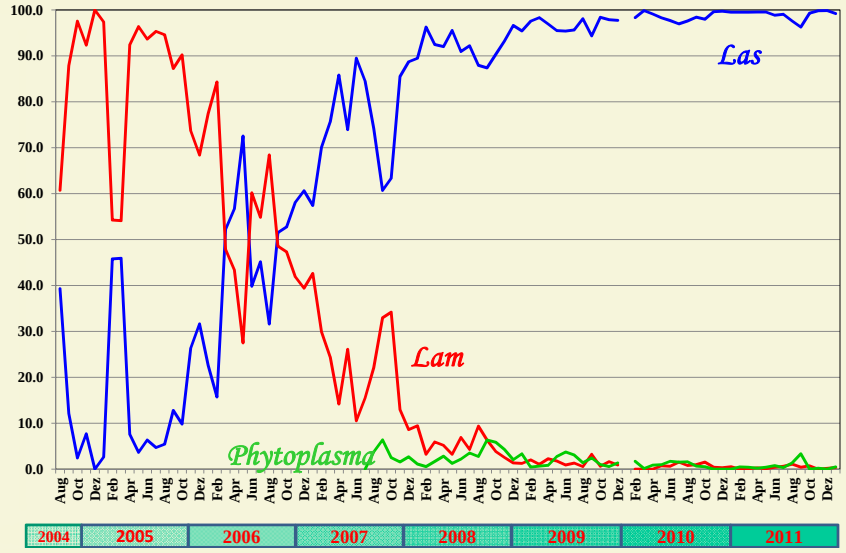
Lam is dying out in Brazil. Why?



--Slide courtesy N. Wulff

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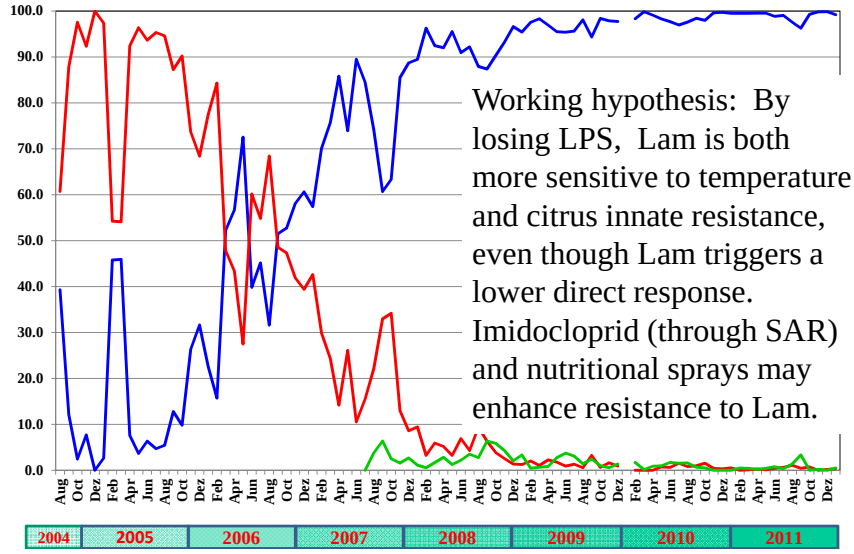
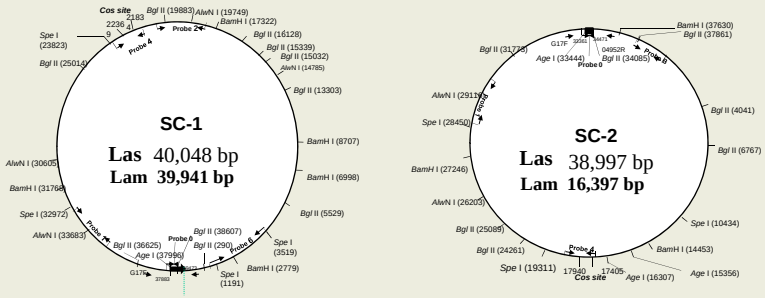


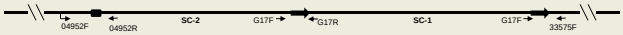
Working hypothesis: By losing LPS, Lam is both more sensitive to temperature and citrus innate resistance, even though Lam triggers a lower direct response. Imidocloprid (through SAR) and nutritional sprays may enhance resistance to Lam.

Both Las and Lam carry two prophages; one replicating as an excision plasmid, allowing amplification of lysogenic conversion genes.

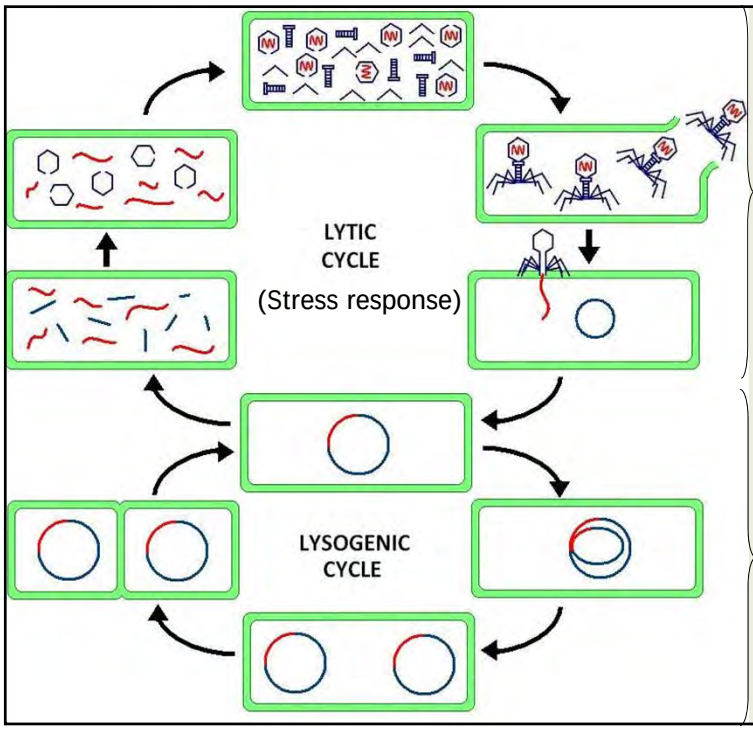
(Las and Lam also both carry suicide programs; Dr. Y.P. Duan has evidence for heat activation).



--Zhang et al. 2011 MPMI 24:458–468.

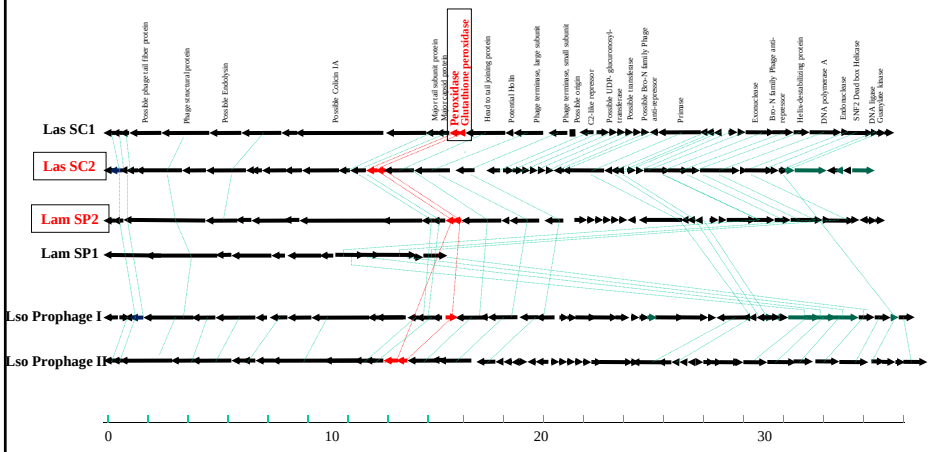


Wulff, N.A. (Fundecitrus), Zhang, S. (UF), Gabriel, D.W. (UF)



Only in plants

In psyllids and also plants, but not 100%



In red:

Glutathione peroxidase

Peroxidase

Conclusions

- Liberibacters show large scale degeneration of core genomes; relatives are all biotrophs.
- Some surface polysaccharide enzymes are unique.
- No obvious citrus host range offensive effectors shared by Lam and Las.
- Liberibacters keep disproportionate # of defense genes
- Liberibacters try to shed (elicitor) PAMPs
  - No flagella seen
  - Lam is missing nearly all LPS genes (scattered).
  - Lam retains OM

Speculation & Potential Directions

- Loss of Lam LPS explains Lam: 1) heat sensitivity (OM fragile); 2) lower titer (OM lets phytoalexins through), and 3) one aspect of poor performance as a pathogen in Brazil.
- Peroxidases are lysogenic conversion genes replicating in high number on excision plasmids. Las has 2X as many as Lam, resulting in better control of host programmed cell death.
- Las has superior strategy at moment: better host PCD control; keep LPS. But existence of Lam implies adaptive flexibility.
- Strains of both Las and Lam carry lytic phage (what % of strains?) Can be (safely) activated with chemicals?

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