

A known Q-bacterium in a new host:  
*Clavibacter michiganensis* ssp. *sepedonicus* in tomato



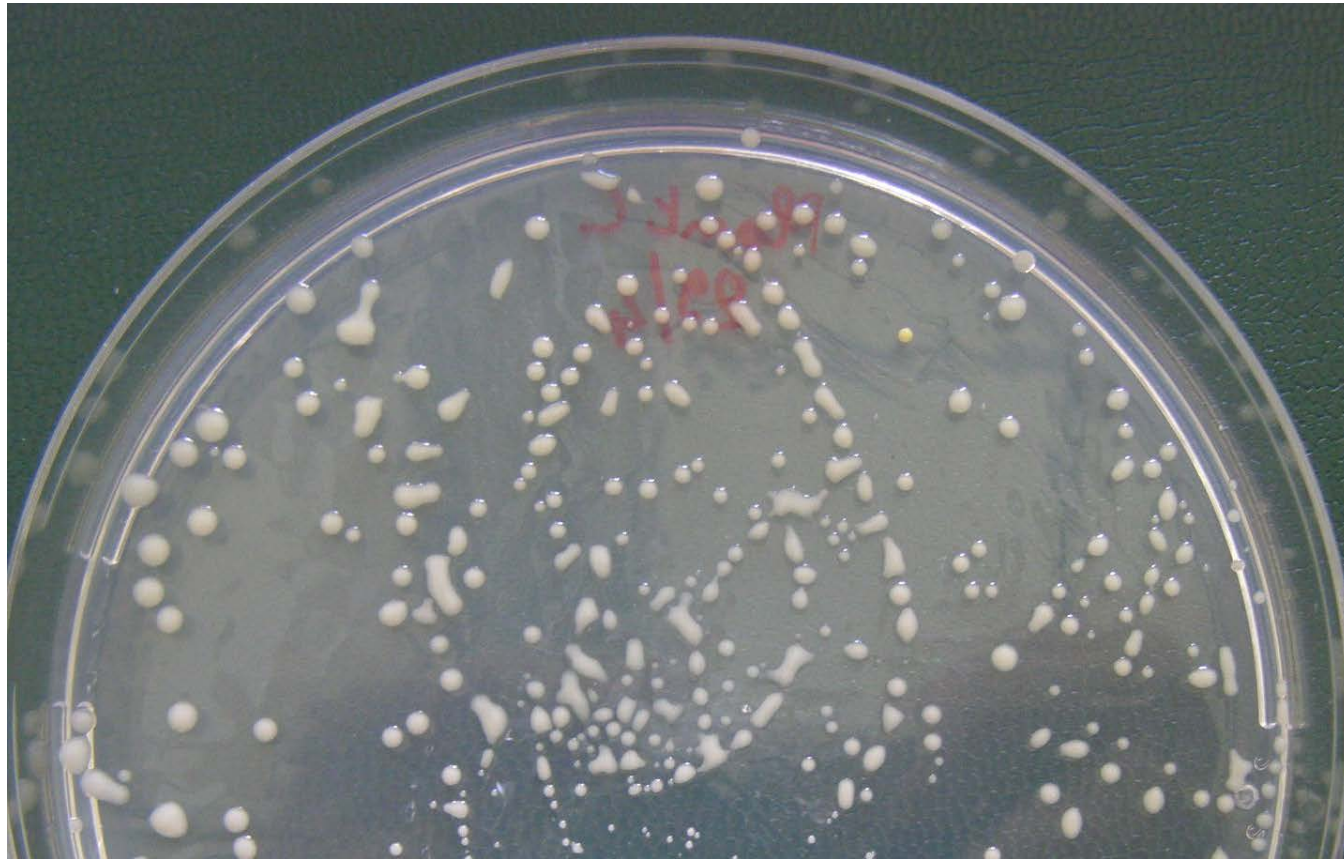
Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus*  
in tomato (*Solanum lycopersicum*)



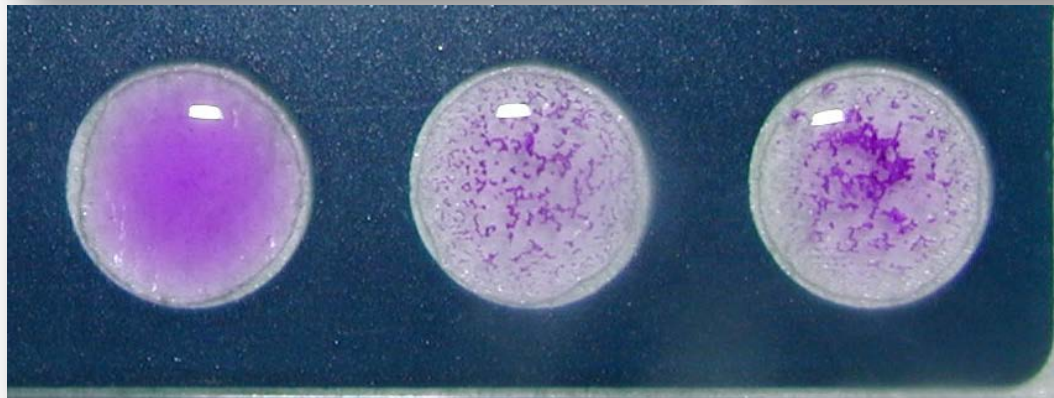
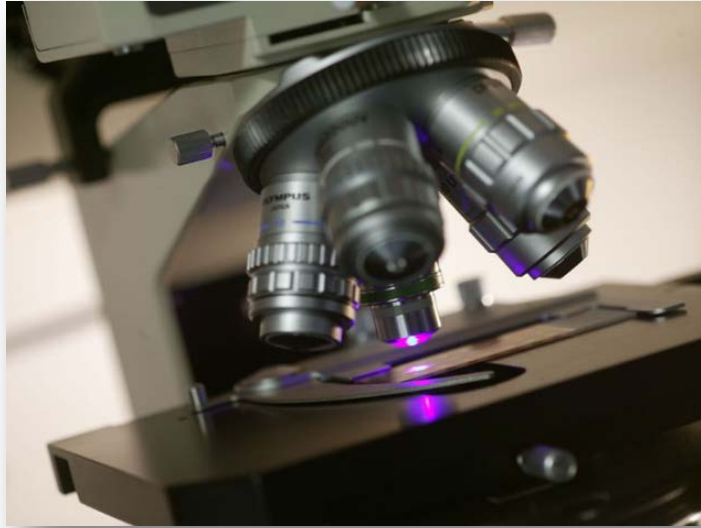
Road map of analysis:

- Isolation on culture media
- Pure culture
- Identification
- Pathogenicity

Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus*  
in tomato (*Solanum lycopersicum*)  
Isolation

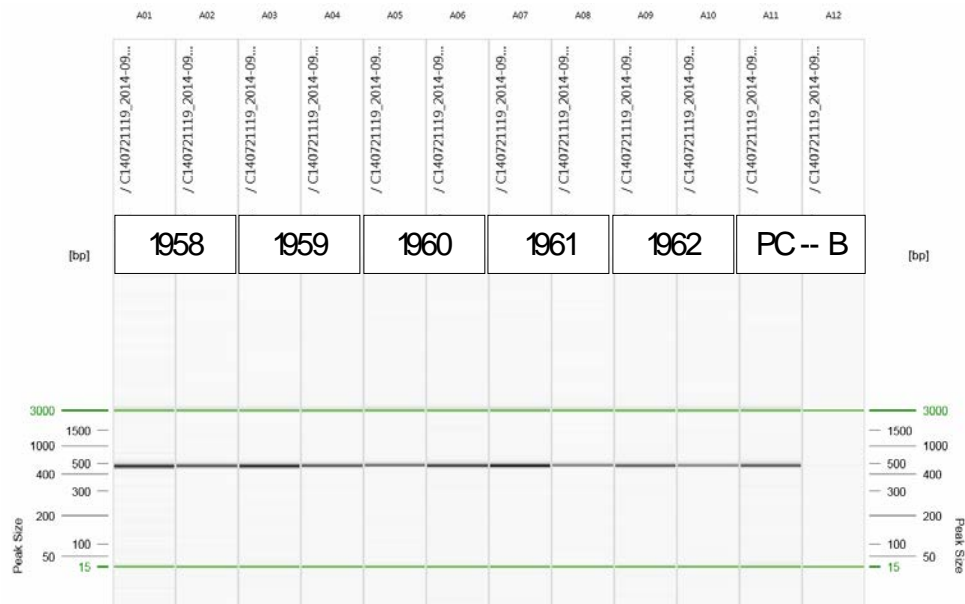


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Serological identification



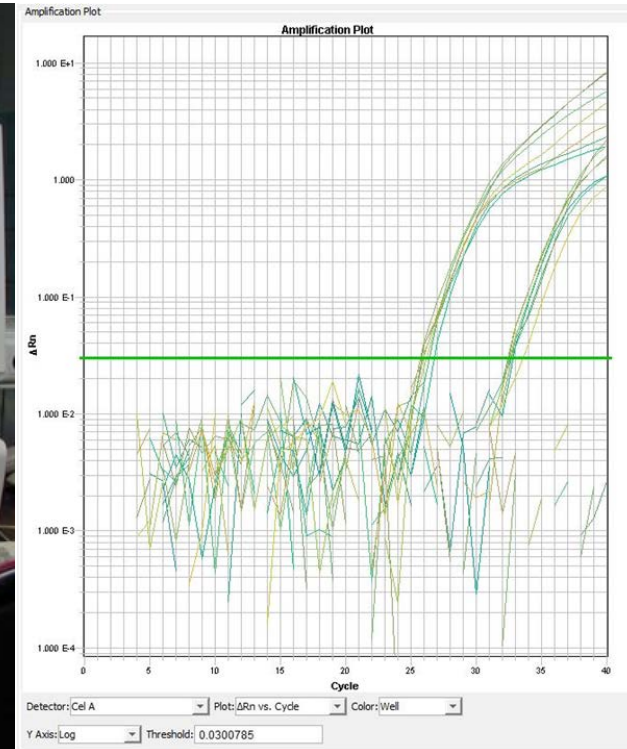
# Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus* in tomato (*Solanum lycopersicum*)

## Identification by taxon-specific PCR (2006/ 56/ EC)



# Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus* in tomato (*Solanum lycopersicum*)

## Identification by taxon TaqMan PCR (*cel A* – Gudmestad *et al.*, 2009)



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Confirmation of pathogenicity



# Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus* in tomato (*Solanum lycopersicum*)

Identification by DNA barcode: *gyr B* (Zaluga *et al.*, 2011)

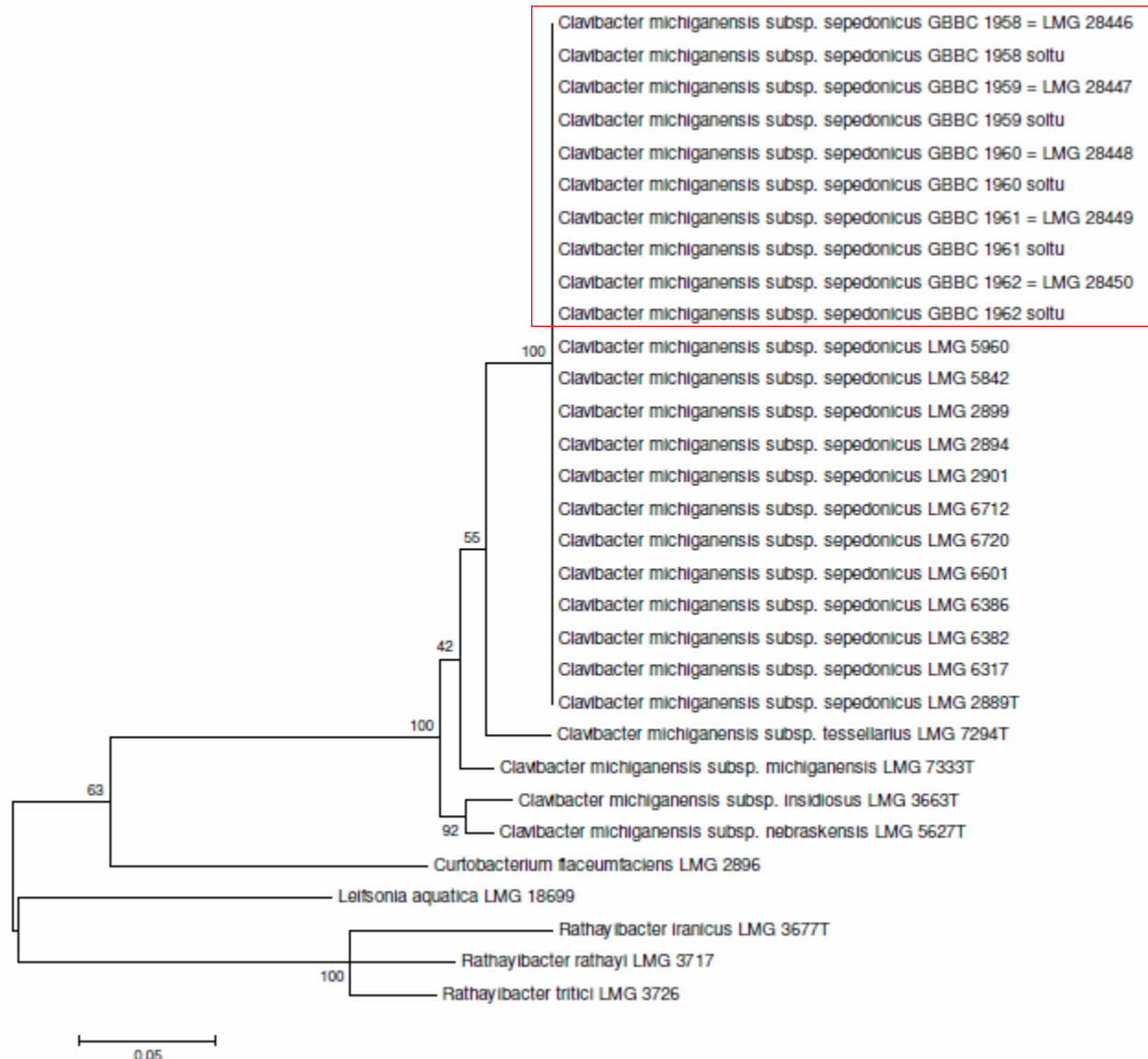


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AGTAATCAGCTACCTTTATCCCTAGCCCCCTGCGCCCCGCGCCCTTGGA  
GTATGGTGAGGTCTGCTCTGGGTCCCTCGCCACCATGTACGTGAGC  
GTGACCCTAGCTCCGTGCGCCACTCCGGCGGCCCTAACCTGGCGC  
GTACCCGGACTACGGTGGTTACCACGTGGCGGCCGCGCTGCTGC  
GTCCCGAGGGCCATCCTGGCCACCGCGTACGGCGCCCTCTCCGC  
CGGGGCGCTGCCGAGCCAACGCGGTAGCCCACGGCCTCAATGGT  
SGCTACAGCAGCCCCGCCGAATACCACGCACACCATCACCCGCATCAC  
GCTGCGCTCCGGATTGCTGCAGACGCTCAACCCCGGCCCTCCAI  
CAGCTGTCCCCAGCGGCCAGCGGCGAAACCTTTGTGAATGGA  
GGAAGCCAAGGTATGCGGTGCTGGGGCGCGCCCCCTGGTCCCGC  
TAGACAGGACAGGAGAAGGGACAAGGGGAGAAATATGGGGGTGC  
TGTATATTTGTGCCTCGCACCCCCAGGTTTGTAGGGCAGAAGAGT  
ACCTCGTTGGCAACCTCGGGACCCCTTCGGAAGCTCCCGGTAGTGTC  
TAGCAGAATCGCGTCCTTTAGTTGGCTGCCACCAGCATTCTTACACCI  
GGGTGATTGGATTCCCTTGCCGAGCCTACTCAACTTAGCTACCCACTCT
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# Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus* in tomato (*Solanum lycopersicum*)

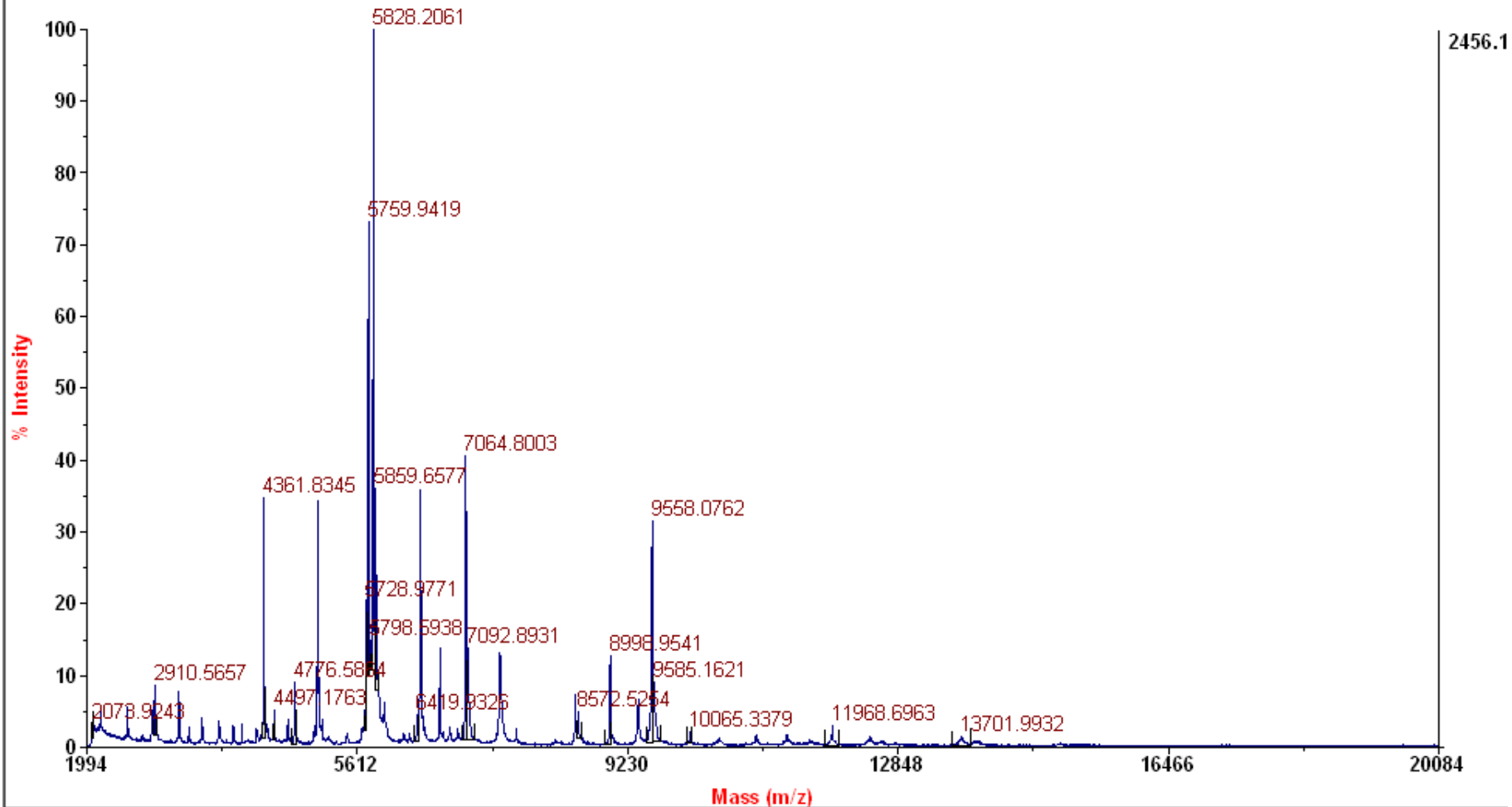
## Identification by DNA barcode: *gyr* B



# Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus* in tomato (*Solanum lycopersicum*)

## Identification by MALDI-TOF: biomarker proteins

4700 Linear Spec #1 MC[BP = 5828.3, 2456]



# Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus* in tomato (*Solanum lycopersicum*)

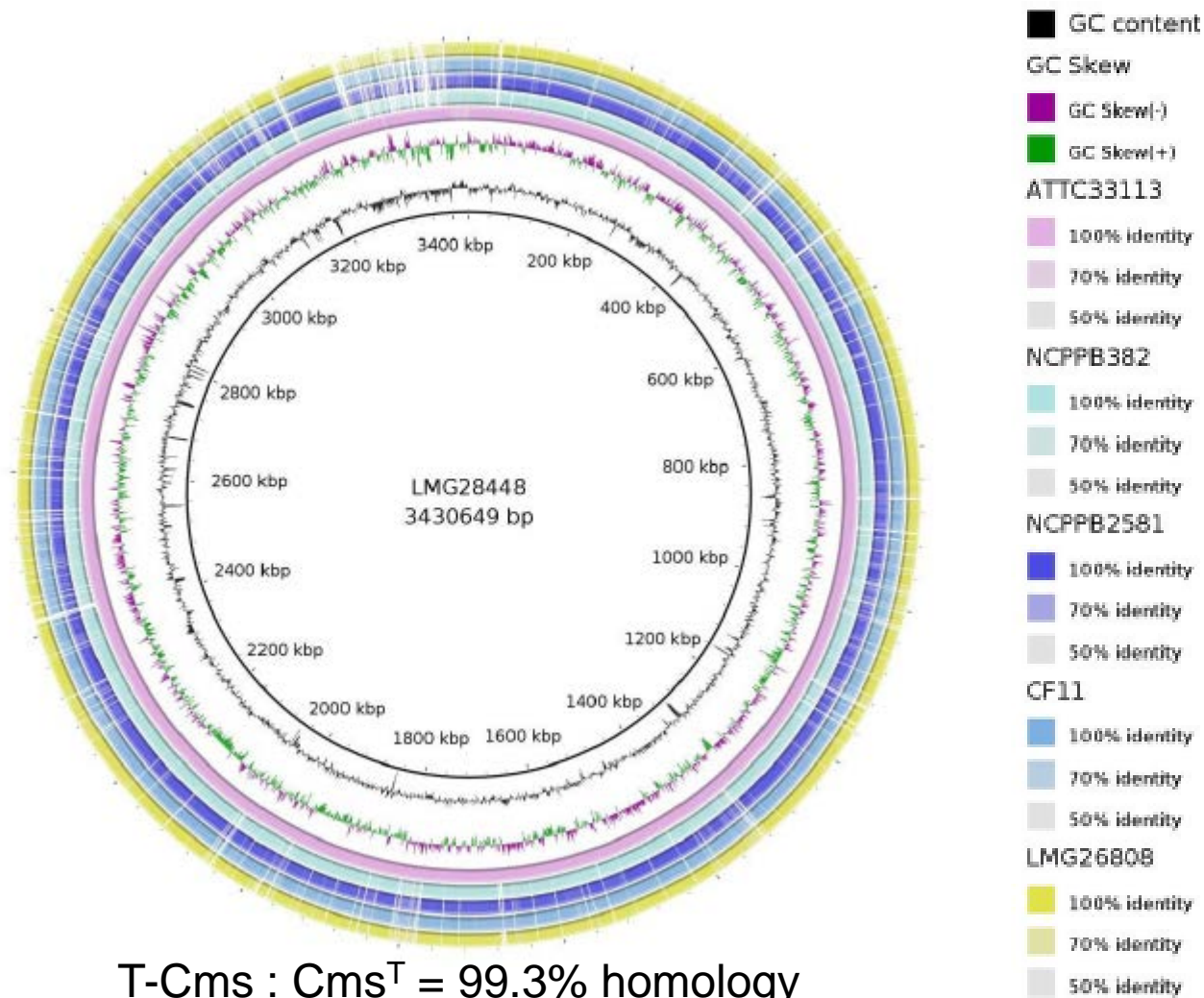
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1. T-Oms has genetic features that determine infection of tomato
2. T-Oms is a P-Oms that accidentally infected tomato (as in sugar beet – Bugbee *et al.*, 1987)

Genome sequencing

# Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus* in tomato (*Solanum lycopersicum*)

Genome sequencing: is T-Oms a different genotype?



Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus*  
in tomato (*Solanum lycopersicum*)

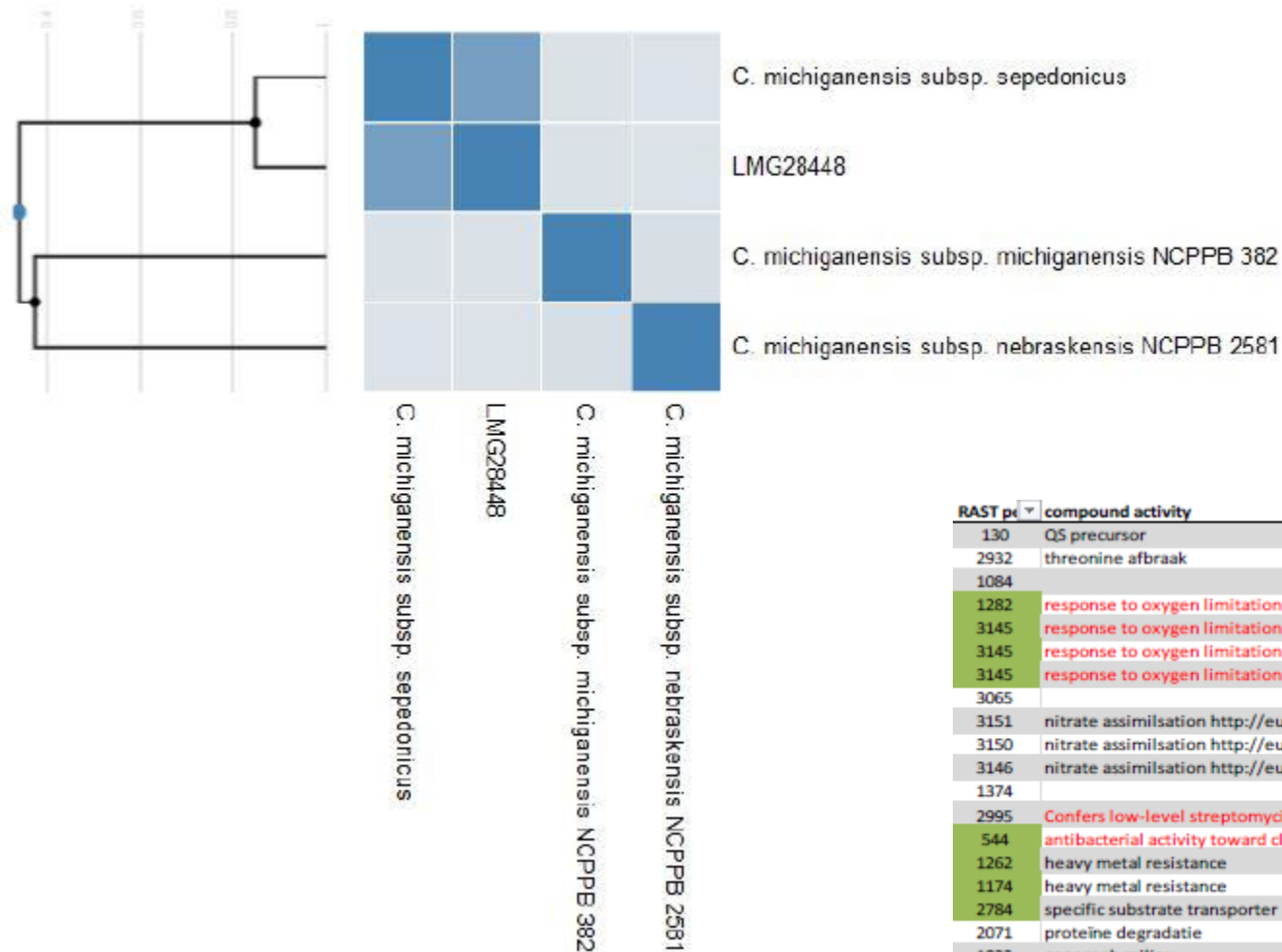
Genome sequencing: is T-Oms a different genotype?

Name	Contig	
	Length	
Chromosome	3258628	
Contig 172-unique	21914	
Contig 174-unique	1349	
Contig 175-unique	1576	
Contig 200-unique	1508	
pCS1	52244	
pCSL1	95848	
totals 7	3433067	

# Natural infection of *Cavibacter michiganensis* subsp. *sepedonicus* in tomato (*Solanum lycopersicum*)

Genome sequencing: is T-Oms a different genotype?

## metaproteome clustering



RAST pt	compound activity
130	QS precursor
2932	threonine afbraak
1084	
1282	response to oxygen limitation or oxidative and nitrosative stress
3145	response to oxygen limitation or oxidative and nitrosative stress
3145	response to oxygen limitation or oxidative and nitrosative stress
3145	response to oxygen limitation or oxidative and nitrosative stress
3065	
3151	nitrate assimilation <a href="http://europepmc.org/articles/PMC4272520">http://europepmc.org/articles/PMC4272520</a>
3150	nitrate assimilation <a href="http://europepmc.org/articles/PMC4272520">http://europepmc.org/articles/PMC4272520</a>
3146	nitrate assimilation <a href="http://europepmc.org/articles/PMC4272520">http://europepmc.org/articles/PMC4272520</a>
1374	
2995	Confers low-level streptomycin resistance
544	antibacterial activity toward closely related Gram-positive bacteria
1262	heavy metal resistance
1174	heavy metal resistance
2784	specific substrate transporter
2071	proteine degradatie
1823	anaeroob milieu

# Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus* in tomato (*Solanum lycopersicum*)

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1. Homologues of virulence genes of *Omm* are in T-*Oms* and in *Oms*<sup>T</sup>
2. Not on PI – some are pseudogenes
3. T-*Oms* has no specific genetic features that determine infection of tomato
4. T-*Oms* is a P-*Oms* that accidentally infected tomato:  
how and from where?
  - transmission from tomato seed
    - MLVA

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姦  
姦女

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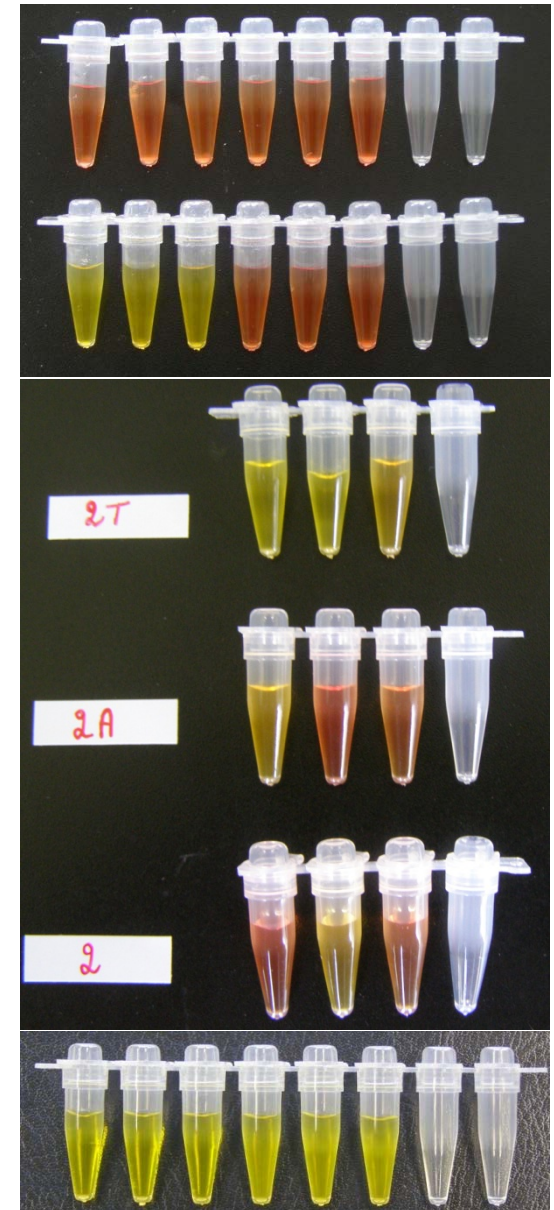
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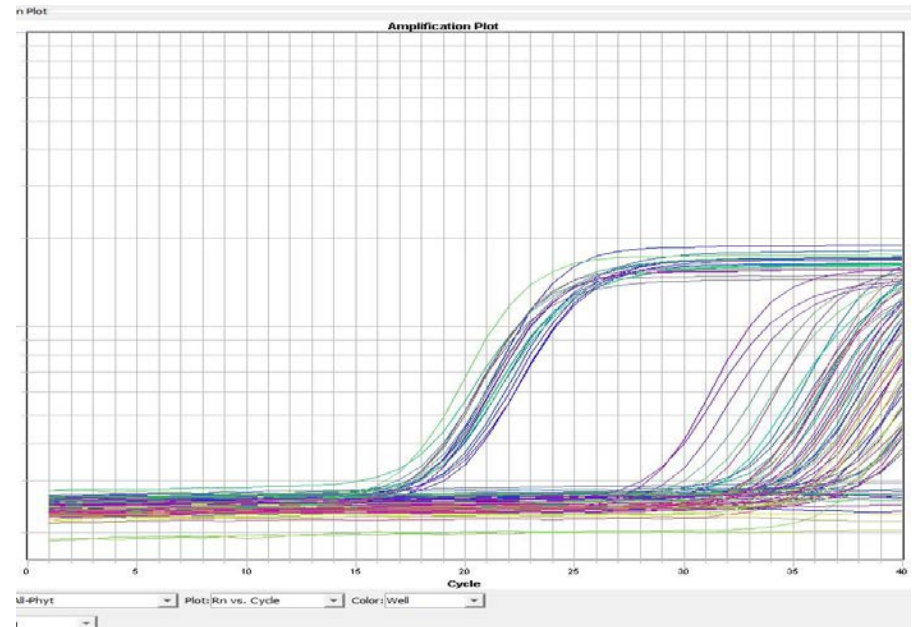
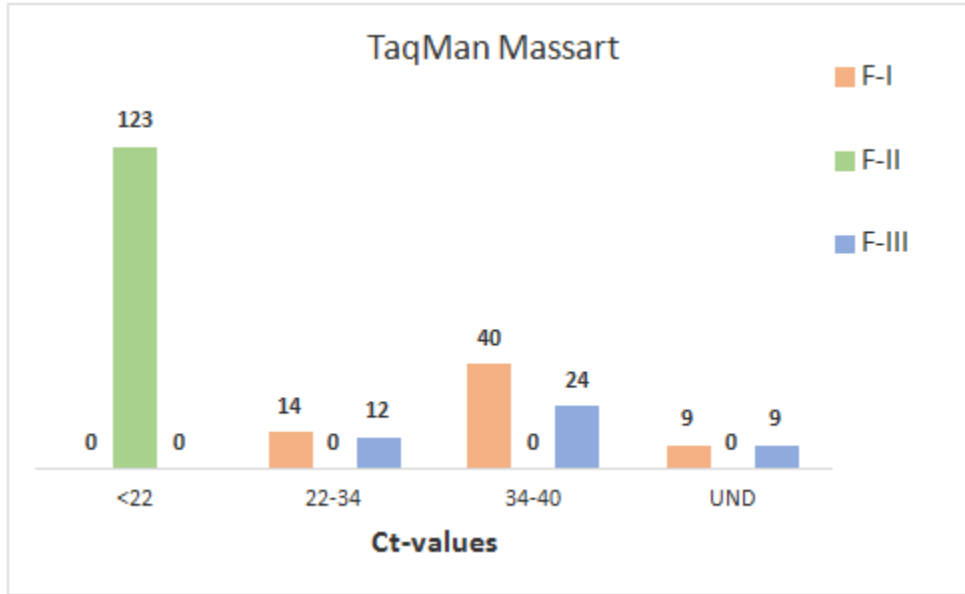


# ILC on identification of non-european *Ralstonia solanacearum*

- 231 cultures  
63 F-I, 123 F-II, 45 F-III
- biovar
- TaqMan Weller (all variants)
- TaqMan Massart (R3bv2 / F-IIIb, seq. 1)
- Huang (race 1)
- *egl* barcode



# ILC on identification of non-european *Ralstonia solanacearum*



# ILC on identification of non-european *Ralstonia solanacearum*. eg/ barcode

	Endo-F: ATGCATGCCGCTGGTCGCCGC						
Ph I	GMI1000	ATGCATGCCGCTGGT <b>T</b> GCCGC	+/+	188596	188616	Megaplasmid	
Ph II	IPO 1609	ATGCATGCCGCTGGTCGCCGC	+/-	3217320	3217300	Megaplasmid	
	NCPPB 909	ATGCATGCCGCTGGTCGCCGC	+/+	219	239	scaffold 75	
	K60-1	no functional match in NCBI					
Ph III	CMR15	ATGCATGCC <b>A</b> ATGGTCGCCGC	+/+	137436	137456	Megaplasmid	
Ph IV	PSI07	ATGCATGCCGCTGGTCGCCGC	+/+	123846	123866	Megaplasmid	
	Endo-R: GCGTTGCCCGGCACGAACACC						bp
Ph I	GMI1000	GCGTTGCC <b>G</b> GGCACGAACACC	+/-	189432	189412	Megaplasmid	837
Ph II	IPO 1609	GC <b>A</b> TTGCCCGGCACGAACACC	+/+	3216475	3216495	Megaplasmid	846
	NCPPB 909	GC <b>A</b> TTGCCCGGCACGAACACC	+/-	1064	1044	scaffold 75	846
	K60-1	no functional match in NCBI					x
Ph III	CMR15	GCGTTGCC <b>G</b> GGCACGAACACC	+/-	138278	138258	Megaplasmid	843
Ph IV	PSI07	GCGTTGCC <b>G</b> GGCACGAACACC	+/-	124688	124668	Megaplasmid	843

# ILC on identification of non-european *Ralstonia solanacearum*

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- 20 DNA F-I, F-IIA/ B, F-III, (F-IV)
- TaqMan identification
- *egl*/ barcode
  
- early spring 2016
- isolates?