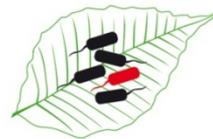


Diversity of seed-borne bacteria and consequences for detection strategies

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Emergence, systématique
et écologie des bactéries
associées aux plantes



Management strategies for seed-borne diseases

Exclusion of the pathogens:

-Chemical treatments: hot water, chlorine treatment



Management strategies for seed-borne diseases

Exclusion of the pathogens:

-Chemical treatments: hot water, chlorine treatment

-Seed health testing



Method for the Detection of *Clavibacter michiganensis* subsp. *michiganensis* on Tomato seed

Crop: Tomato (*Lycopersicon esculentum* L. now *Solanum lycopersicum*)
Pathogen: *Clavibacter michiganensis* subsp. *michiganensis*
Revision history: Version 4.1, January 2014

Sample and sub-sample size
The recommended minimum sample size is 10,000 seeds, with a maximum sub-sample size of 10,000 seeds.
Note: Sub-samples larger than 2,000 seeds require an additional concentration step of the extracted bacteria.

Principle

- o Extraction from the seed of externally and internally located bacteria
- o Isolation of viable *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) bacteria by dilution plating seed extract on two different semi-selective media



International Rules for Seed Testing
Annexe to Chapter 7: Seed Health Testing Methods



7-021: Detection of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* on *Phaseolus vulgaris* (bean)

Important notice

The pathogenicity test for this method is under review due to the occurrence of false positive results. Please see the ISTA website for more details.

Published by: International Seed Testing Association (ISTA), Bassersdorf, Switzerland
2014

DISCLAIMER: whilst ISTA has taken care to ensure the accuracy of the methods and information described in this method description ISTA shall not be liable for any loss damage etc., resulting from the use of this method.



Reliable seed health tests

Diversity of seed-borne bacteria and consequences for detection strategies

Specificity of *Clavibacter michiganensis* subsp. *michiganensis* detection methods and existence of look-alikes

Transmission of Cmm look-alikes to tomato seeds

Bacterial wilt and canker of tomato

Clavibacter michiganensis subsp. *michiganensis*

– Actinobacteria

• Microbacteriaceae

- Enters the plant by wounds and stomata
- Gets into the xylem: systemic infection
- Plugs the xylem vessels causing the wilting of the leaves
- May affect growth of leaves and petioles
- Induces canker on the stems



Seedborne pathogen

Infected seeds = major inoculum source and the cause for Cmm outbreaks

Quarantine pathogen in EU (A2)



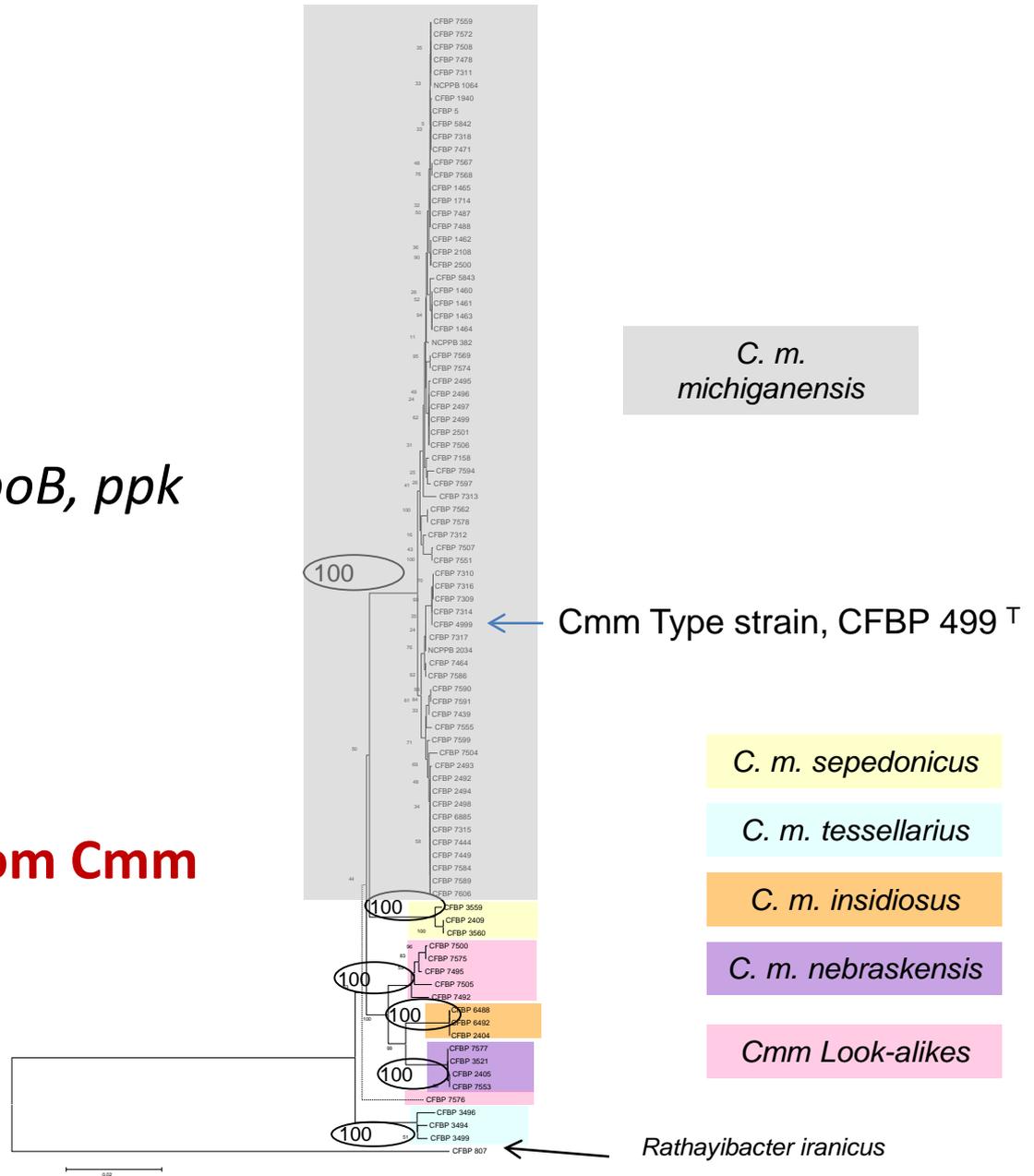
Look-alikes of *Cmm* isolated from tomato



atpD, *dnaK*, *gyrB*, *recA*, *rpoB*, *ppk*
ML, 1000 bootstraps

Look-alikes

- are clearly distinct from *Cmm*
- form several phyla



Characterization of *Clavibacter*-like strains isolated from tomato

Pathogenicity on tomato

5 PCR-based identification tests

IF: 2 antisera compared



Characterization of *Clavibacter*-like strains isolated from tomato

Nb of strains	Pathogenicity
129	+
11	+/-
1	(+/-)
1	-
1	-
2	-
6	-
2	-
4	-
4	-
2	-
2	-
32	-

141 Cmm

**56 avirulent
Strains: look-alikes**

+ Canker
Wilting



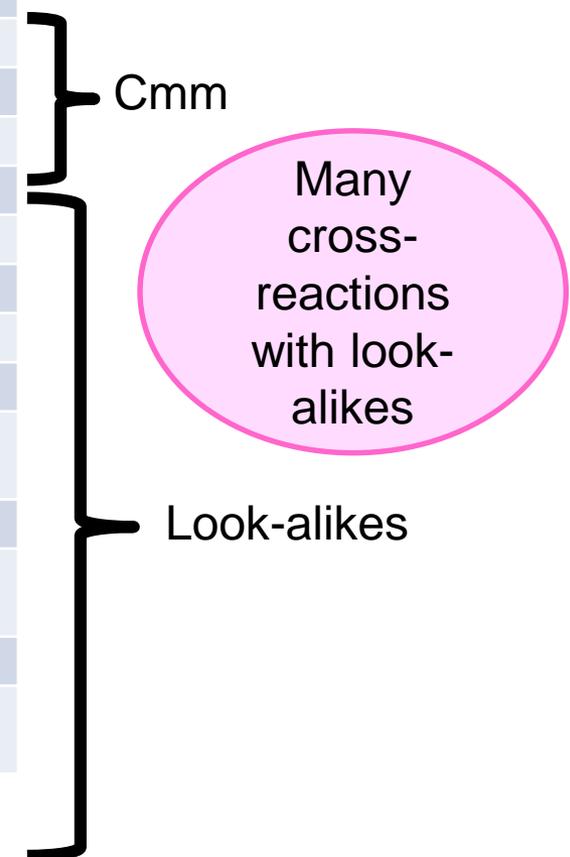
+/- (small) canker
(+/-) No wilting



197 strains

Characterization of *Clavibacter*-like strains isolated from tomato

Nb of strains	Pathogenicity	IF		PCR-based identification				
		IF (PRI)	IF (Loewe)	ZTO	Cmm F/R	Psa	PsaR/8	Ptssk
129	+	+	+	+	+	+	+	+
11	+/-	+	+	+	+	+	+	+
1	(+/-)	+	+	+	+	+	+	+
1	-	+	+	+	+	+	-	-
1	-	+	+	+	+	-	+	-
2	-	+	+	+	+	-	-	-
6	-	+	+	+	+	trace	-	-
2	-	+	+	-	-	trace	+	-
4	-	+	+	-	-	-	-	-
4	-	+	+	-	-	trace	-	-
2	-	-	+	-	-	-	-	-
2	-	-	-	+	+	-	-	-
32	-	-	-	-	-	-	-	-



197 strains

(Nunhems, 2008; Rijlaarsdam *et al*, 2004; Pstrik & Rainey, 1999; Berendsen *et al.*, 2011)

Jacques *et al.*, 2012

Characterization of *Clavibacter*-like strains isolated from tomato

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129	+	+	+	+	+	+	+	+
11	+/-	+	+	+	+	+	+	+
1	(+/-)	+	+	+	+	+	+	+
1	-	+	+	+	+	+	-	-
1	-	+	+	+	+	<1%	+	-
2	-	+	+	+	+	-	-	-
6	-	+	+	+	+	trace	-	-
2	-	+	+	-	-	trace	+	-
4	-	+	+	-	-	-	-	-
4	-	+	+	-	-	trace	-	-
2	-	-	+	-	-	-	-	-
2	-	-	-	+	+	-	-	-
32	-	-	-	-	-	-	-	-

Cmm

Look-alikes

Many cross-reactions with look-alikes

Ptssk : specific and sensitive

197 strains

(Nunhems, 2008; Rijlaarsdam *et al*, 2004; Pstrik & Rainey, 1999; Berendsen *et al.*, 2011)

Jacques *et al.*, 2012

Role of look-alikes in seed transmission of pathogens

Look-alikes were isolated from seeds.
No report of crossed contaminations.

Do look-alikes have a role in seed transmission of pathogenic strains?
antagonist or facilitator ?

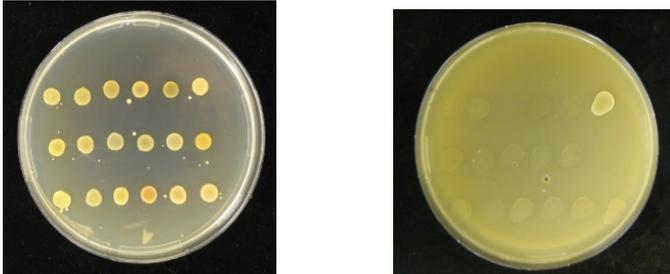
22 “Cmm look-alikes”



1. **In vitro tests to select look-alikes that interfere with Cmm**
2. **Transmission to seed for candidate look-alikes individually, and in mix with the pathogen, C144**

Role of look-alikes in seed transmission of pathogens

In vitro antibiosis tests

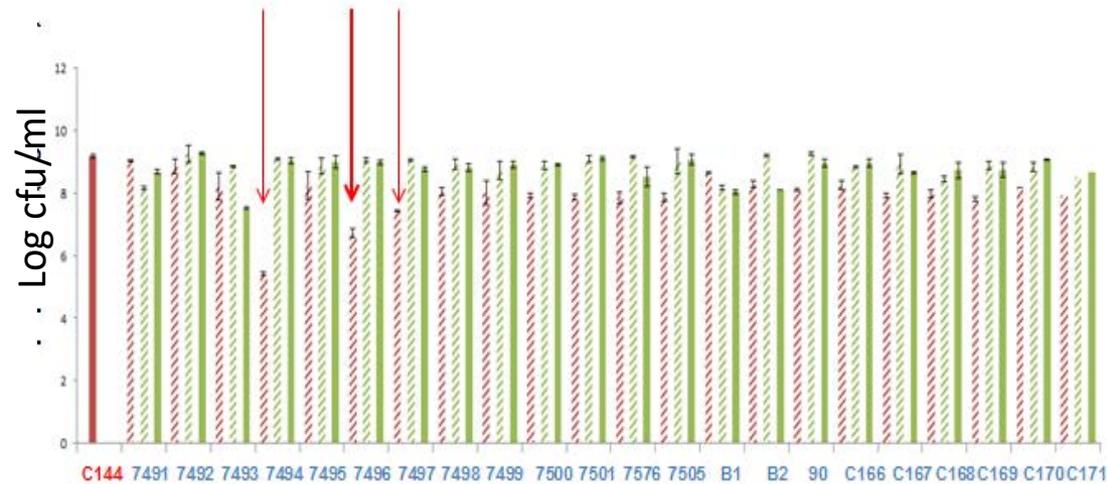


In vitro competition test



Antibiosis of C144 toward 21 look-alikes
CFBP 7496 is not sensitive to C144

■ Cmm alone ■ Look-alike alone
▨ Cmm in mix ▨ Look-alike in mix



Cmm: C144 **CFBP 7494**
CFBP 7496
CFBP 7497



Role of look-alikes in seed transmission of Cmm pathogenic strain

Pathogen alone

Look-alike alone

Mixtures P-NP

5 plants / treatment

3 flower bud/ plant

Cmm: C144	7494
	7496
	7497



Spray inoculation (1×10^6 ufc/mL)

Flower

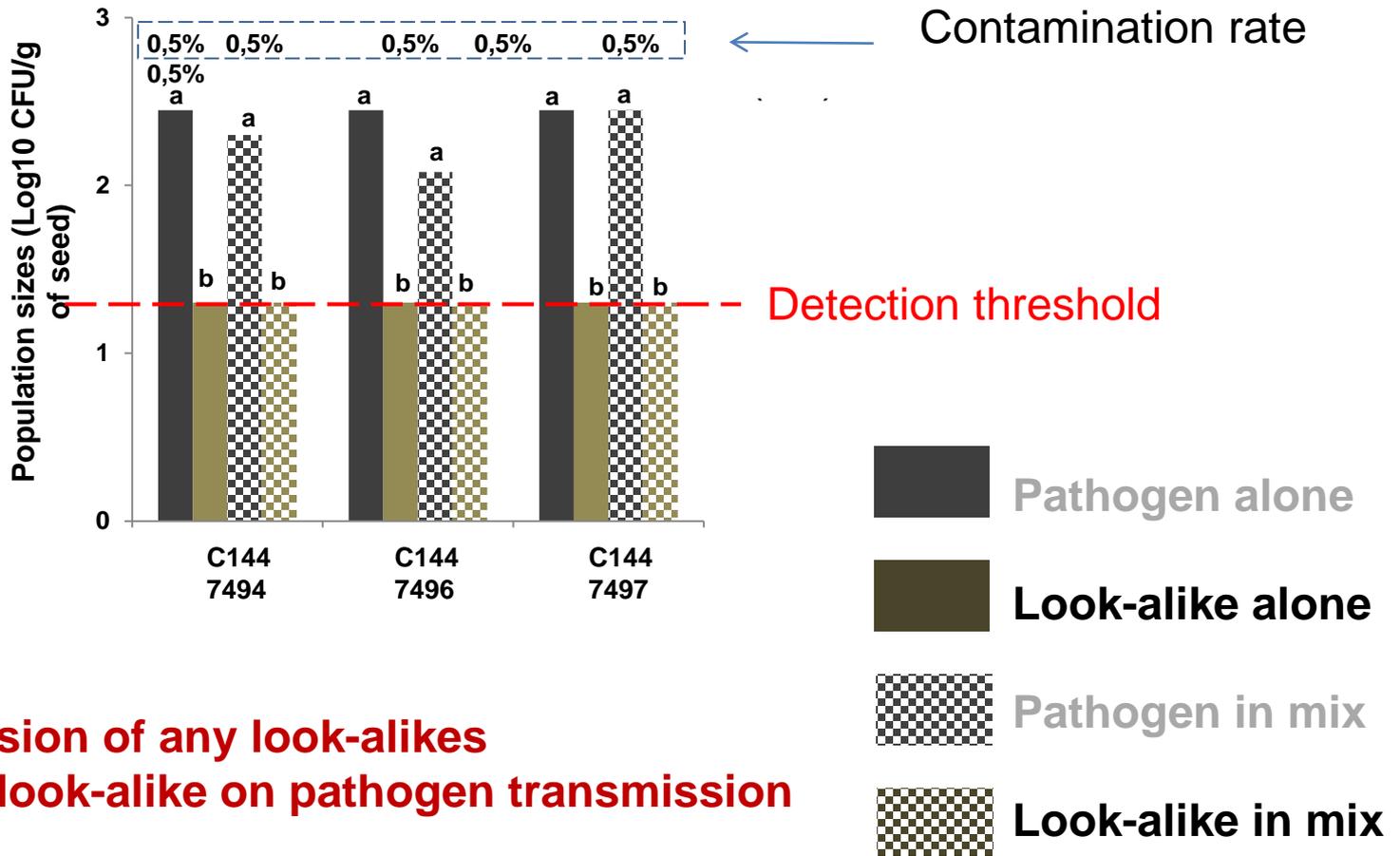
Flower bud

Fruit

Analysis 2 mo after inoculation



Role of look-alikes in seed transmission of Cmm pathogenic strain



No transmission of any look-alikes

No effect of look-alike on pathogen transmission

Summary

Look-alikes

- are responsible for false positive results in identification tests
- are phylogenetically diverse, but could be closely related to pathogens
- are not transmitted to seeds of their host of isolation
- do not interact with pathogens for transmission to seeds

 **Some look-alikes are commensals**

Summary

Look-alikes

- are responsible for false positive results in identification tests
- are phylogenetically diverse, but could be closely related to pathogens
- are not transmitted to seeds of their host of isolation
- do not interact with pathogens for transmission to seeds
- are efficient xylem colonizers

 Some look-alikes are commensals

Could other look-alikes be pathogens carried by non-host material?

Identification of *C. michiganensis* pathogenic on bean (Gonzalez and Trapiello, 2014)

International Journal of Systematic and Evolutionary Microbiology (2014), **64**, 1752–1755

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Clavibacter michiganensis subsp. *phaseoli* subsp. nov., pathogenic in bean

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A yellow Gram-reaction-positive bacterium isolated from bean seeds (*Phaseolus vulgaris* L.) was identified as *Clavibacter michiganensis* by 16S rRNA gene sequencing. Molecular methods were employed in order to identify the subspecies. Such methods included the amplification of specific sequences by PCR, 16S amplified rDNA restriction analysis (ARDRA), RFLP and multilocus sequence analysis as well as the analysis of biochemical and phenotypic traits including API 50CH and API ZYM results. The results showed that strain LPPA 982^T did not represent any known subspecies of *C. michiganensis*. Pathogenicity tests revealed that the strain is a bean pathogen causing a newly identified bacterial disease that we name bacterial bean leaf yellowing. On the basis of these results, strain LPPA 982^T is regarded as representing a novel subspecies for which the name *Clavibacter michiganensis* subsp. *phaseoli* subsp. nov. is proposed. The type strain is LPPA 982^T (=CECT 8144^T=LMG 27667^T).

Identification of *C. michiganensis* pathogenic on bean

Some look-alikes are very close to this new bean pathogen

But, pathogenicity on bean is difficult to evidence

P. vulgaris, cv. Flavert, 5 plants/strain

Stem injection + sponge inoculation on leaves



Cm CFBP 8016



Cmp LPPA 982

Identification of *C. michiganensis* pathogenic on bean

Some look-alikes are very close to this new bean pathogen

But, pathogenicity on bean is difficult to evidence



Cm CFBP 8016



Cmp LPPA 982

↳ Test other bean cv.

Carriage of pathogen by seeds of non-host plants

(Darrasse *et al.*, 2010)

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Thank you for your attention