

How to name those to blame ?

A practical taxonomical study of the *Xanthomonas* causing bacterial leaf spot of lettuce has major consequences for the boundaries delimitation of *X. hortorum*

Lucas Morinière



Sun Zi statue, Yorihama, Japan

“知彼知己，百戰不殆”

*“If you know your enemies and know yourself,
you will not be imperiled in a hundred battles”*

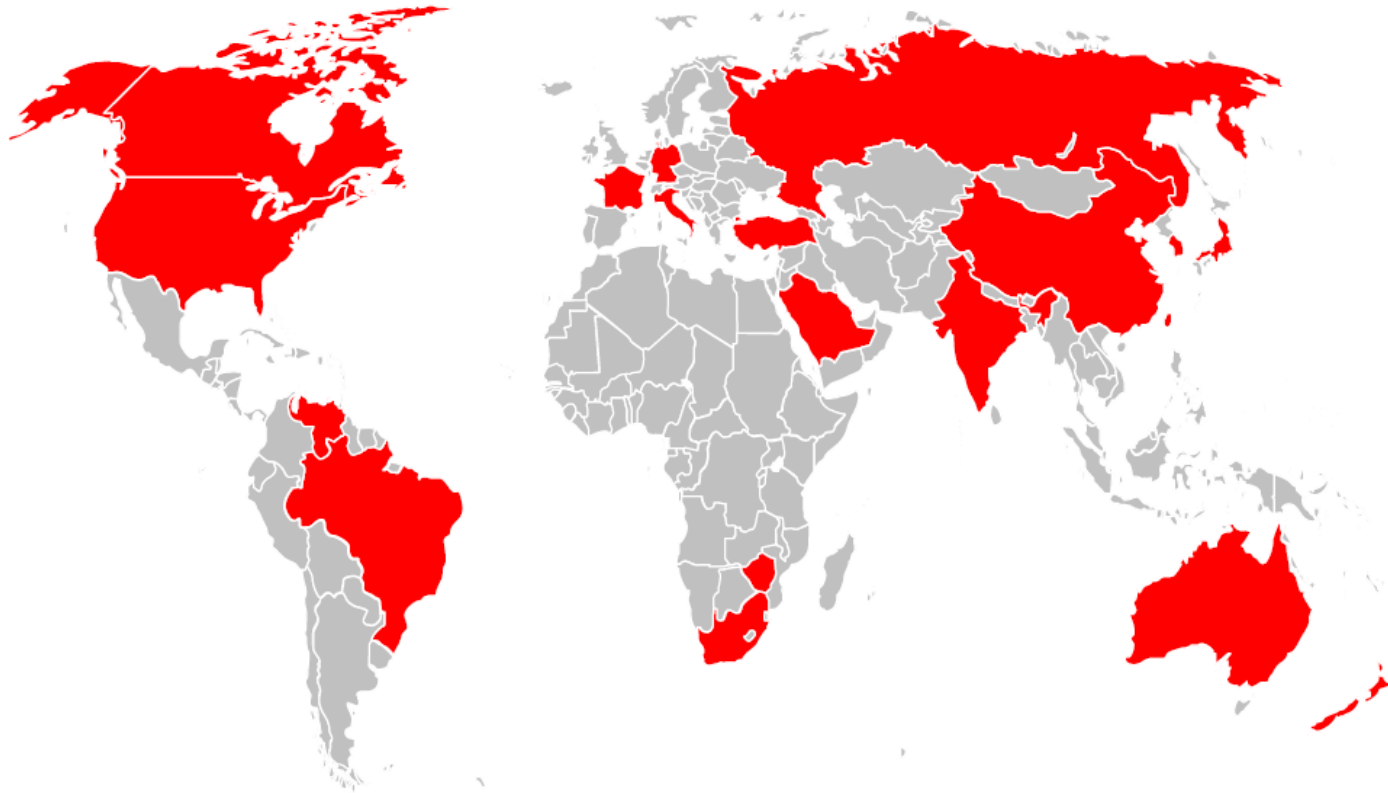
The Art of War
Sūn Zǐ (孫子), 6th century BCE

(introduction of Bull and Koike 2015)

Bacterial leaf spot of lettuce

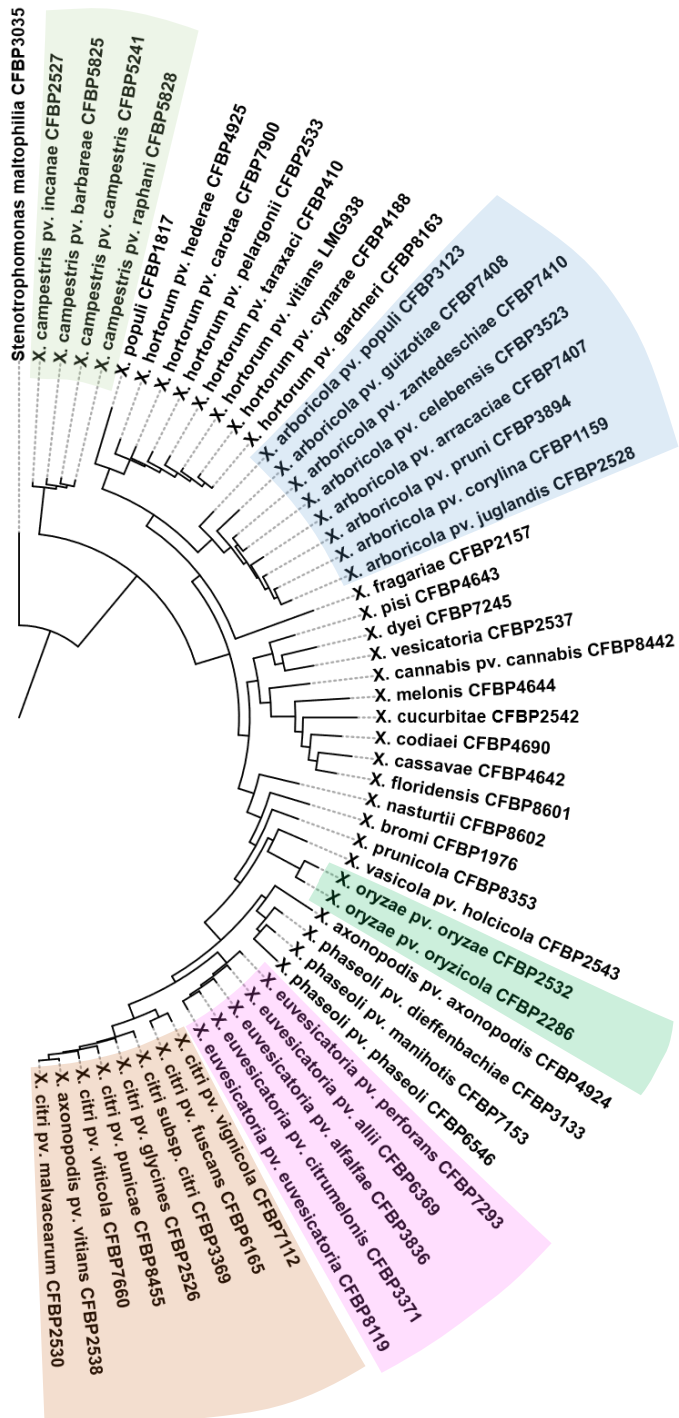
- Worldwide lettuce disease
- Important economic losses
- No cure / prophylaxis measures

Worldwide distribution (reported in the scientific literature)



Infected lettuces in field (France)





X. campestris



X. arboricola



X. oryzae



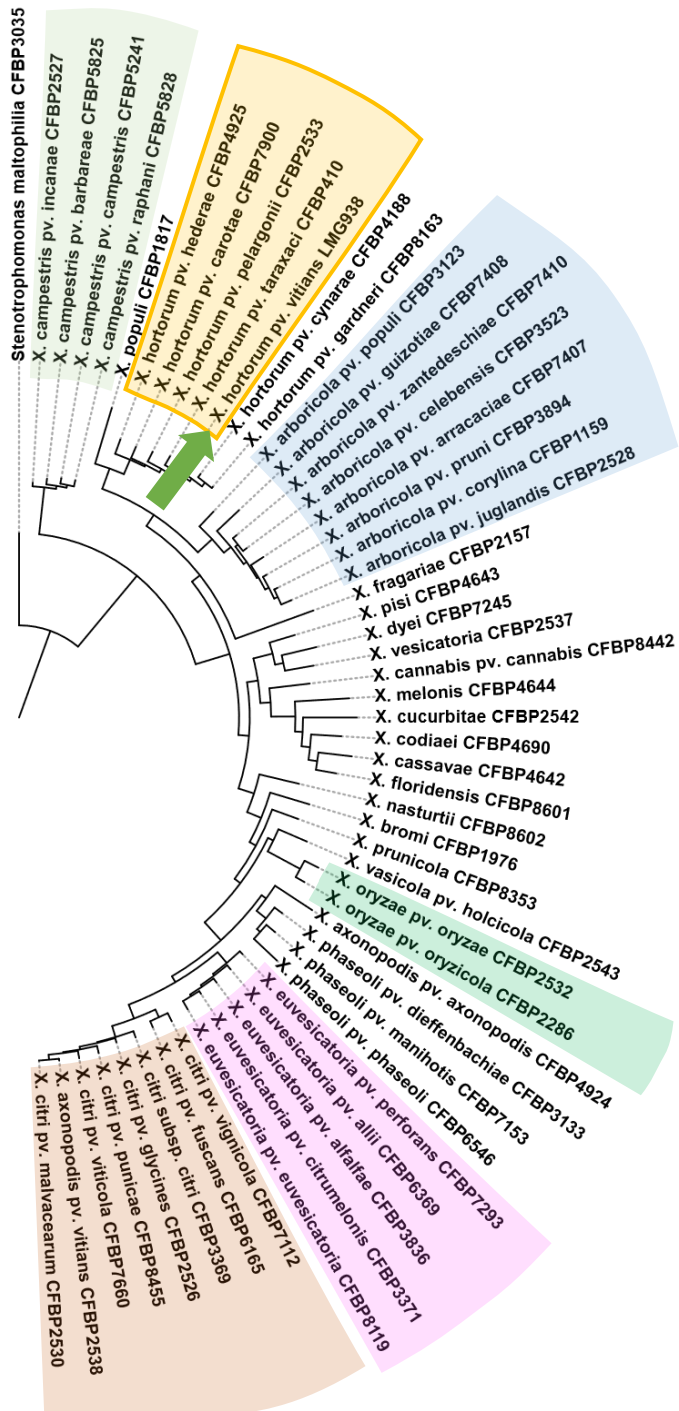
X. euvesicatoria



X. citri



WGS-based phylogeny of *Xanthomonas* clade II



Xanthomonas hortorum Vauterin *et al.* 1995



T
 pv. *hederae*
 English Ivy



pv. *taraxacii*
 Russian dandelion



pv. *pelargonii*
 Geranium



pv. *carotae*
 Carrot



pv. *vitians*
 Lettuce

Creation of *Xanthomonas hortorum* sp. nov.
 based on DNA-DNA hybridizations
 (Vauterin *et al.* 1995)

X. campestris



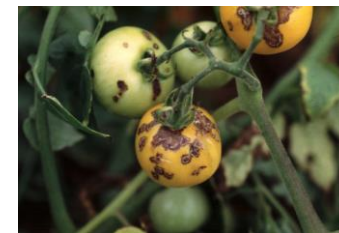
X. arboricola



X. oryzae



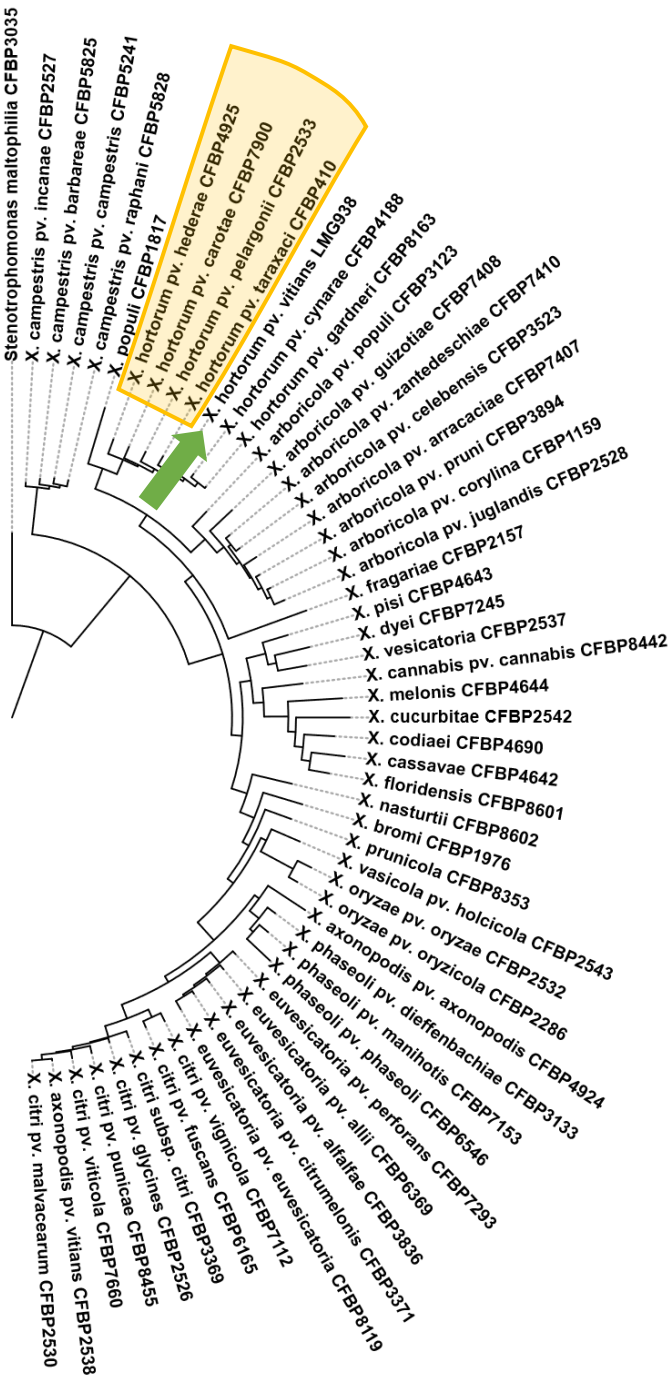
X. euvesicatoria



X. citri



WGS-based phylogeny of *Xanthomonas* clade II



X. hortorum Vauterin *et al.* 1995



pv. hederarum
English Ivy



pv. taraxacii
Russian dandelion



pv. pelargonii
Geranium



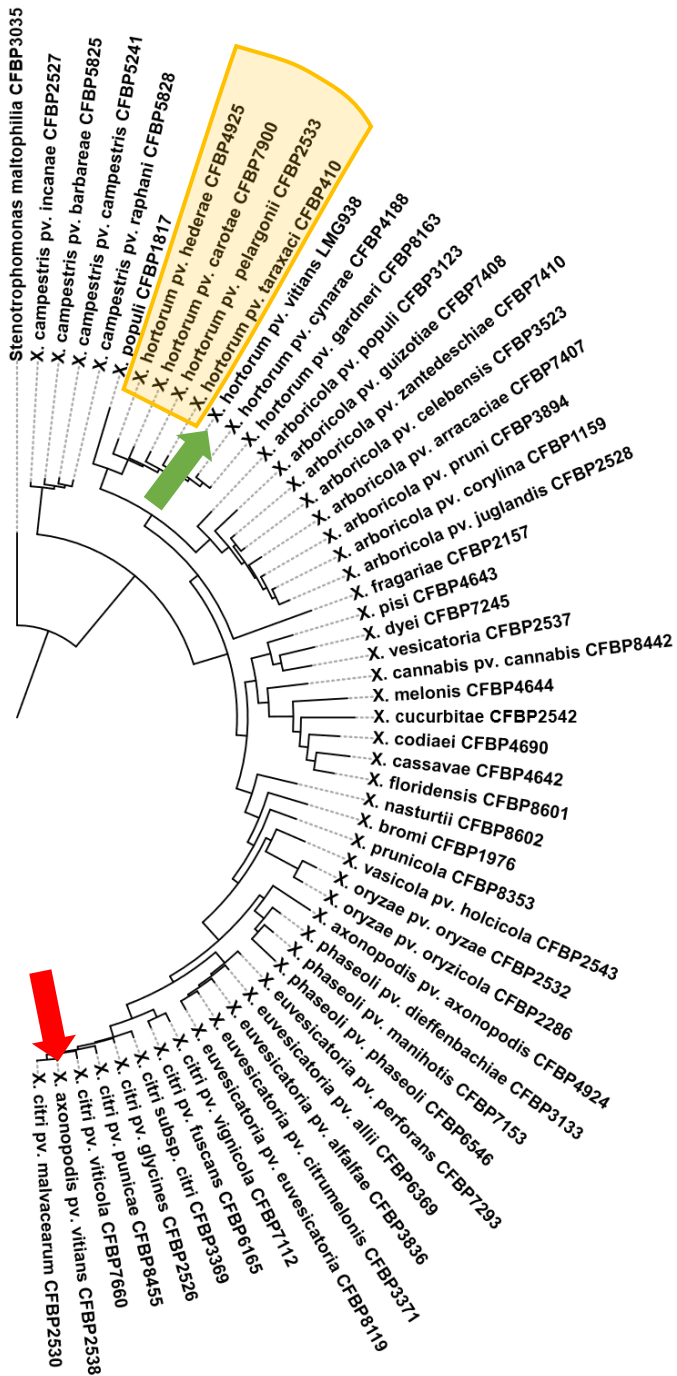
pv. carotae
Carrot



pv. vitians
Lettuce

ICPP-Committee on the Taxonomy of Plant Pathogenic Bacteria refused the name *X. hortorum* *pv. vitians*.

(Young *et al.* 1996)



X. hortorum Vauterin *et al.* 1995



pv. *hederae*
English Ivy



pv. *taraxacii*
Russian dandelion



pv. *pelargonii*
Geranium



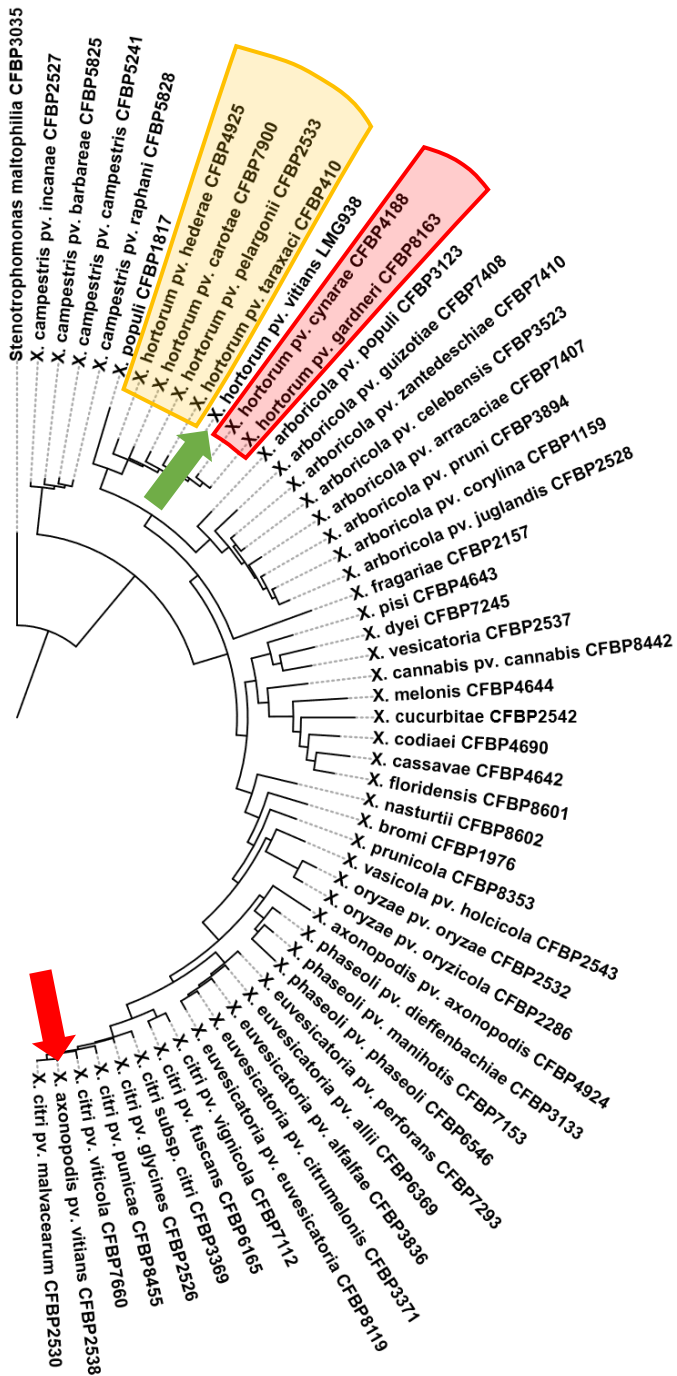
pv. *carotae*
Carrot



pv. *vitians*
Lettuce

ICPP-Committee on the Taxonomy of Plant Pathogenic Bacteria refused the name *X. hortorum* pv. *vitians*.

- 1) No description of the **new taxon** (protologue)
- 2) Pathotype strain is ***X. axonopodis* pv. *vitians* CFBP 2538^{PT}** (Young *et al.* 1996)



X. hortorum Vauterin *et al.* 1995



T
pv. *hederae*
English Ivy



pv. *taraxacii*
Russian dandelion



pv. *pelargonii*
Geranium



pv. *carotae*
Carrot



pv. *vitians*
Lettuce

Combination of *X. cynarae* Trébaol *et al.* 2000 and *X. gardneri* Jones *et al.* 2006 based on OGRI* calculations (Timilsina *et al.* 2018)



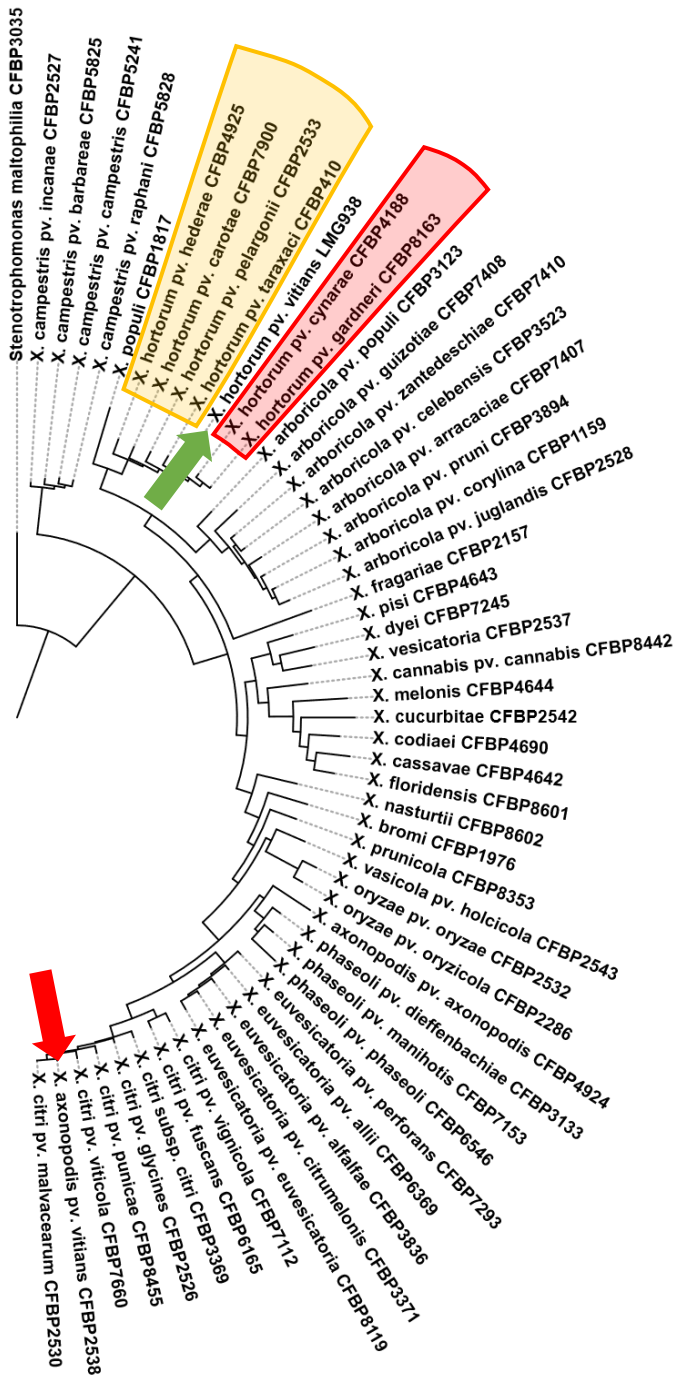
T
pv. *cynarae*
Artichoke



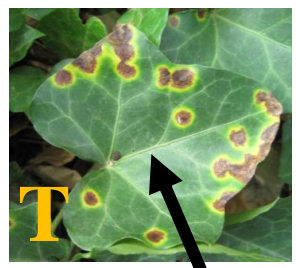
pv. *gardneri*
Tomato and pepper

X. cynarae (Trebaol 2000) Timilsina *et al.* 2018

*OGRI = Overall Genome Relatedness Indices (ANI, *is*DDH...)



X. hortorum Vauterin *et al.* 1995



pv. *hederae*
English Ivy



pv. *taraxacii*
Russian dandelion



pv. *pelargonii*
Geranium

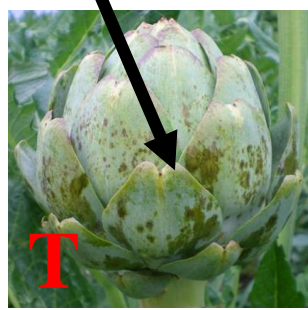


pv. *carotae*
Carrot



pv. *vitians*
Lettuce

ANI = ~ 96 % and isDDH = ~ 65 %
(Timilsina *et al.* 2018)

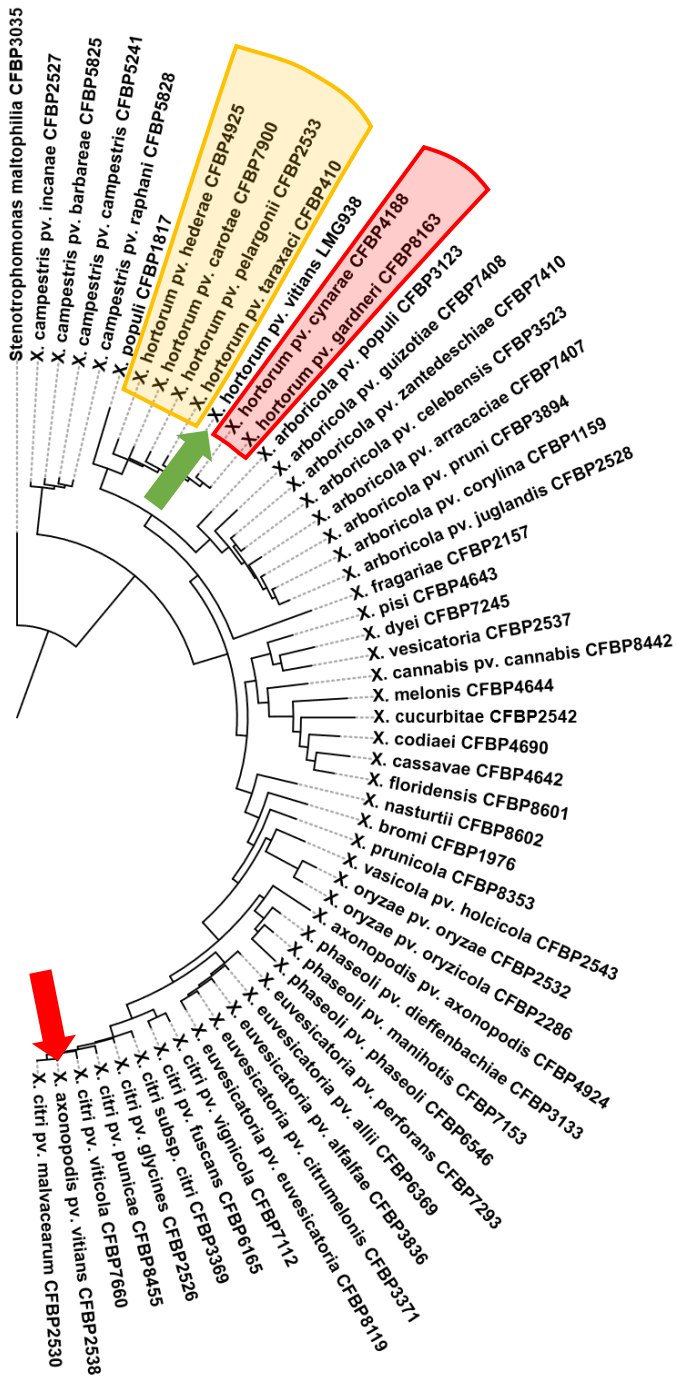


pv. *cynarae*
Artichoke



pv. *gardneri*
Tomato and pepper

X. cynarae (Trebaol 2000) Timilsina *et al.* 2018



X. hortorum Vauterin *et al.* 1995



T
 pv. *hederae*
 English Ivy



pv. *taraxacii*
 Russian dandelion



pv. *pelargonii*
 Geranium



pv. *carotae*
 Carrot



pv. *vitians*
 Lettuce

No comprehensive comparison including all the taxonomically relevant strains (*i.e.* types / pathotypes) of all the pathovars ever performed.



T
 pv. *cynarae*
 Artichoke



pv. *gardneri*
 Tomato and pepper

X. cynarae (Trebaol 2000) Timilsina *et al.* 2018

HYPOTHESES

X. hortorum Vauterin *et al.* 1995



pv. *pelargonii*
Geranium



pv. *carotae*
Carrot



pv. *taraxacii*
Russian dandelion



pv. *hederae*
English Ivy

X. campestris



pv. *vitians*
Lettuce

X. cynarae

(Trebaol 2000) Timilsina *et al.* 2018



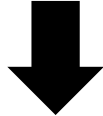
pv. *cynarae*
Artichoke



pv. *gardneri*
Tomato and pepper

- I) Bacterial leaf spot of lettuce causing strains all belongs to *X. hortorum* pv. *vitians*.
- II) *X. cynarae* is a later heterotypic synonym of *X. hortorum*.

54 strains isolated from lettuce during sampling campaigns or acquired from collections (CFBP & LMG)



Multi-Locus Sequence Analysis on 3 housekeeping genes (*gyrB*, *gapA*, *rpoD*)

+

Pathogenicity assays on 2 cultivars



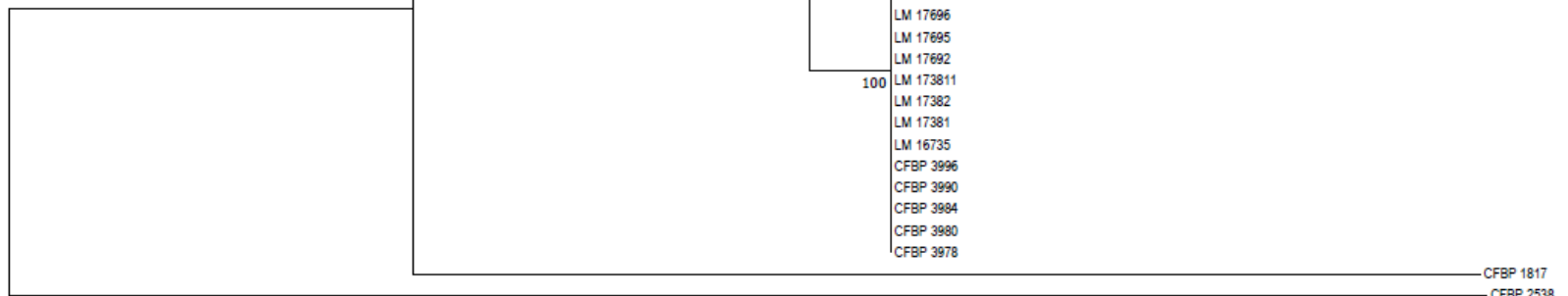
1

2

3

4

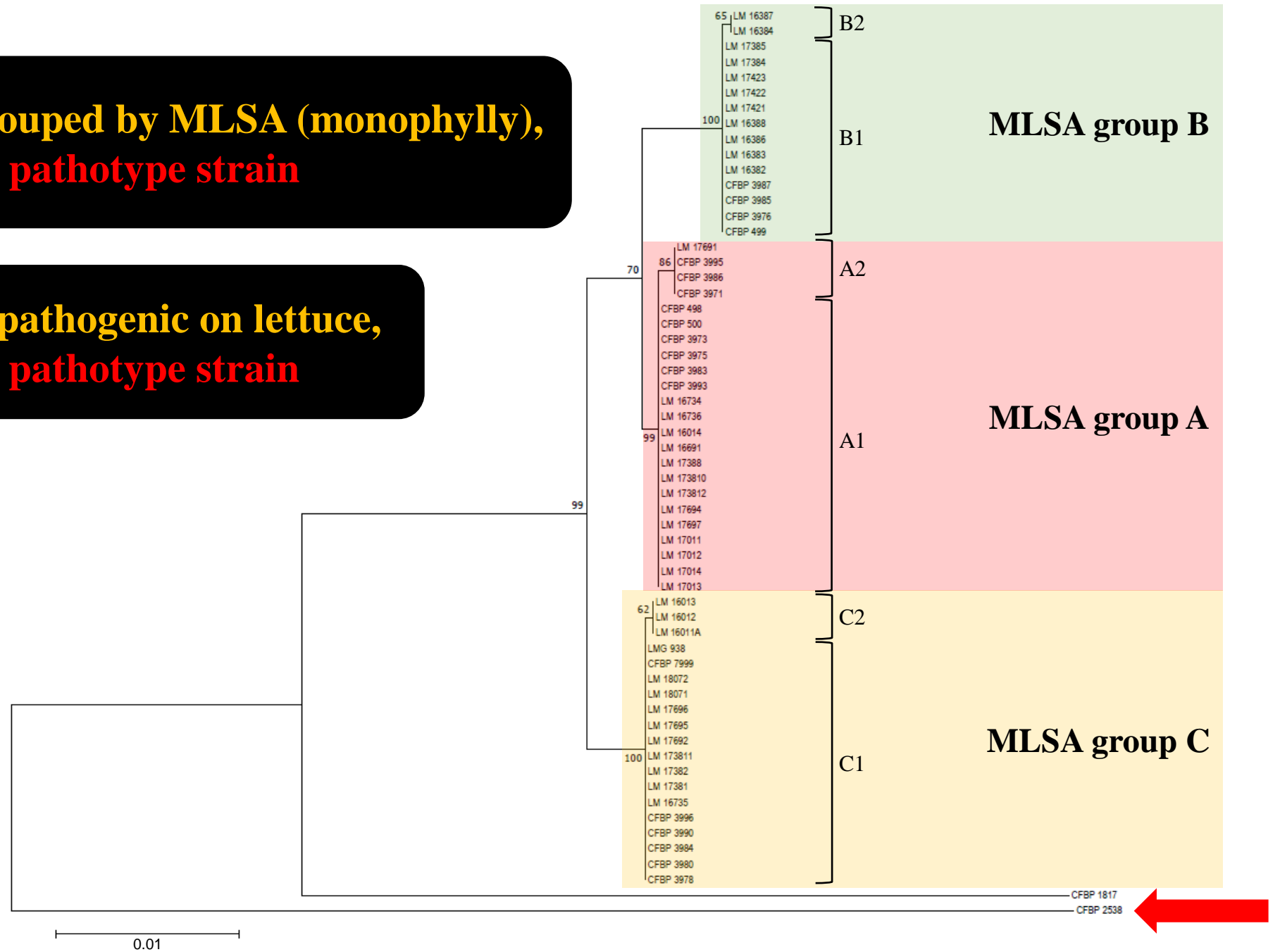
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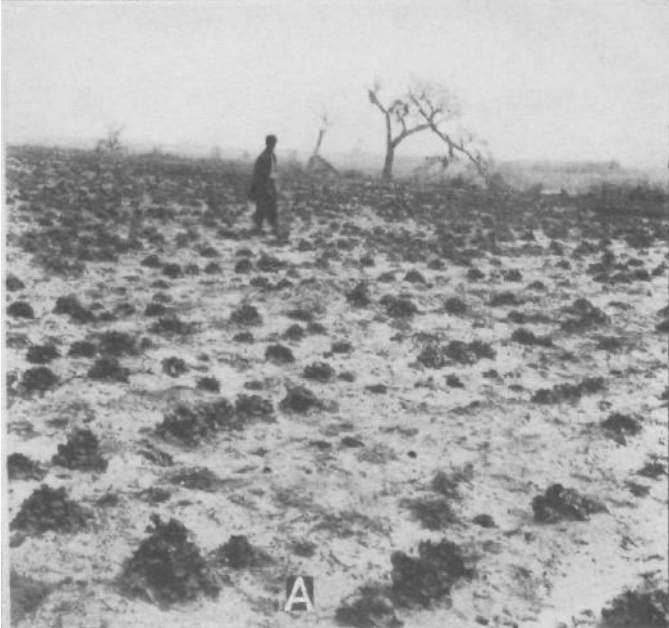
0.01

**All strains tightly grouped by MLSA (monophyly),
except pathotype strain**

**All strains pathogenic on lettuce,
except pathotype strain**



Outbreak in Beaufort, SC, USA, 1916
(adapted from Brown 1918)



Nellie A. Brown
(1876-1956)
American phytopathologist

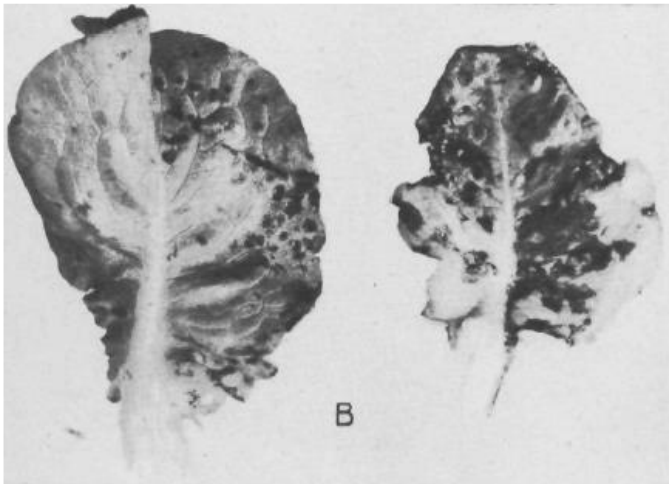
SOME BACTERIAL DISEASES OF LETTUCE

By NELLIE A. BROWN

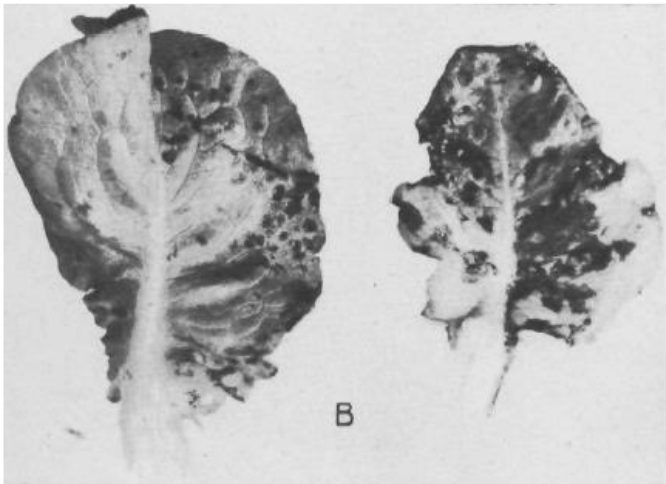
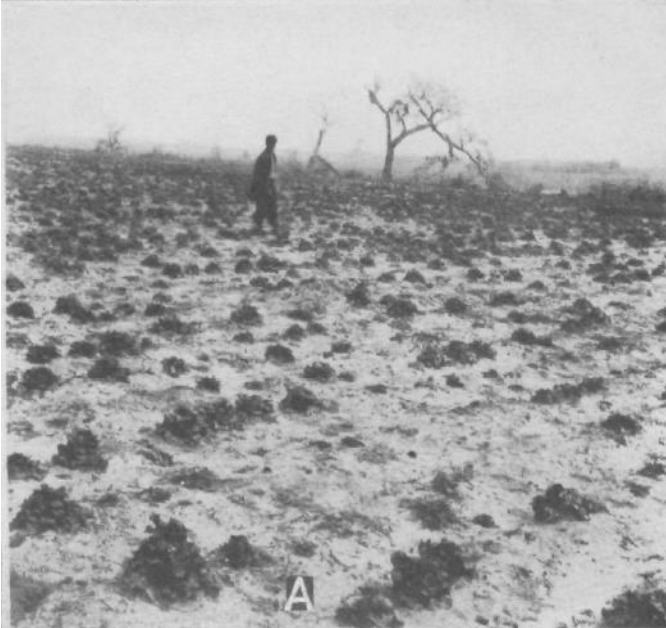
*Assistant Pathologist, Laboratory of Plant Pathology, Bureau of Plant Industry,
United States Department of Agriculture*

DESCRIPTION OF THE SOUTH CAROLINA ORGANISM

The organism is a bacterium, a short rod with rounded ends, occurring singly or in pairs, occasionally in short chains. In stained host tissue the measurements of single rods vary from 0.62 to 1.04 μ long, and 0.42 to 0.83 μ wide (Pl. 41, A). Grown for one day on beef agar and stained with Loeffler's flagella stain, they vary from 0.62 to 1.24 μ long and 0.42 to 0.83 μ wide.



Outbreak in Beaufort, SC, USA, 1916
(adapted from Brown 1918)



Brown's original description in 1918

Alkaline reaction in
Litmus Milk
(Hydrolyzation and
precipitation of casein)

Gelatin hydrolyzation
(slow)

Motility

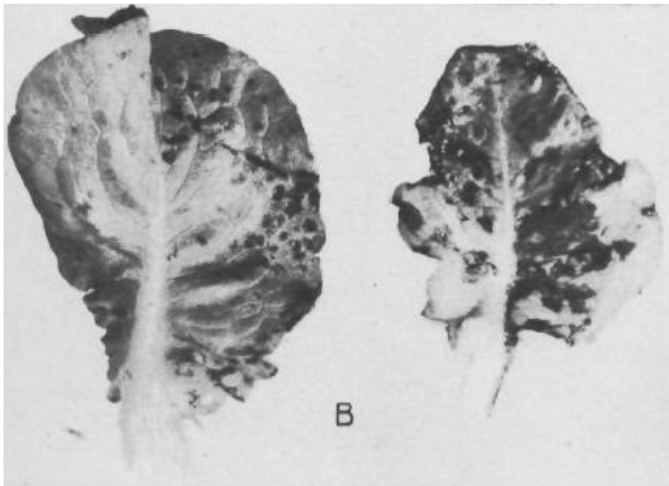
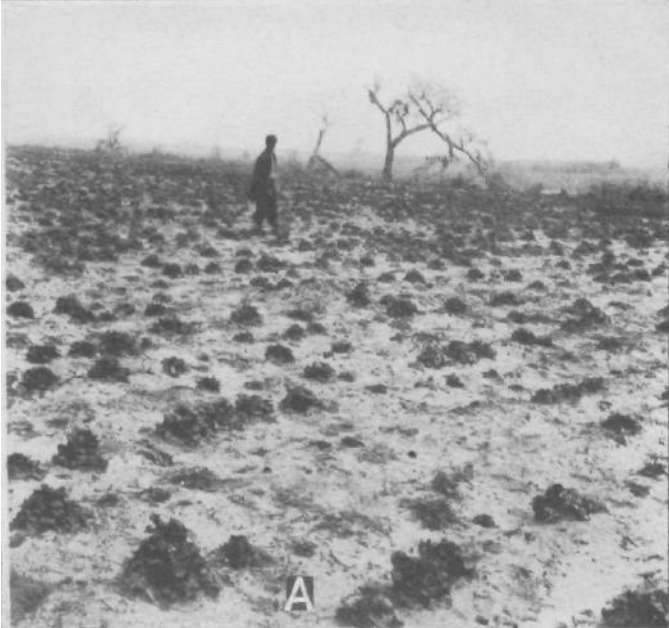
Hydrogen sulphide
production

Indols production
(slight)

Pathotype strain
CFBP 2538

Reference strain
LMG 938

Outbreak in Beaufort, SC, USA, 1916
(adapted from Brown 1918)



Brown's original description in 1918

Alkaline reaction in Litmus Milk
(Hydrolyzation and precipitation of casein)

Gelatin hydrolyzation
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Motility

Hydrogen sulphide production

Indols production
(slight)

Pathotype strain CFBP 2538

-

-

+

+

+

Reference strain LMG 938

+

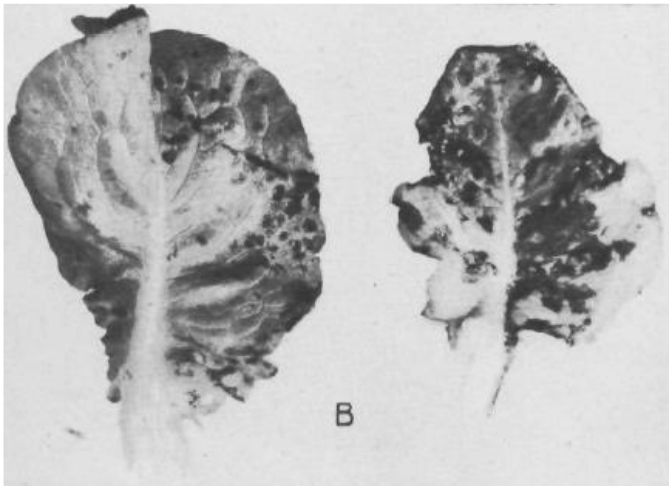
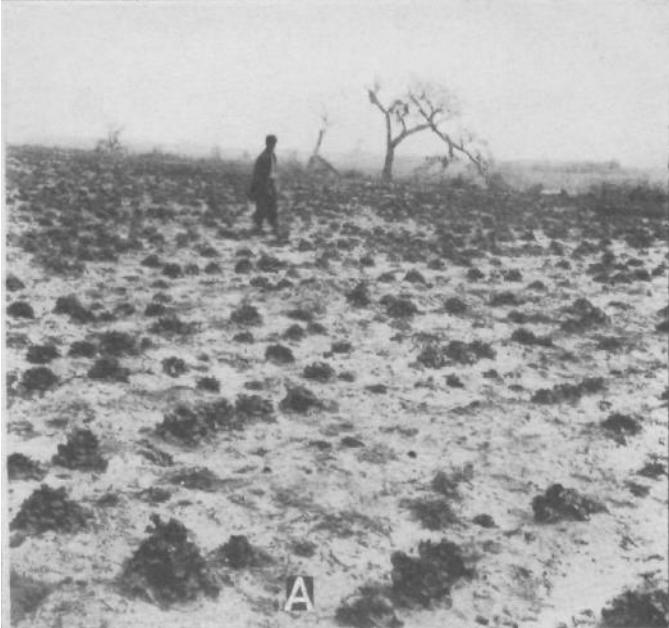
+

+

+

+

Outbreak in Beaufort, SC, USA, 1916
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Brown's original description in 1918

Alkaline reaction in Litmus Milk
(Hydrolyzation and precipitation of casein)

Gelatin hydrolyzation
(slow)

Motility

Hydrogen sulphide production

Indols production
(slight)

Pathotype strain CFBP 2538

-

-

+

+

+

Reference strain LMG 938

+

+

+

+

+

Cancellation of pathotype strain CFBP 2538

Proposal of replacement by LMG 938^{neoPT}

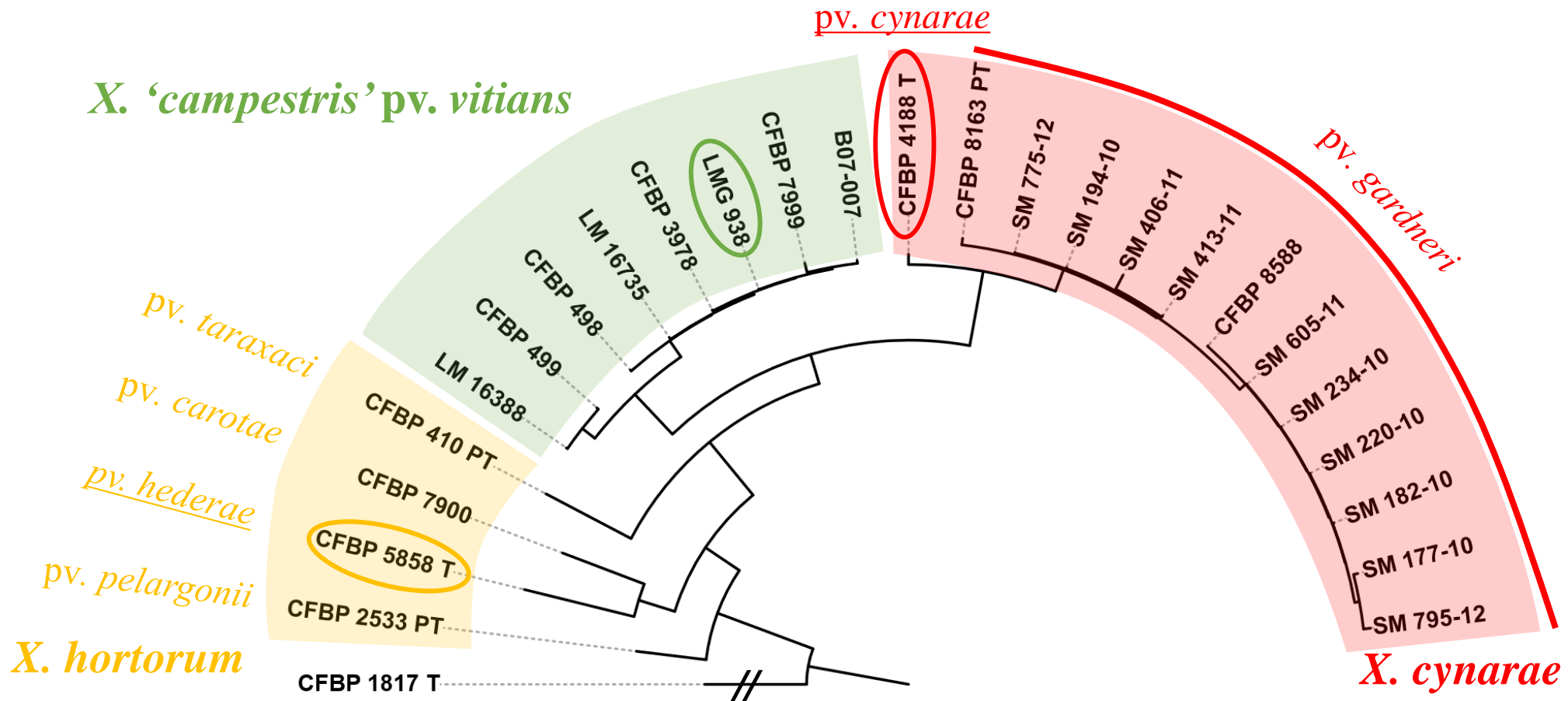
WGS-sequencing of :

7 strains of *X. 'campestris' pv. vitians*

2 pathotypes of *X. hortorum*
(pvs. *taraxaci* & *pelargonii*)

Pathotype of *X. cynarae* pv. *gardneri*

WGS-based phylogeny of all genomes available for *X. hortorum*, *X. cynarae* and *X. campestris* pv. *vitians*



OGRI calculations

Neopathotype LMG 938^{neoPT}

vs

Type of *X. hortorum* CFBP 5858^T

None of the pathovars of *X. hortorum* is pathogenic on lettuce (excludes synonymy)

Observed

Theoretical thresholds
(Rossello-Mora 2015)

ANIm

96.1 %

95 ~ 96 %

isDDH

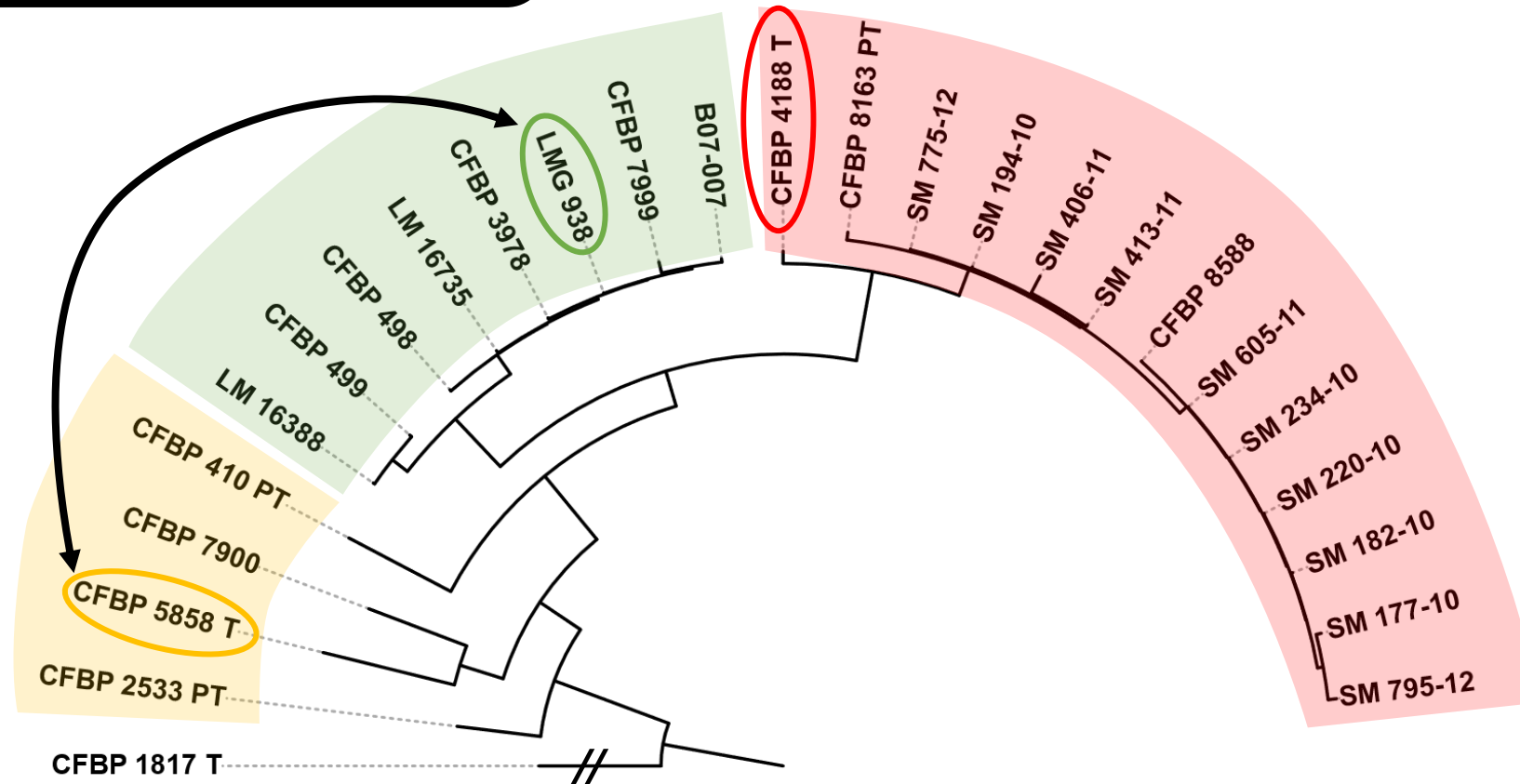
68.2 %

~ 70 %

Tetra

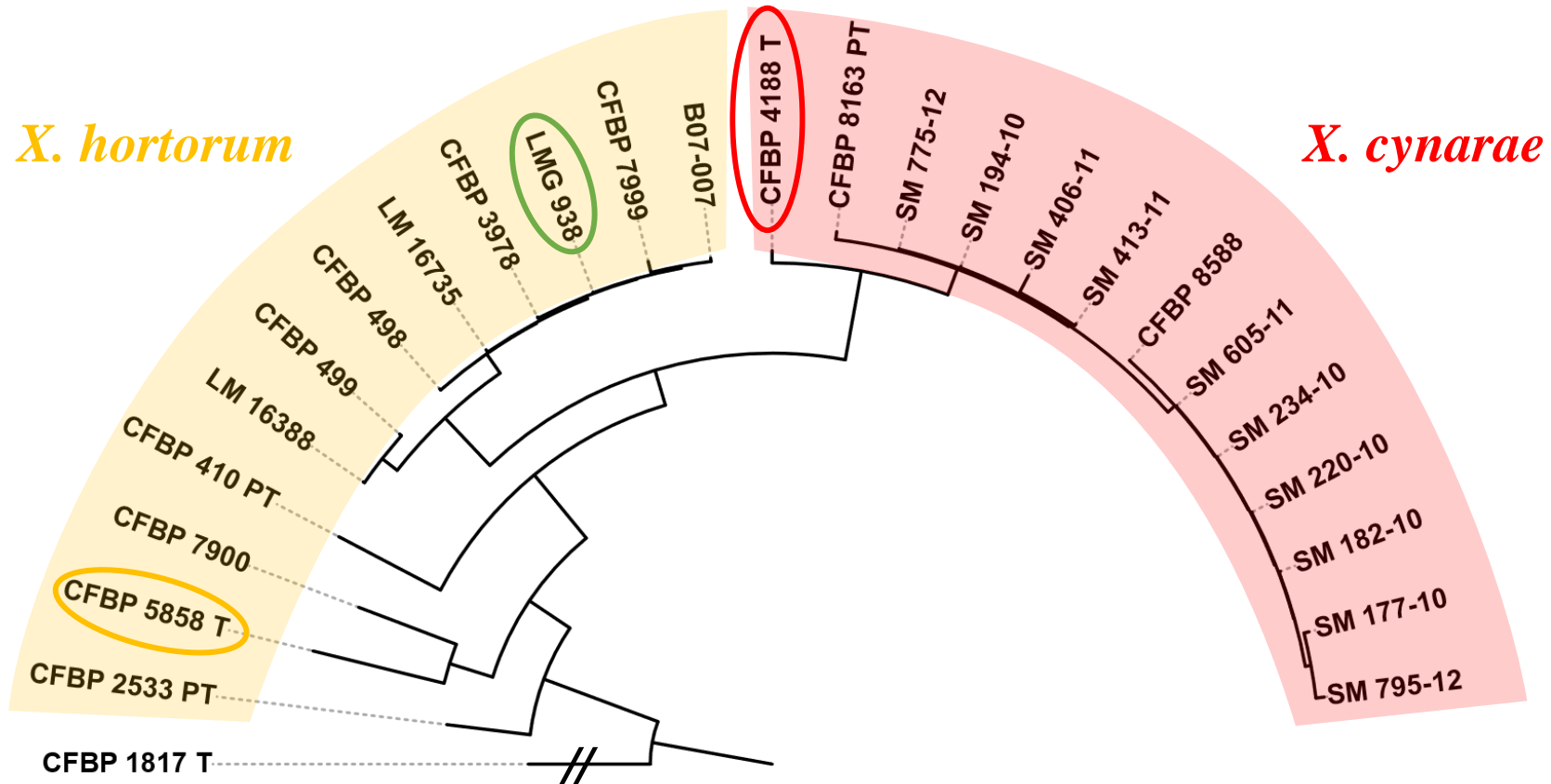
0.99872

0,99000



X. hortorum pv. *vitians* comb. nov.

LMG 938^{neoPT}



OGRI calculations

All vs. all

Observed

Theoretical thresholds
(Rossello-Mora 2015)

ANIm

> 95 %

95 ~ 96 %

isDDH

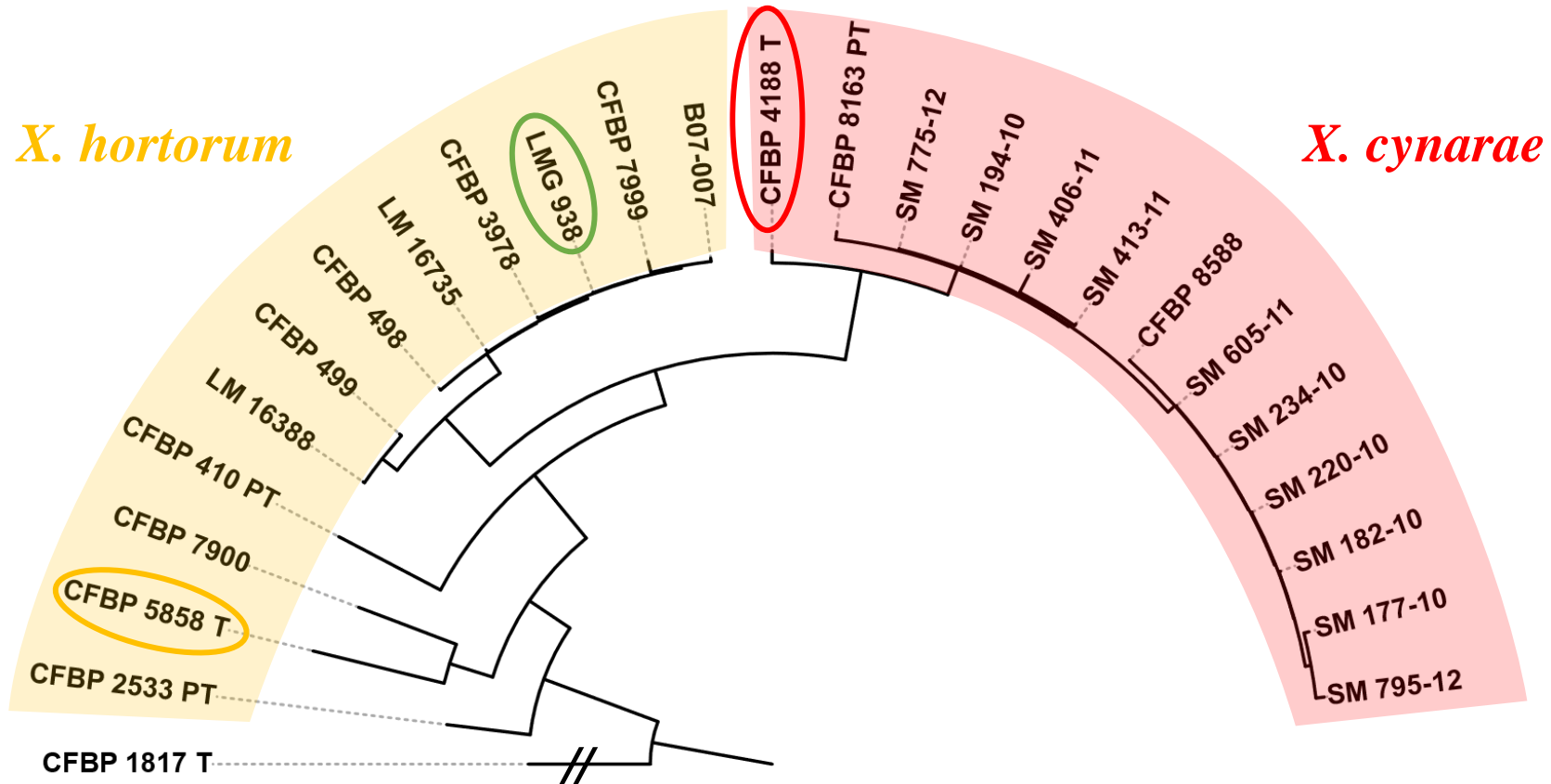
> 64 %

~ 70 %

Tetra

> 0.998

0,990

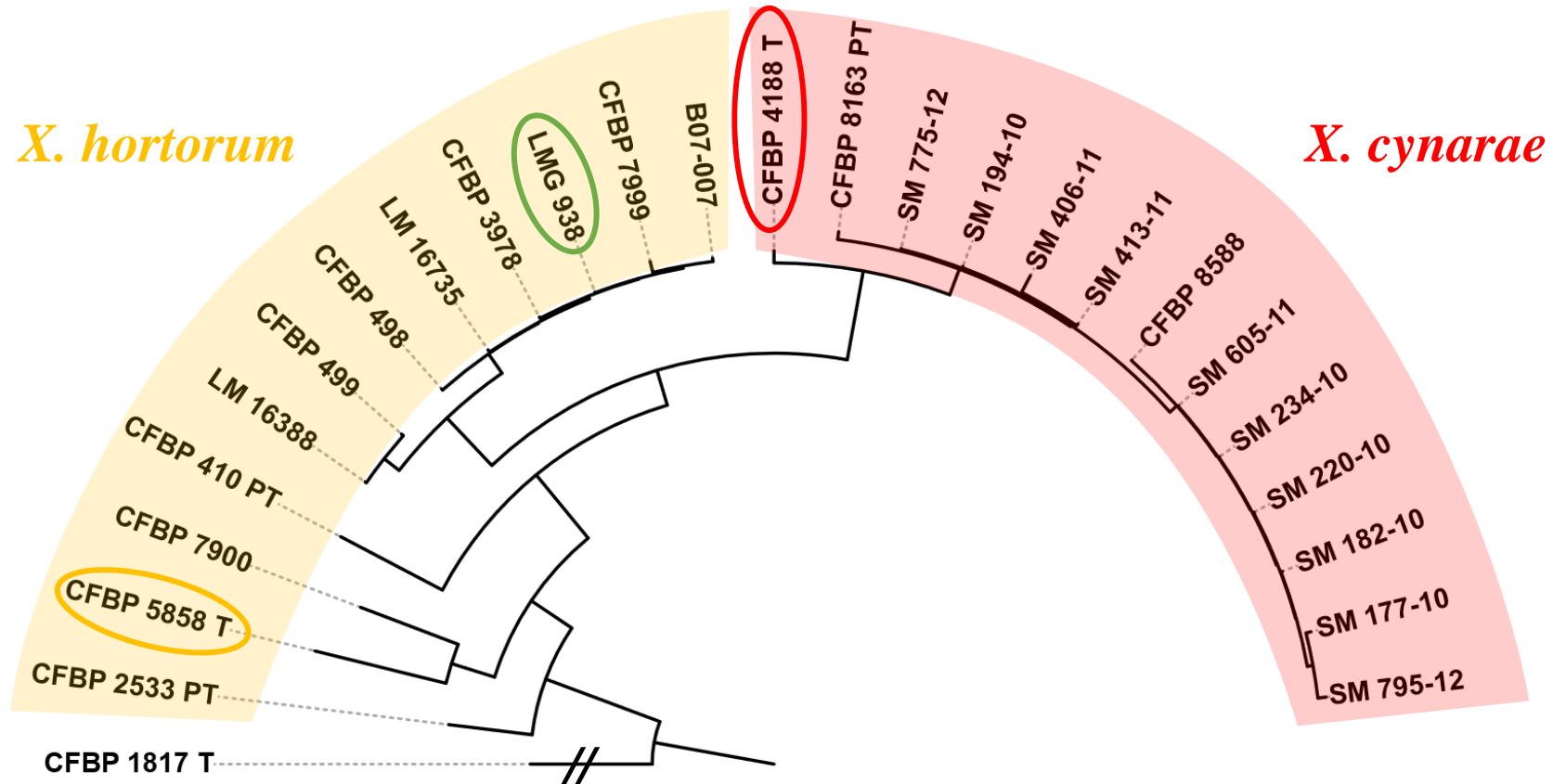


Ambiguous ANI and *is*DDH results

Falls in the “transition zone”

(Richter and Rossello-Mora 2009)

(Rossello-Mora 2015)

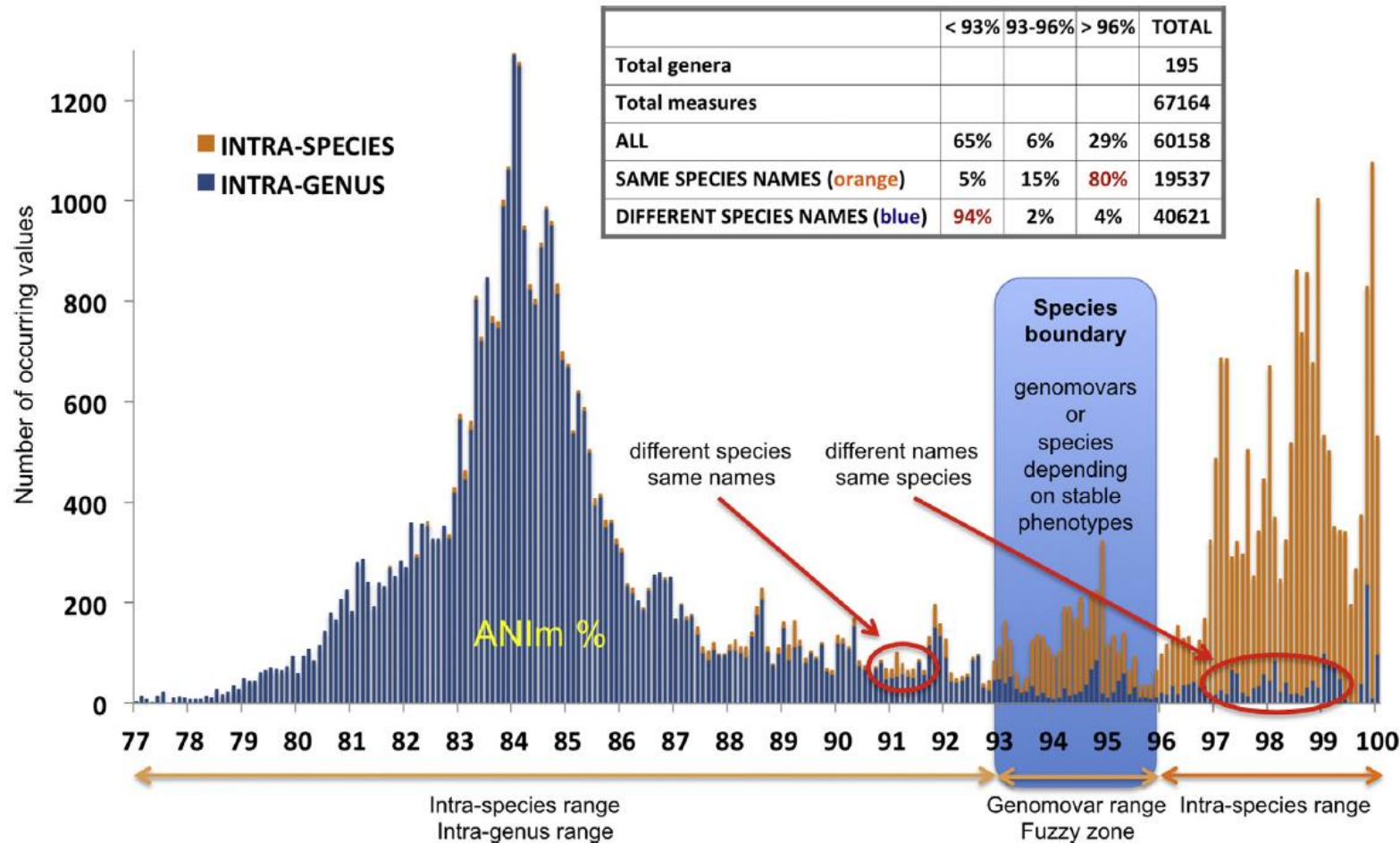


Past and future species definitions for *Bacteria* and *Archaea*

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^a Marine Microbiology Group, Department of Ecology and Marine Resources, Mediterranean Institute for Advanced Studies (IMEDEA, CSIC-UIB), E-07190 Esporles, Illes Balears, Spain

^b Max Planck Institute for Marine Microbiology, D-28359 Bremen, Germany



‘Transition zone’

Boundaries of species

93 % < ANIm < 96 %

60 % < isDDH < 70 %

“An ANI range between 93 and 96% should be treated cautiously, and implies that the **phenotypic diagnostic traits** that would serve as a reason for splitting strains into two or more species need to be **as robust as possible disregarding unstable or unclear phenotypes.**”

Phenotypic profiling



Biolog GEN III microplates
(3 biological replicates)

Core phenotype (stable)

Highly used carbon sources

Variable phenotype (unstable)

Weakly used carbon sources
Chemical compounds resistances

 = Carbon sources utilization
 = Chemical compounds resistances

Pv. hederæ CFBP 5858

Pv. pelargonii CFBP 2533

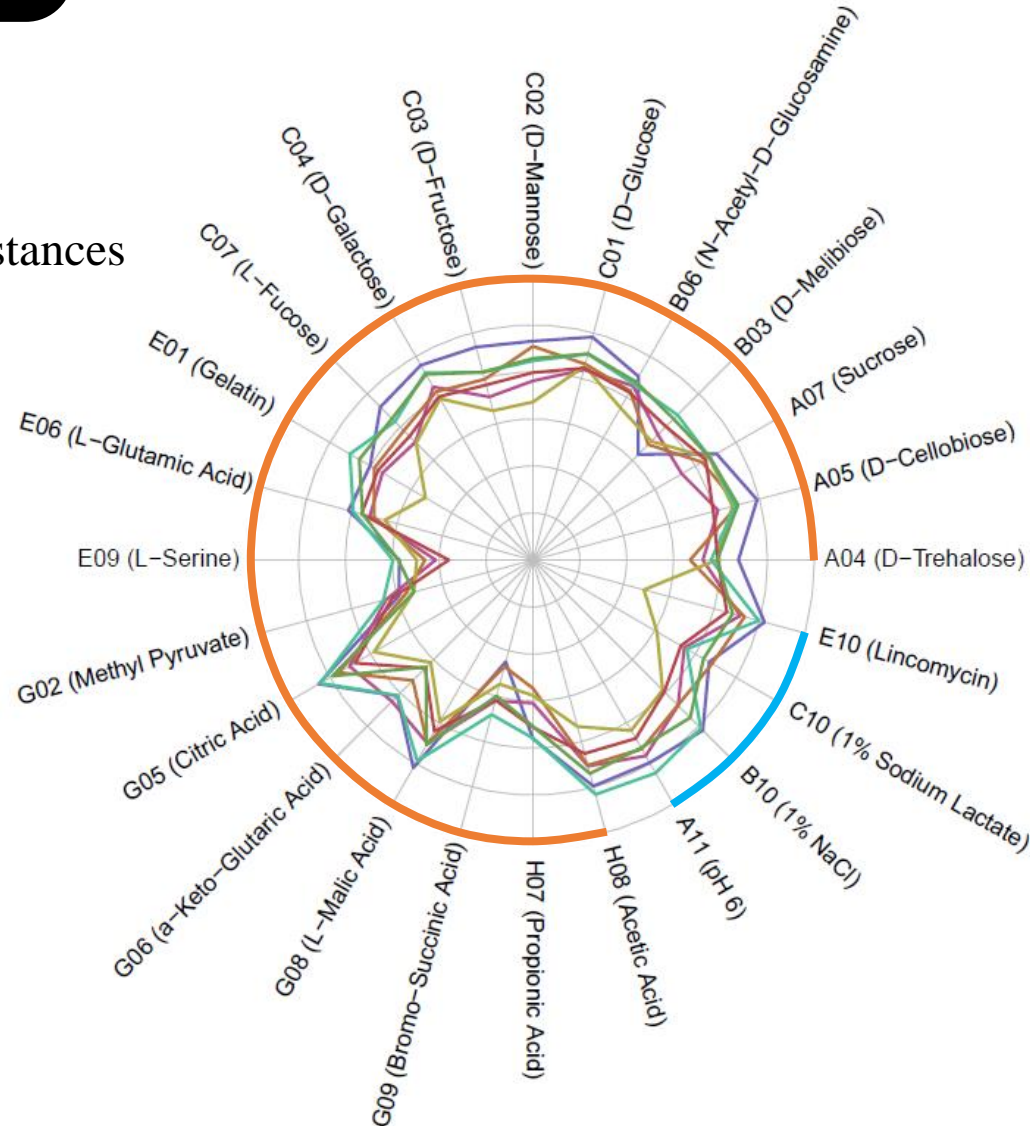
Pv. carotæ CFBP 7900

Pv. taraxaci CFBP 410

Pv. vitians LMG 938

Pv. cynaræ CFBP 4188

Pv. gardneri CFBP 8163



No stable discriminative diagnostic trait observed between *X. hortorum* and *X. cynaræ* strains.

Phenotypic profiling



Biolog GEN III microplates
(3 biological replicates)

Core phenotype (stable)

Highly used carbon sources

Variable phenotype (unstable)

Weakly used carbon sources
Chemical compounds resistances

 = Carbon sources utilization
 = Chemical compounds resistances

Pv. hederæ CFBP 5858

Pv. pelargonii CFBP 2533

Pv. carotæ CFBP 7900

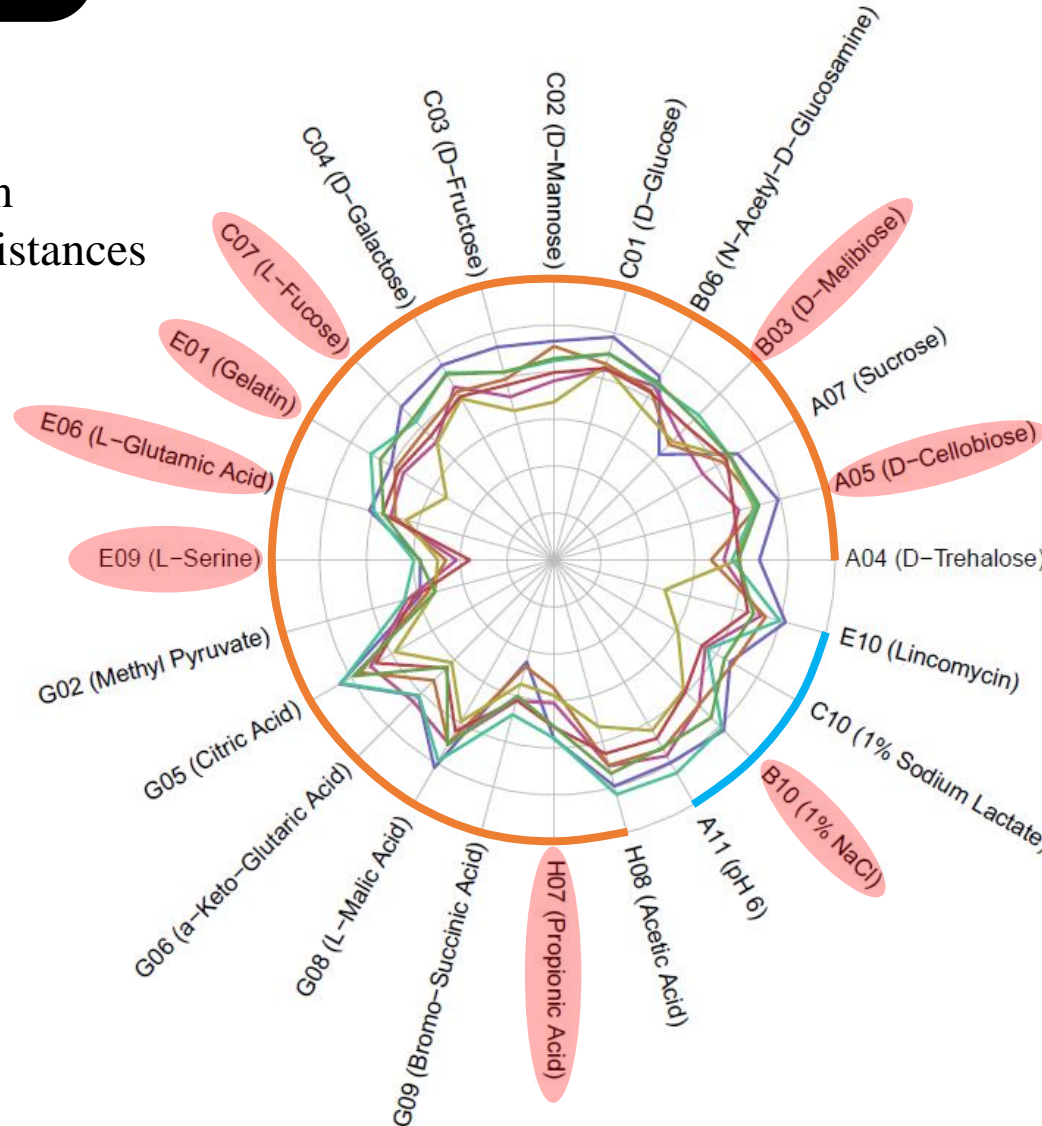
Pv. taraxaci CFBP 410

Pv. vitians LMG 938

Pv. cynaræ CFBP 4188

Pv. gardneri CFBP 8163

 = Not used by *X. populi*



Differentiation with *X. populi* (closest species described)

7 carbon sources not used
Dextrin used as a carbon source
No growth with 1% NaCl
Optimum growth T°C: 20°C

(Van den Mooter and Swings 1990,
Ridé and Ridé 1992,
Vauterin *et al.* 1995)

Xanthomonas hortorum emend.



pv. hederae
English Ivy

Type strain
CFBP 5858^T



pv. cynarae
Artichoke



pv. gardneri
Tomato



pv. vitians
Lettuce

Neopathotype
LMG 938^{neoPT}



pv. pelargonii
Geranium



pv. carotae
Carrot



pv. taraxacii
Russian dandelion

**Combination of *X. cynarae* and *X. hortorum*
in *X. hortorum* emend.**

Xanthomonas hortorum emend.



pv. hederae
English Ivy

Type strain
CFBP 5858^T



pv. cynarae
Artichoke



pv. gardneri
Tomato



pv. vitians
Lettuce

Neopathotype
LMG 938^{neoPT}



pv. pelargonii
Geranium



pv. carotae
Carrot



pv. taraxacii
Russian dandelion



?

Lavender
(Roberts and
Parkinson 2014)



?

Radicchio
(Zacaroni *et al.*
2012)



?

Artemisia
(Ssekiwoko *et al.*
2009)



?

Peony
(Klass *et al.*
2019)



?

Pot marigold



?

Greater burdock



?

Avocado

(Parkinson *et al.* 2009)

Acknowledgments



MINISTÈRE DE
L'ENSEIGNEMENT
SUPERIEUR ET DE
LA RECHERCHE



Financial support institutions



Elise Lacroix



Franck Bertolla
Laurène Mirabel
Céline Lavire
Xavier Nesme
Danis Abrouk



Marion Fischer-Le Saux
Perrine Portier
Martial Briand
Matthieu Barret
Géraldine Taghouti



Alexandre Burlet
Luc Veyron
All the lettuce producers



PennState

Carolee T. Bull
Emma Rosenthal