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Back to basics : Rethinking the diversity and pathogeneity of the causal agent of Bacterial Leaf Spot of Lettuce

Lucas MORINIERE¹, Alexandre BURLET², Perrine PORTIER³, Céline LAVIRE¹, Xavier NESME¹, Franck BERTOLLA¹

¹Ecologie Microbienne Lyon, UMR CNRS 5557 INRA 1418, Université de Lyon, Villeurbanne Cedex, France

²Station d'Expérimentation Rhône-Alpes Information Légumes SERAIL, Brindas, France

³IRHS, INRA, AGROCAMPUS-Ouest, Université d'Angers, SFR4207 QUASAV, CIRM-CFBP, 42 rue Georges Morel, 49071 Beaucauzé cedex, France

Bacterial Leaf Spot of Lettuce (BLSL) is a major threat to lettuce fields worldwide due to the lack of knowledge on its causal agent, *Xanthomonas hortorum* pv. *vitians* (*Xhv*). Moreover, this disease seems to have emerged since the last few years in the Rhône-Alpes region, a major area of lettuce production in France.

Characterization of accurate genetic diversity and taxonomy is a necessary step preliminary to epidemiological studies. However, genetic diversity of *Xhv* has been described only on northern-american strains so far and its taxonomy remains

unclear (Fayette *et al.* 2016). Indeed, this pathogen is currently divided in two groups : group B includes all the *Xhv* strains except for the pathotype strain (CFBP2538). This last strain is registered as *X. citri* pv. *vitians* and considered as group A.

Furthermore, the pathway this pathogen follows to invade the plant and its histological localization during infection have not been yet fully described (Barak *et al.* 2002). Such parameters are determinant to understand the symptomatology and can be valuable to ensure the pathovar homogeneity (Hugouvieux *et al.* 1998).

Objectives of this study

- Investigate the genetic diversity of strains from various origins and their phylogenetic relatedness with other pathovars and close *Xanthomonas* species.
- Search for potential virulence variation and cross these phenotypic data with phylogenetic analyses to redefine the limits of the *vitians* pathovar.
- Characterize the entry gate(s) of *Xhv* in planta.



BLSL on a Leaf Lettuce (Rhône-Alpes, summer 2016)

Field sampling and strains recovery

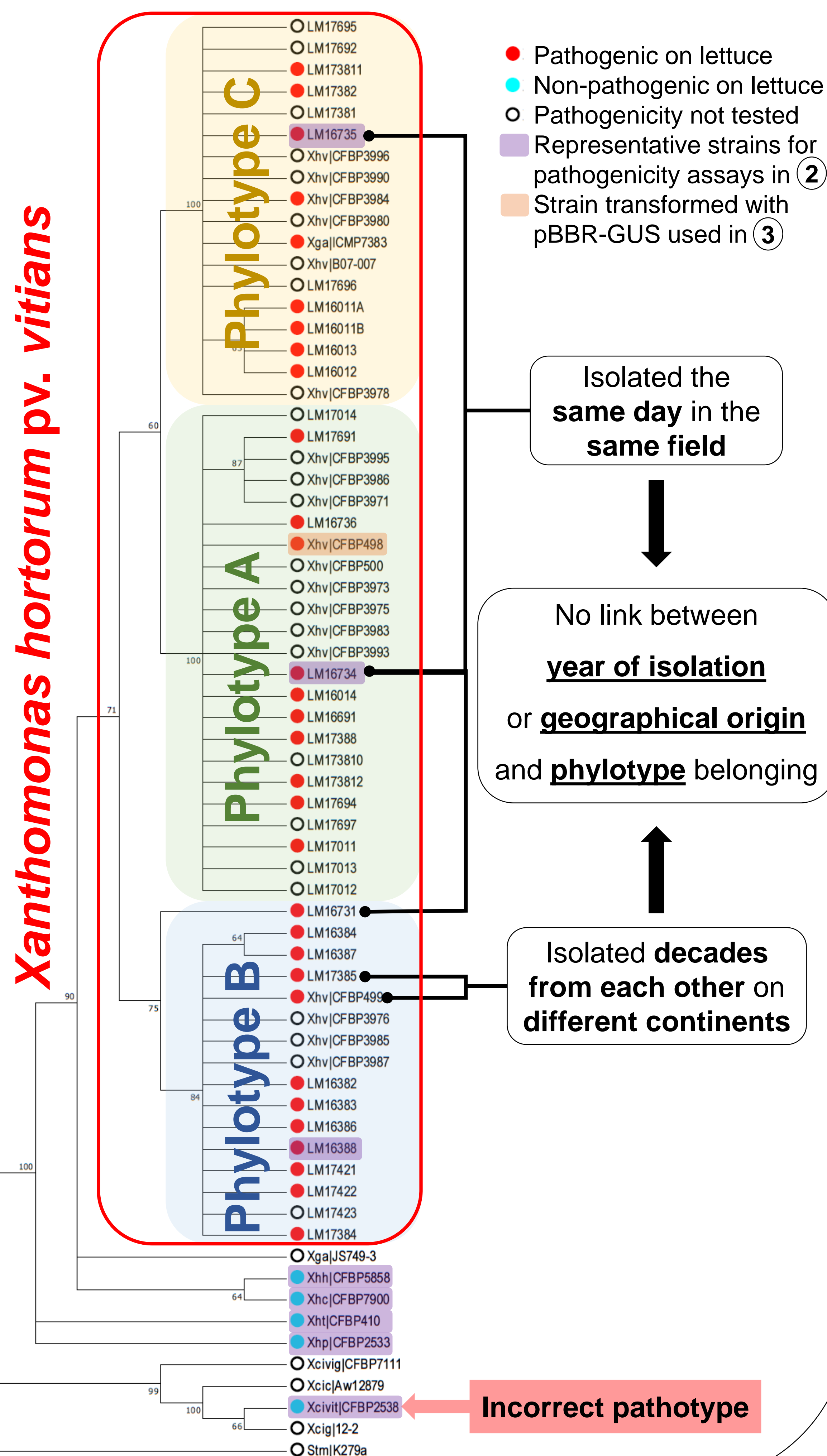
MLSA typing on partial *gyrB* and *gapA* and crossing with isolation data

Pathosystem and disease index design
Pathogenicity assays on Oakleaf lettuce

Histochemical localization of transformed CFBP498 strain with GUS reporter system

1 Phylogenetic analyses

- Maximum Likelihood phylogeny (GTR + γ + I) on 1640 positions (1000 bootstraps, branches condensed if supported by < 60 % bootstraps)

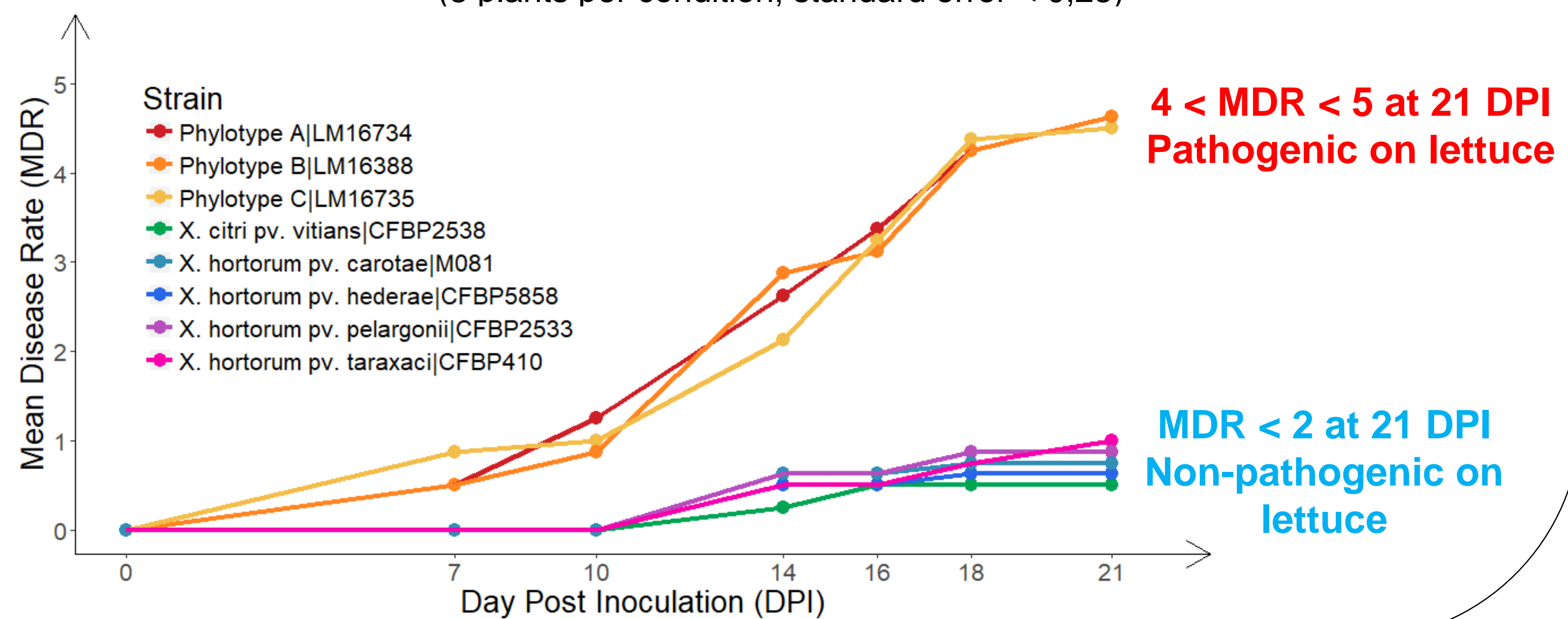


2 Pathogenicity assays

- Disease Index on a 5-point scale, based on number of spots and lesions size (on Oakleaf lettuce cv. Kirinia, adapted from Bull & Koike 2005)

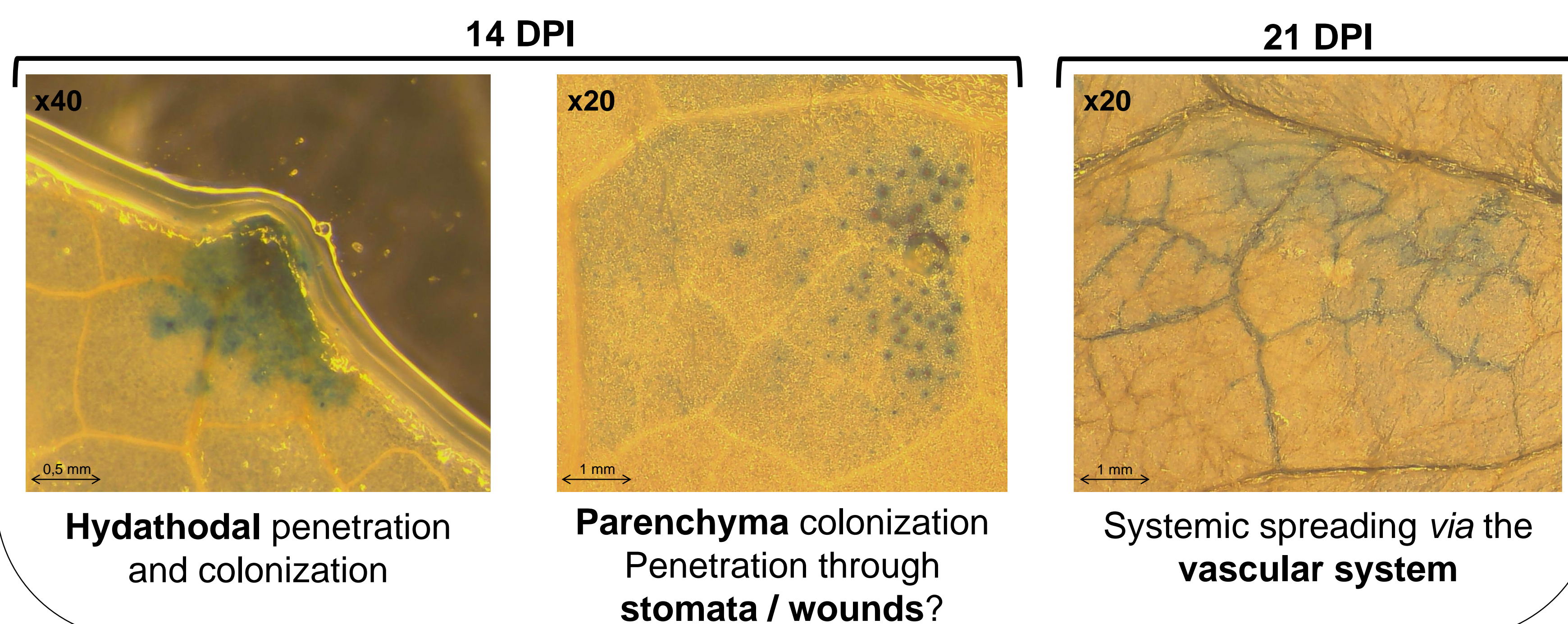


- Mean Disease Rate (MDR) evolution from 0 to 21 days post-inoculation (8 plants per condition, standard error < 0,25)



3 Histochemical localization with GUS reporter

- Binocular magnifier observations of blue final reaction product accumulation in leaf tissues (X-gluc substrate overnight bath at 37°C followed by chlorophyll extraction in 70% ethanol bath at 80°C)



Conclusions

As expected, the pathotype strain *X. citri* pv. *vitians* CFBP2538 differs from the other *Xhv* strains and had been shown to be non-pathogenic on lettuce. Thus, a more suitable pathotype strain should be described. Consequently the *vitians* pathovar appears to be monophyletic, all strains sharing the same direct common ancestor.

Moreover, we described three phylotype in the *Xhv* taxa without any visible influence of geographical origins or time of isolation. These results are consistent with a recent similar study on northern-American strains by Fayette *et al.* in 2016, suggesting that these three phylotypes are prevalent worldwide.

Phenotypically, *Xhv* seems able to penetrate the leaf indistinctly through hydathodes and stomata causing vascular and parenchyma infection. However, stomatal penetration and parenchyma infection should be confirmed by microscopy experiments.

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Literature cited

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