Genetic diversity of *Pseudomonas syringae* causing bacterial leaf spot on table beet (*Beta vulgaris*) and Swiss chard (*Beta vulgaris* subsp. *cicla*)

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Introduction

Table beet and Swiss chard are grown throughout the US, with a significant proportion of baby leaf production in California and 90% of US seed production in western Oregon and Washington. Bacterial leaf spot on beet and Swiss chard, caused by *Pseudomonas syringae* pv. *aptata*, has been reported from California and Oregon, but not yet from Washington.

Pseudomonas syringae sensu lato is a heterogeneous species that consists of at least 8 distinct genomospecies which are further subdivided into pathovars based on the host range and disease manifestation (Gardan et al., 2000; Bull and Koike 2015). Genomospecies 1, also referred to as *Pseudomonas syringae sensu stricto,* consists of organisms from a broad range of hosts. *Pseudomonas syringae* pv. *aptata* (the pathogen causing bacterial leaf spot on beet and chard) belongs to genomospecies 1. However, several studies have indicated that pathovar designations do not accurately represent the pathogenic and genetic diversity within *Pseudomonas syringae sensu stricto* (genomospecies 1). In particular, the pathogens previously identified as *Pseudomonas syringae* pv. *aptata* appear to have host ranges that do not fit the pathovar concept (Berge et al., 2014; Newberry et al., 2015). Since 1999, pseudomonads have been isolated from symptomatic beet and chard plants (Fig. 1) grown in the western US. The goal of this research is to determine the genetic diversity of pathogens causing bacterial leaf spot on beet and chard and to determine whether *P. syringae* pv. *aptata* causes bacterial leaf spot of beet or chard in Washington.

Methods

Experiments were conducted as previously published (Bull et al., 2011). Strains were isolated from extracts of surface-disinfested symptomatic beet or chard tissue by spreading on KMB agar. Cultures derived from single colonies were stored at -80ºC and used for further study. The fluorescence phenotype was determined by observation of colonies grown on KMB agar. Rep-PCR (using the BOXA1R primer) was used to categorize strains into different genotypes. Representative strains from each rep-PCR genotype were evaluated further.

1. Pathogenicity tests: 2 or 4-week-old beet and chard plants were inoculated with bacterial suspensions adjusted to 10^8 CFU/ml. *P. syringae* pv. *aptata* and buffer were used as positive and negative control treatments, respectively. Plants were incubated in a greenhouse or growth chamber. The identity of strains reisolated from symptomatic tissue was confirmed by rep-PCR assay.
2. Multilocus sequence analysis (MLSA) was used to determine the genetic diversity of the strains causing bacterial leaf spot on chard and beet from the western coastal growing regions. These strains were compared to the pathotype strains of *P. syringae sensu stricto* (genomospecies 1) and *P. syringae* pv. *lachrynans* (genomospecies 2). MLSA was conducted using sequenced fragments amplified from *gyrB*, *rpoD*, *gap1*, and *gltA,* concatenated in this order and analyzed as previously described.

Conclusions

- Pathogens from three distinct genotypes have caused bacterial leaf spot on beet and chard since 1999 in the western US.
- The majority of pathogens isolated from diseased beet and chard were non-fluorescent and belonged to a genotype distinct from the pathotype strain of *P. syringae* pv. *aptata.*
- Because pathovars are delineated according to pathogenic reaction and not genotype, the new clade would also named *P. syringae* pv. *aptata.*
- *P. syringae* pv. *aptata* has, therefore, been identified from Washington, Oregon, and California from either diseased chard or beet.
- Two distinct clades causing the same disease further support the conclusion that pathovar concept does not fit pathogens in *P. syringae sensu stricto* (genomospecies 1).

Acknowledgements and Citations

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- Berge et al., 2014,PLoS One 10.1371/journal.pone.010554
- Bull and Koike 2015, Annu. Rev. Phytopathol. 53:8.1–8.24
- Bull et al., 2011, Phytopathology 101:847-858.
- Gardan et al., 2000, IJSB 49:469-478
- Newberry et al., 2015, Plant Disease http://dx.doi.org/10.1094/PDIS-11-15-1332-RE

Results

86 *Pseudomonas* strains were evaluated from 11 disease outbreaks in California or infected seed lots (see Derie et al. 2016 Poster 682-P) produced in Washington and Oregon from 1999 to 2016. Of the strains evaluated, many were fluorescent on KMB but were not pathogenic on either beet or chard and were not studied further. An additional 4 strains (3 of which BP1002, BS3826, and BS3829 are shown here) obtained off seed lots grown in Washington and Oregon were fluorescent and were weakly to moderately pathogenic on beet but not on chard. These strains were more closely related to *P. syringae* pv. *syringae* and *P. syringae* pv. *lapsa.*

The genetic distance between the type strain of *P. syringae* and the beet and chard pathogens was ≤0.01, indicating that the pathogens were all members of *P. syringae sensu stricto* (genomospecies 1). The MLSA (Fig. 2) revealed that only two strains isolated since 1999 (one from western Europe) were genetically identical to the pathotype strain of *P. syringae* pv. *aptata* (CFBP1617), the named beet and chard pathogen. These strains as well as the four strains (discussed above) were the only pathogenic strains that were also fluorescent on KMB. The majority of pathogenic strains were not fluorescent and belong to a new clade within genomospecies 1 that was most closely related to *P. syringae* pv. *atrofaciens* (highlighted in yellow).

Figure 1. Bacterial leaf spot on beet (left) and chard (right). Photos by M. Derie.

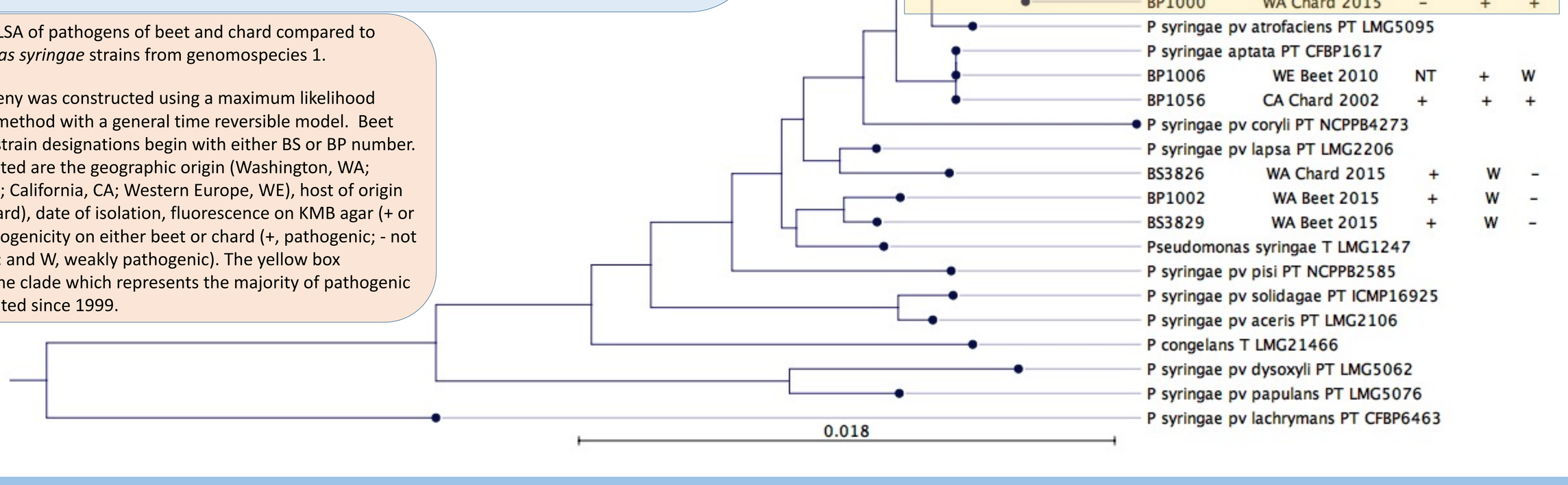

Figure 2. MLSA of pathogens of beet and chard compared to *Pseudomonas syringae* strains from genomospecies 1.

The phylogeny was constructed using a maximum likelihood phylogeny method with a general time reversible model. Beet and Chard strain designations begin with either BS or BP number. Also presented are the geographic origin (Washington, WA; Oregon, OR; California, CA; Western Europe, WE), host of origin (beet or chard), date of isolation, fluorescence on KMB agar (+ or -), and pathogenicity on either beet or chard (+, pathogenic; - not pathogenic; and W, weakly pathogenic). The yellow box highlights the clade which represents the majority of pathogenic strains isolated since 1999.