Comparative genomics to explore host specificity in *Pseudomonas syringae*

Michelle T Hulin[1], Andrew D Armitage[1], John W Mansfield[2], Robert W Jackson[3] and Richard J Harrison[1]

[1]East Malling Research, New Road, East Malling, Kent [2] Imperial College London, South Kensington, London [3]University of Reading, Reading, Berkshire

Introduction

The bacterial phytopathogen *Pseudomonas syringae,* encompasses pathovars that infect over 180 plant species[1]. Individual pathovars show specificity for one or a few hosts. Despite this specialisation, host jumps have occurred frequently within *P. syringae*. Effector repertoires have been linked to host range and therefore genetic alteration of these repertoires may enable host range expansion.

The pathovars *morsprunorum* (Psm) (which is differentiated into two races) and *syringae* (Pss) have convergently evolved to cause canker of cherry and plum (Figure 1). These strains were genome sequenced using Illumina Mi-Seq to search for factors important in disease. Comparative analysis between and within pathovars was used to study the virulence factor repertoires of these strains in order to explore their mechanisms of pathogenicity on *Prunus*.

STUDY AIM: To identify genes involved in pathogenicity and host specificity in *P. syringae* strains that cause bacterial canker of *Prunus* and close outgroups. Putative effector genes modulating specificity will then be transferred between strains to manipulate host range in the laboratory, providing an insight into bacterial host range evolution.

Figure 1: Napoleon cherry tree infected with canker. Red arrow shows disease lesion and white arrow shows gummosis. Picture taken at East Malling (2014)

Methods

- DNA libraries were prepared for genome sequencing using the NEXTflex Rapid DNA-Seq kit and sequencing was performed using Illumina Mi-Seq 300bp paired-end reads
- Genomes were assembled using SPAdes[2] and annotated using the RAST annotation server[3]
- Bayesian phylogenetic analysis was performed using MrBayes[4] software (Figure 2)
- Effector genes were identified by blasting effector sequences against the genomes using tblastn (Figure 3)

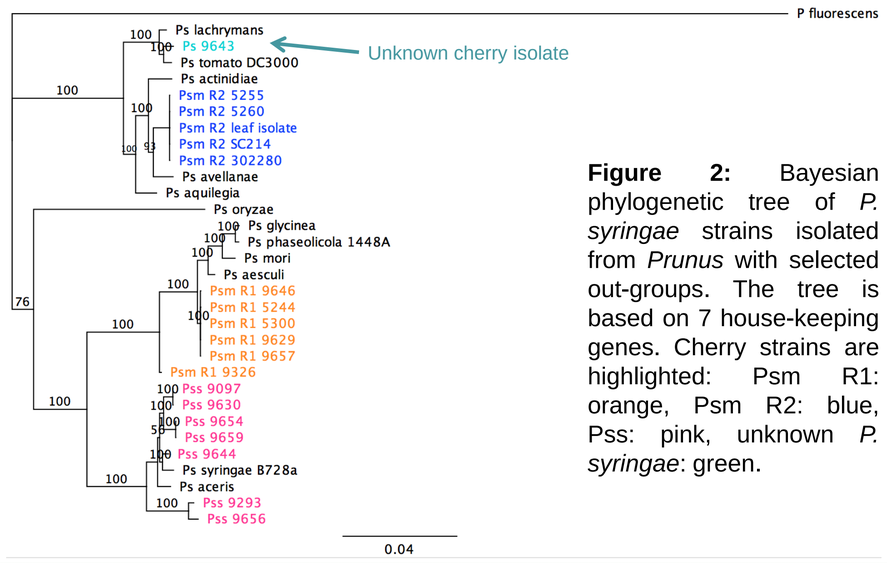

Figure 2: Bayesian phylogenetic tree of *P. syringae* strains isolated from *Prunus* with selected out-groups. The tree is based on 7 house-keeping genes. Cherry strains are highlighted: Psm R1: orange, Psm R2: blue, Pss: pink, unknown *P. syringae*: green.

Results

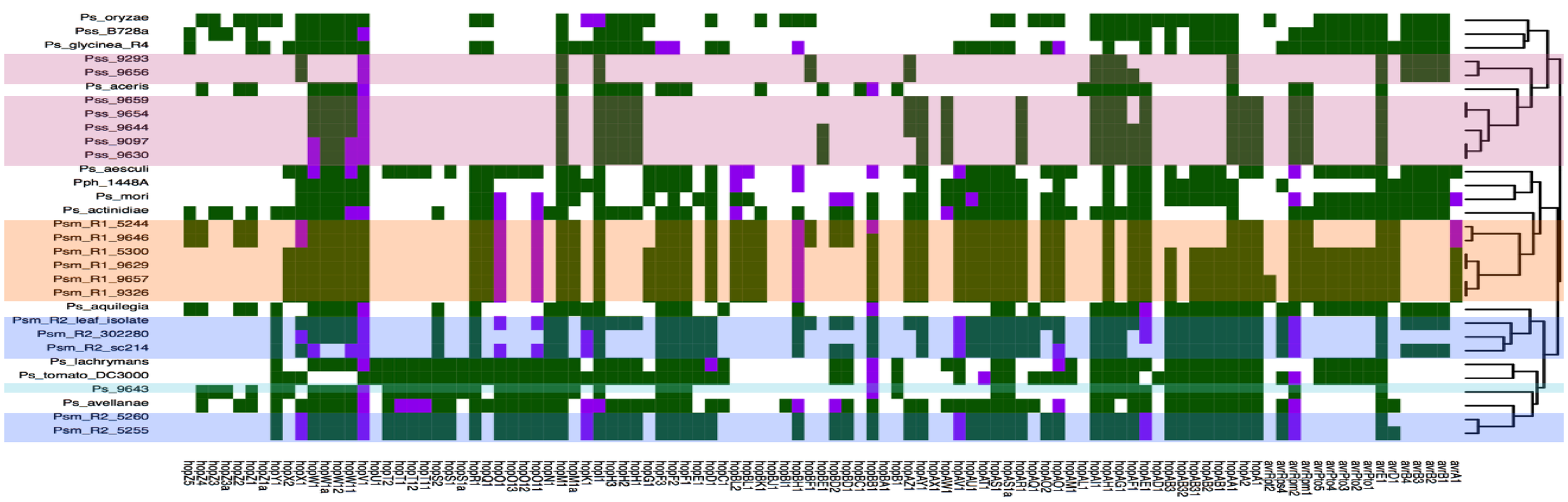

Figure 3: Heatmap of **effector presence** for each *P. syringae* strain. Green: Present with hit length over 40% of the query (likely a real gene), Purple: Present with a hit length of less than 40% of the query. White: Not present. Heatmap was generated using R software[5]. The strains were clustered using UPGMA[6]. *Prunus* strains are highlighted: Psm R1: orange, Psm R2: blue, Pss: pink, unknown *P. syringae*: green.

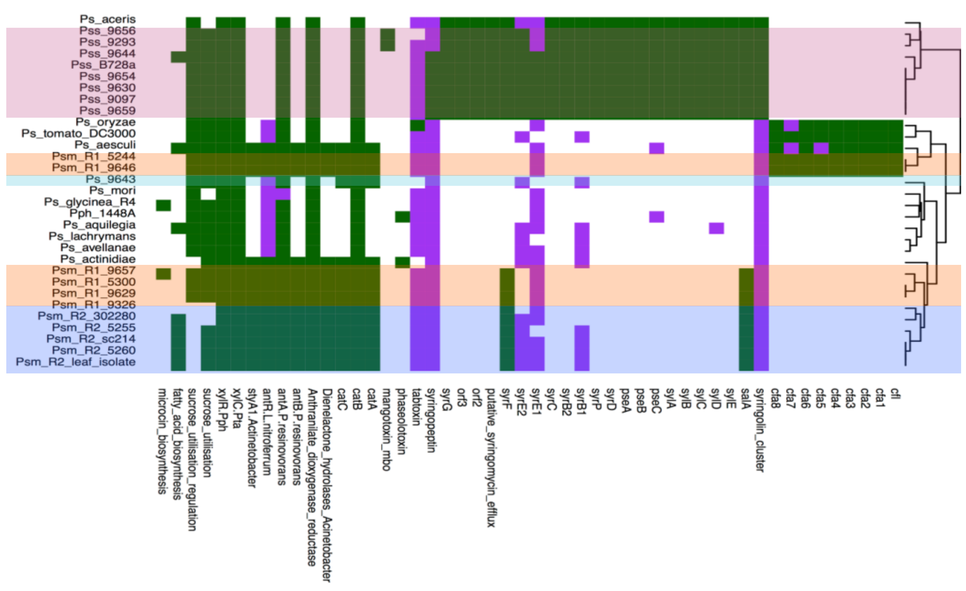

Figure 4: Heatmap of genes associated with **wood degradation** and **toxins** for each *P. syringae* strain. Labelling as above.

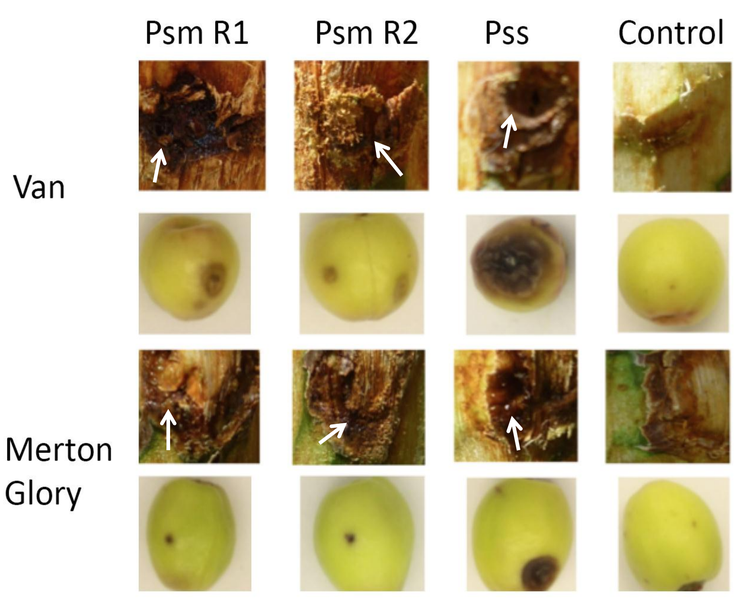

Figure 5: Whole tree and cherry fruit **pathogenicity assays** of two cherry cultivars (Van: susceptible, Merton Glory: tolerant). Whole trees were inoculated in the field and left for two months. Cherry fruits were inoculated in the laboratory and left for one week. Arrows show gummosis at infected sites.

Conclusions

- Bioinformatics analysis has revealed that the different strains possess highly divergent toxin and effector repertoires (Figure 3 and Figure 4). Strains of Pss have reduced effector repertoires and more toxins indicating that they may rely more on toxins for virulence. The two pathovars may therefore use different pathogenicity mechanisms to cause bacterial canker
- Genes involved in the degradation of woody compounds are present in Psm but not in Pss. Indicating Psm may be better adapted to woody environments, or that Pss uses a different strategy to survive in this niche
- The 9643 strain was not positioned phylogenetically in Psm R1, R2 or Pss so may belong to an uncharacterised group of strains that infect *Prunus*

Future work

- The pathogenicity of the different pathovars will be assessed using a combination of cut shoots, cherry fruit, micropropagated plantlets and whole trees (e.g. Figure 5)
- Candidate effectors identified via bioinformatics will be studied in more details
- Genomic libraries will be generated for selected strains and screened to find regions important in pathogenicity
- Genes identified to be important for pathogenicity will then be transferred into phylogenetically related strains isolated from other hosts to see if they confer host specificity for *Prunus* species

1: Dudnik and Dudler, 2014. Genomics-based exploration of virulence determinants and host-specific adaptations of Pseudomonas syringae strains isolated from grasses. *Pathogens*, **3**(1)
2: Bankevich *et al.* 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, **19**(5)
3: Aziz *et al.* 2008. The RAST annotation server: Rapid annotations using subsystems technology. *BMC Genomics,* **9**(75)
4: Huelsenbeck, J.P., and Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**(8)
5: R Development Core Team, 2005. *R: A language and environment for statistical computing, reference index version 3.1.0*. Vienna, Austria
6: Michener, C.D., Sokal, R.R.,1957. A quantitative approach to a problem of classification. *Evolution*, **11**(2)