Genetic diversity of *Pseudomonas syringae* pv. *actinidiae*: seasonal and spatial population dynamics

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State of the Art

Pseudomonas syringae pv. *actinidiae* (Psa) is a gram-negative bacterium responsible for the bacterial canker in *Actinidia deliciosa* and *A. chinensis*, a quarantine disease threatening the kiwifruit industry sustainability. No curative methods have yet been described and control strategies are based mostly on preventive measures (1). Psa has been the subject of several studies in attempt to know the population structure and currently the existence of at least six different Psa types (also known as biovars) capable of infecting *Actinidia spp.*, with different levels of virulence was confirmed (2, 3). The predominant Psa population isolated since the 2008 outbreak in Italy, and currently spread throughout European orchards, belongs to the pandemic biovar 3, responsible for substantial economic losses worldwide (4). This pandemic biovar derived from a diverse Psa population in China, presenting a highly stable core genome and having recently undergone clonal expansion (5). Recent studies in biovar 3 strains isolated in Europe suggested the existence of variant strains, with reduced virulence, co-existing with virulent strains in mixed populations (6). The knowledge of the structure and evolution of Psa populations present in the orchards is fundamental for the development of disease control strategies.

The aim of this study was to determine the genetic structure of endophytic and epiphytic population of *Pseudomonas syringae* pv. *actinidiae* (Psa).

Materials & Methods

Four orchards of *A. deliciosa* from different areas of continental Portugal (Figure 1) were selected for the presence of Psa based on the region, age, degree of Psa severity and cultivar (Table I). The orchards were sampled twice in 2016, once during the spring and again in the autumn. Leaves were collected from the same kiwifruit plants and the endophytic and epiphytic Psa diversity was assessed independently.

Psa isolates were identified with duplex-PCR (7) and the DNA fingerprinting performed using BOX-PCR (8). Molecular tests, such as multiplex-PCR (9), phytotoxins - coronatin (10) and phaseolotoxin (11) and MLST (12) were performed for biovar identification and characterization.

Orchard	Localization	Cultivar	Age (years)	Psa detection	Psa disease severity degree[1]	Average annual temperature (T°)[2]	Annual cumulative rainfall (mm)[2]	Number of cold hours (h)[2]
A	North	Erica	7	2010	1	13.5	1800	478
B	North	Hayward	5	2015	2	12.5	1800	1031
C	Centre	Hayward	4	2015	3	14.5	1100	541
D	Centre	Hayward	30	2016	2	14.5	1100	440

1) Adapted from a symptomatology scale (0 – asymptomatic plants to 4 – completely dry plants) used in pathogenicity assays by Cunty *et al.*, 2015; **2)** Average annual temperature, annual cumulative rainfall: normal of 1961/90; Number of cold hours: total number of hours of T°C below 7.2°C between 01/10/2015 to 30/04/2016. Accessed online: www.ipma.pt - Portuguese Institute for Sea and Atmosphere, I.P. (IPMA, I.P.).

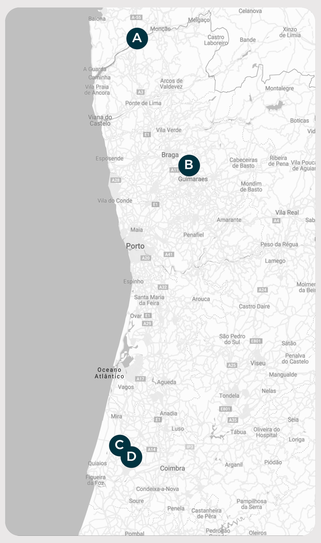

Figure 1 Geographical localization of the four selected kiwifruit orchards in Portugal. A) Valença do Minho (Viana do Castelo; B) São Esteves de Briteiros (Guimarães); C) Catarruchos (Coimbra); D) Cabeça-Alta (Coimbra). Source: https://www.google.pt/maps

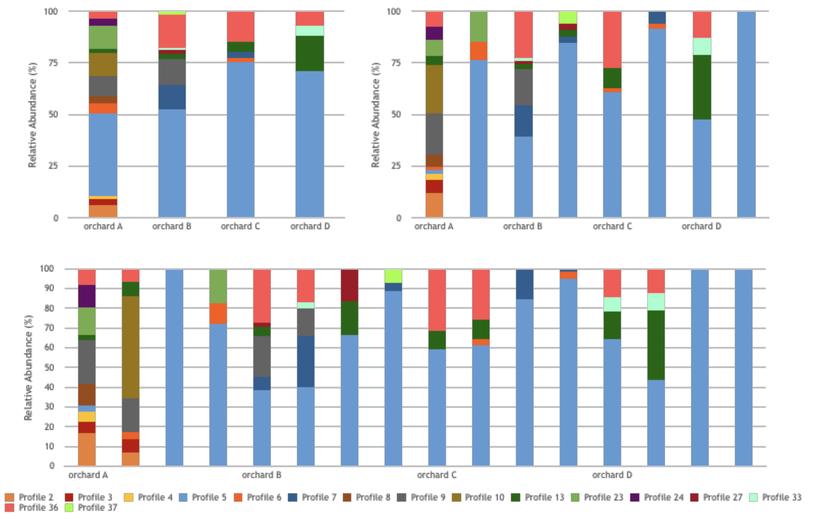

Figure 2 Diversity of Psa isolates determined by BOX PCR profiling. A – in orchard A to D; B – in spring and autumn from the analysed orchards; C – in epiphytic (EP) and endophytic (EN) isolates from spring and autumn from each of the analysed orchards; D – in epiphytic (EP) and endophytic (EN) isolates from spring and autumn from the three representative plants from each orchard.

Results

In this context Psa populations seem to be selected overtime from a diverse genetic pool according to their fitness.

Diversity of Psa populations in Portuguese kiwifruit orchards

The Psa population genetic structure was characterized from 600 isolates obtained from several Portuguese orchards with distinct abiotic conditions in consecutive seasons. Based on BOX-PCR fingerprinting analysis we determined that Psa population was highly heterogeneous with several co-existing populations (Figure 2). Our data supports the existence of mixed Psa populations within each orchard (Figure 2A, 3A). In addition, the overall Psa diversity was remarkably distinct between kiwifruit orchards, since only three Psa profiles (P) were common to all, namely P5, P13 and P36 (Figure 2A, 3A).

Biovar 3 and a new polyphyletic lineage

Psa stains were identified has biovar 3 but our phylogenetic analysis revealed an unreported and highly polymorphic MLST-based lineage (Cluster II, Figure 4).

The diversity of Psa populations varies between seasons and in the phyllosphere

Evident changes occurred in the population structure between seasons translated in a notable decrease in Psa diversity in autumn (Figure 2B, 3B) however some Psa populations were persistently recovered - namely the dominant ones, such as P5 and P13. This trend was observed for all orchards (Figure 2B). Moreover, differences between the epiphytic (EP) and endophytic (EN) population were also observed in samples collected simultaneously (Figure 2C, 3C). This distribution of Psa profiles could be a consequence of changes in abiotic conditions combined with several implemented orchards practices that varied between spring and autumn that could affect directly the Psa population structure, indirectly the community or the physiological status of the plant (6, 13, 14, 15).

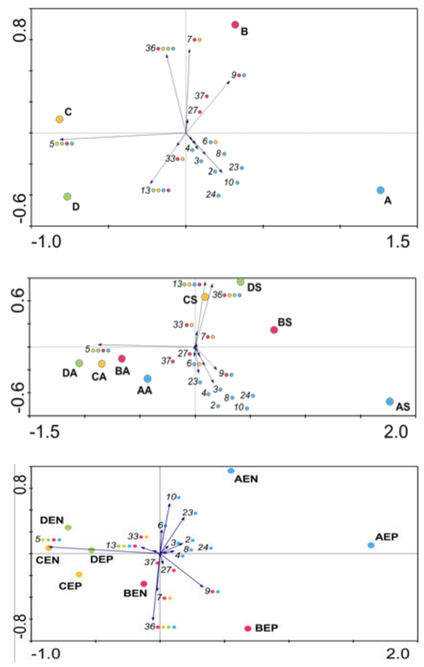

Figure 3 PCoA plots were generated from Psa profiles determined by BOX fingerprinting from the four studied orchards using CANOCO (Šmilauer & Lepš, 2014). PCoA plots were constructed to ascertain: A – the distribution of Psa populations within and between orchards (Blue, profiles isolated from orchard A; Pink, from orchard B; Yellow, from orchard C; Green, from orchard D); B - the distribution of Psa profiles over time [Blue, profiles isolated from orchard A (AA - orchard A, Autumn AS- orchard A Spring); Pink, profiles isolates from orchard B (BA - orchard B Autumn, BS- orchard B Spring); Yellow, profiles isolates from orchard C (CA - orchard C Autumn, CS - orchard C Spring); Green, profiles isolates from orchard D (DA - orchard D Autumn, DS - orchard D Spring)]; C - The distribution of Psa profiles as epiphytic or endophytic profiles in the different orchards [Blue, profiles isolated from orchard A (AEN - orchard A endophytic profiles, AEP - orchard A epiphytic profiles); Pink, profiles isolates from orchard B (BEN - orchard B endophytic profiles, BEP - orchard B epiphytic profiles); Yellow, profiles isolates from orchard C (CEN - orchard C endophytic profiles, CEP - orchard C epiphytic profiles); Green, profiles isolates from orchard D (DEN - orchard D endophytic profiles, DEP - orchard D epiphytic profiles)]. Numbers correspond to Psa profiles and arrows identify the weight that each profile had on the diversity relationship between orchards.

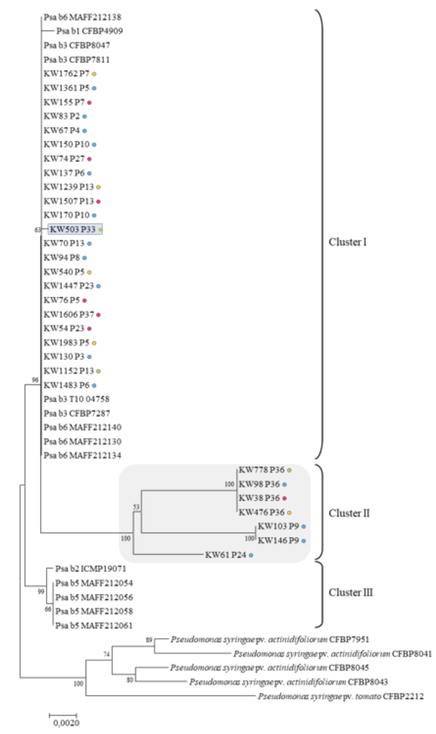

Figure 4 Diversity of Psa isolates determined by BOX-PCR profiling. A – in orchard A to D; B – in spring and autumn from the analysed orchards; C – in epiphytic (EP) and endophytic (EN) isolates from spring and autumn from each of the analysed orchards; D – in epiphytic (EP) and endophytic (EN) isolates from spring and autumn from the three representative plants from each orchard.

Conclusions

Obvious changes in population's structure occurred between leaf niches and seasons favoring the dominance of some Psa strains in autumn. In this context Psa populations seem to be selected overtime from a diverse genetic pool according to their fitness. This perspective is important for the understanding of kiwifruit bacterial canker disease occurrence and Psa evolution and it is also relevant when adopting strategies for epidemics management.

Bibliography:

1. Donati, *et al.* (2014). J Berry Res 4: 53–67; 2. Chapman, *et al.* (2012). Phytopathol 102: 1034-1044; 3. Fujikawa & Sawada (2016). Sci Rep 1-11; 4. Vanneste (2013). N Z Plant Prot 66: 170-177; 5. McCann, *et al.* (2017). Genome Biol Evol 9: 932-944; 6. Firrao, *et al.* (2018). Front Microbiol 9: 1-13; 7. Gallelli, *et al.* (2011). Phytophatol Mediterr 50: 462-472; 8. Louws, *et al.* (1994). Appl Environ Microbiol 60: 2286-2295; 9. Balestra, *et al.* (2013). Plant Dis 97: 472-478.; 10. Bereswill, *et al.* (1994). Appl Environ Microbiol 60: 2924-2930.; 11. Templeton, *et al.* (2005). Biochemistry 44: 4408-4415.; 12. Sarkar and Guttman. (2004). Appl Environ Microbiol 70: 1999-2012; 13. Scortichini (2005). J Plant Pathol 87: 5-12; 14. Prencipe, *et al.* (2017). Eur J Plant Pathol 150: 191-204; 15. Straub, *et al.* (2018). Environ Microbiol 9.

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