GENETIC DIVERSITY AND POPULATION STRUCTURE OF *PSEUDOMONAS* SP. ISOLATED FROM PORTUGUESE KIWI ORCHARDS

Eva Garcia[1,2]; Daniela Figueira[2]; António Veríssimo[2], Joana Costa[1,2]

[1] FitoLab - Instituto Pedro Nunes, 3030-060 Coimbra, Portugal; [2] Centre for Functional Ecology, University of Coimbra, 3001-401 Coimbra, Portugal

STATE OF THE ART

Kiwifruit industry has been growing in Portugal, having increased 1 thousand ha between 2005 and 2014. Portugal was the 11th worldwide kiwifruit producer in 2014, with 12 thousand tones exported, which represented an income of 13 million euros (FAO, 2016). Currently, one of the major threats to Kiwifruit industries worldwide is the bacteria *Pseudomonas syringae* pv. *actinidiae* (Psa), that causes the bacterial canker of kiwifruit. Psa has been the subject of several studies in attempt to know the population structure, development of reliable detection techniques and control strategies of the pathogen. However, the knowledge of the microbial populations present in the orchards is also fundamental for the development of disease control strategies.

The aim of this study was to assess the diversity of *Pseudomonas* sp. present in Portuguese kiwifruit orchards.

MATERIALS & METHODS

During 2016, both epiphytic and endophytic *Pseudomonas* sp. were isolated from *Actinidia deliciosa* orchards (Table 1 and Figure 1).

Non-Psa isolates were confirmed by Gallelli *et al*. (2011), and were identified and characterized by morphological and molecular tests, such as BOX-PCR (Lows *et al*., 1994), MLST (Sarkar and Guttman, 2004) and 16SrRNA gene sequencing.

Table 1. Description of studied orchards

Orchard	Localization	Cultivar	Age (years)	First detection of Psa	Psa disease severity degree
A	Viana do Castelo	*A. deliciosa* cv. "Hayward"	7	2010	1
B	Guimarães	*A. deliciosa* cv. "Erika"	5	2015	2
C	Albergaria-a-Nova	*A. deliciosa* cv. "Hayward"	16	2016*	1
D	Montemor-o-Velho	*A. deliciosa* cv. "Hayward"	4	2015	3
E	Montemor-o-Velho	*A. deliciosa* cv. "Hayward"	30	2016	2

Figure 1. Geographical localization of the five sampling orchards in Portugal. Source: https://www.google.pt/maps

RESULTS & DISCUSSION

A total of 974 *Pseudomonas* sp. were isolated from the 5 studied orchards corresponding to 34 BOX profiles (Figure 2).

Based on the MLST analysis inferred from the partial sequence of *gapA*, *gltA*, *gyrB* and *rpoD* genes, 26 clusters were identified (Figure 3). Representative strains from each group were identified based on the sequence of the 16SrRNA gene. A high diversity was found among isolates since they were phylogenetically distributed by 12 discrete clusters.

Some strains were closely related with *P. viridiflava,* well known by kiwifruit producers due to the leaf necrotic spots and necrotic buds and flowers that causes economical losses (EPPO, 2014). In addition, several isolates were phylogenetic clustered with known plant pathogenic bacteria namely *P. amygdali*, P. *tremae*, *P. savastanoi*, *P. caricapapayae*, *P*. *ficuserectae* and *P*. *cerasi*. Curiously, *P. protegens* known for its biocontrol properties against *Fusarium oxysporum*, *R. solani, P. ultimim* and *Xanthomonas citri* subsp. *citri* (Jara, 2015; Michavila *et al*., 2017) was also found.

In a discrete cluster one strain evidenced high similarity with *P. putida,* whose inhibitory effect against Psa was demonstrated *in vitro* (Tontou *et al*., 2016). Similarly, an isolate clustered with *P. graminis* related with antagonistic activity against *Erwinia amylovora* (Mikiciński *et al*., 2016).

The remaining isolates were scattered through the phylogenetic tree related with other plants associated Pseudomonas sp. with unknown functions.

CONCLUSIONS

Given the high diversity of *Pseudomonas* sp. recovered from *Actinidia* orchards, these bacteria could be involved in important biological and ecological functions that could ultimately be developed and integrated in new management strategies against Psa. Further studies will help clarify the role of these bacteria in kiwifruit plants microbiota.

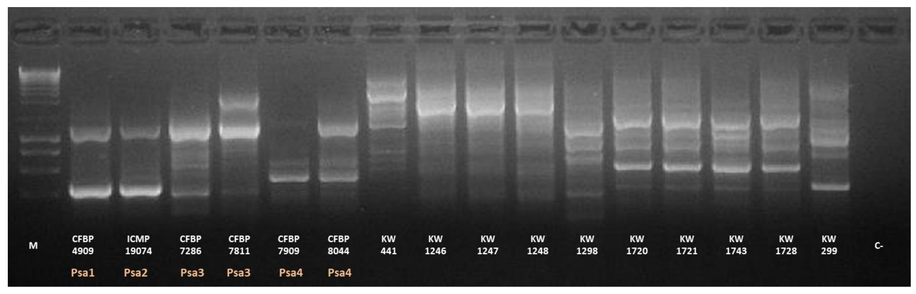

Figure 2. BOX-PCR profiles obtained from *Pseudomonas* sp. strains isolated from plant samples. M: ladder III (Nzytech); Reference strains: CFBP 4909 strain; Psa2b: CFBP 19074 strain; Psa3b: CBFP 7286 strain, CFBP 7811 strain; Psa4b: CFBP 7909 strain, CFBP 8044 strain. KW: isolates in this study; C-: negative control.

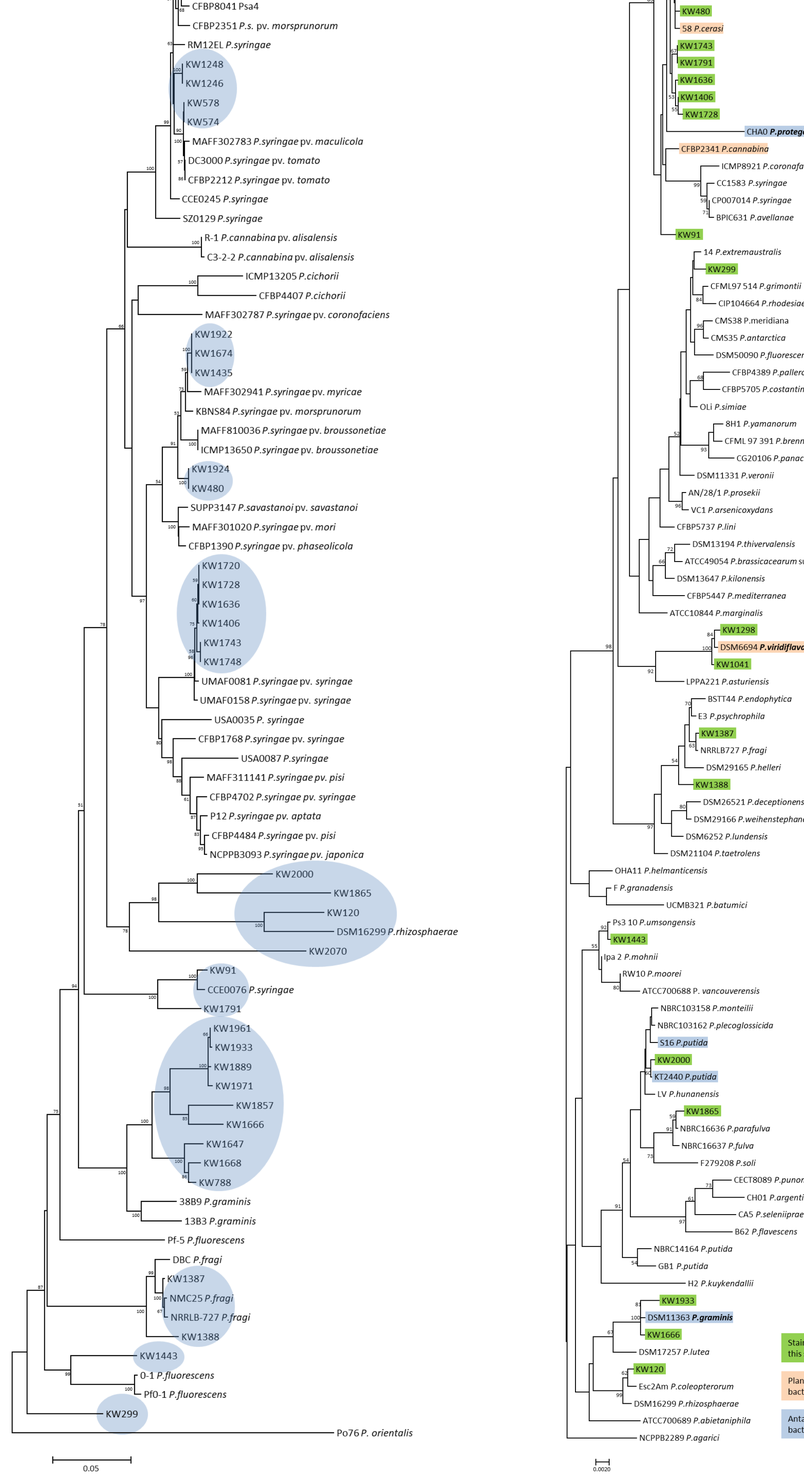

Figure 3. Neighbour joining tree constructed with the concatenated partial sequences of four housekeeping genes (*gapA, gltA, gyrB* and *rpoD*). The scale bar represents the number of nucleotide substitutions per site. Percentage of bootstrap scores obtained for 1000 replicates.

Figure 4: Phylogenetic neighbour joining tree inferred from the 16S rRNA gene sequences showing the relationship of isolates with closely related *Pseudomonas* sp. reference strains retrieved from the database EZTaxon.

BIBLIOGRAPHY:

Food and Agricultural Organization. (2016). http://www.fao.org/faostat/en/#data/QC.

Gallelli, A., L'Aurora, A., and Loreti, S. (2011). Gene sequence analysis for the molecular detection of *Pseudomonas syringae* pv. *actinidiae*: developing diagnostic protocols. Journal of Plant Pathology. *93*, 425–35.

Louws, F.J., Fulbright, D.W., Stephens, C.T., and De Bruijn, F. (1994). Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. Applied and Environmental Microbiology, *60*, 2286–95.

Sarkar, S.F., and Guttman, D.S. (2004). Evolution of the core genome of *Pseudomonas syringae*, a highly clonal, endemic plant pathogen. Applied and Environmental Microbiology. *70*, 1999–2012.

EPPO (2014). PM 7/120 (1) *Pseudomonas syringae* pv. *actinidiae* . EPPO Bulletin, *40* (3), 360-375.

Tontou, R., Giovanardi, D., Ferrari, M., Stefani, E. (2016). Isolation of bacterial endophytes from *Actinidia chinensis* and preliminary studies on their possible use as antagonists against *Pseudomonas syringae* pv. *actinidiae*. Journal of Berry Research.

Michavila, G., Adler, C., De Gregorio, P. R., Lami, M. J., Caram Di Santo, M.C., Zenoff, A.M., Cristobal, R.E., Vincent, P. A. (2017). *Pseudomonas protegens* CS1 from the lemon phyllosphere as a candidate for citrus canker biocontrol agent. Plant biology, 19, 608-617.

Mikiciński, A., Sobiczewski, P., Puławska, J., Maciorowski, R. (2016). Control of fire blight (Erwinia amylovora) by a novel strain 49M of Pseudomonas graminis from the phyllosphere of apple (Malus spp.). Plant Pathol, 145, 265-276.

Jara, H. (2015). Relaciones entre comunidades fúngicas de la corona de trigo y genotipos de *Pseudomonas* spp. productoras de 2,4-diacetilfloroglucinol (2,4 DAPG) y fenazina (PCA), en predios comerciales del sur de Chile. (Master's thesis). Retrieved http://agrarias.uach.cl/egresado/herman-alberto-doussoulin-jara-rindio-examen-de-grado-de-la-estudiante-del-programa-de-magister-en-ciencias-vegetales/.

Tribelli PM1, Raiger Iustman LJ, Catone MV, Di Martino C, Revale S, Méndez BS, López NI. (2012). Genome sequence of the polyhydroxybutyrate producer *Pseudomonas extremaustralis*, a highlystress-resistant Antarctic bacterium. Journal of Bacteriology. 194 (9) 2381-2382.

FUNDING

Trabalho realizado no âmbito da Ação 1.1 Grupos Operacionais "I9K – InovKiwi - Desenvolvimento de estratégias que visem a sustentabilidade da fileira do kiwi através da criação de um produto de valor acrescentado" promovida pelo PDR2020 e co-financiada pelo FEADER, no âmbito do Portugal 2020.

ACKNOWLEDGES