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Biodiversity and Biogeography of Three *Pseudomonas syringae* Pathovars which Affect Kiwi Fruit Cultivation

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Submission: September 10, 2018; **Published:** November 15, 2018

Abstract

Kiwi fruit is an economically important crop in several countries and it can be affected by several bacterial pathogens. Three pathovars of *Pseudomonas syringae* have been reported affecting kiwi fruit plants: *Pseudomonas syringae* pv. *actinidiae* causal agent of bacterial canker of kiwi fruit; *P. syringae* pv. *actinidifoliorum,* causal agent of leaf spot of kiwi fruit, and *P. syringae,* pv. *syringae* causal agent of floral buds necrosis. Since there is a high phenotypic and genetic diversity among strains inside these pathovars here are briefly described the main differences found in the kiwi fruit strains of these three pathovars to help in developing preventive and curative control strategies to efficiently manage the bacterial diseases that affect kiwi fruit.

Keywords: Biovar; Populations; Phenotypic diversity; Genetic diversity

Introduction

The kiwi fruit is a climbing plant of the genus *Actinidia,* family *Actinidiaceae*. Its cultivation is economically important in many countries being the main world producers China, Italy and New Zealand but in many European and South American countries it is currently considered an emerging crop. According to the latest data available 4.274.870 tons of kiwi fruit were produced in 2016 (http://www.fao.org/). The main cultivated species are *A. deliciosa* and *A. chi*nensis due to its high fruit production and quality. This emerging crop like many others is not exempt from diseases caused by phytopathogenic agents such as fungi and bacteria.

Among bacteria, three pathovars of the plant pathogen *Pseudomonas syringae* have been reported affecting kiwi fruit plants. Infact the most important disease of kiwi fruit is bacterial canker caused by *P. syringae* pv. *actinidiae* (Psa). which is a limiting factor for the cultivation of susceptible cultivars and has caused important economical losses around the world since 2008. The bacterium was reported for the first time in 1989 in Japan. [1] and then in China [2], Korea and Italy [3,4]. From 2008 onward more aggressive strains of Psa were detected in several countries like Italy [5], New Zealand [6], France [7], and Australia [8] affecting especially some golden yellow fleshed cultivars of *A. chinesis*. Psa is currently considered the most threatening pathogen of some *Actinidia* crops worldwide.

Therefore, it was included in the list of A2 quarantine organisms of the European and Mediterranean Plant Protection Organization (EPPO) and was the topic of a decision of the european commision (2012) to prevent its introduction in new areas and its dissemination in the European Union. The characteristic symptoms of this disease usually appear in early spring: angular brown spots surrounded by a yellow halo in the leaves and in the branches moreover if the environmental conditions are appropriate trunk cankers with white to reddish exudates can also appear. These symptoms are usually accompanied by wilting and in very susceptible cultivars like 'Hort 16A' even plant death can occur.

There are different populations or biovars of Psa distributed around the world. The characterization and classification in biovars has been designed according to the phenotypic and genetic characterization of the strains from different areas. The principal phenotypic tests used for the characterization of Psa populations are: fluorescence on King´s medium B, LOPAT tests (levan production oxidase potato rot arginine dihydrolase and tobacco hypersensitivity), ice nucleating activity (INA), GATTA tests (gelatin liquefaction, aesculin hydrolysis, tyrosinase activity and tartrate utilization) and plant susceptibility. And the principal genetic tests for the characterization are based on the multilocus sequence analysis (MLSA), the type III secretion system effector genes, the repetitive genome sequences (rep-PCR) and the analyses of complete genomes [9,10]

Until 2014 four Psa biovars were described biovar 1 (Psa1) strains were isolated in Japan and Italy before 2008 and were characterized by their low virulence in kiwi plants biovar 2 (Psa2) was only reported in Korea and biovar 3 (Psa3) responsible for

important damages in kiwi crops worldwide is characterized by highly virulent strains [6] Different studies of the complete genome of several strains show that Chinese strains are the origin of this pandemic lineage [11]

Strains of Psa3 from different countries have been deeply studied at the genetic and phenotypic levels and recently certain differences among strains of this biovar 3 were observed. In Portugal by morphological biochemical physiological and molecular tests two genetically different subpopulations of Psa3 have been identified [12]. Moreover an analysis of New Zealand and European strains of Psa3 using single molecule real-time (SMRT) showed molecular evidences of frequent mobilization and loss of the transposon Tn6212 (without any change in the phenotype) as well as large chromosome inversions and ectopic integration of IS sequences that affect genes *hrp* and is associated with a negative hypersensitivity response (HR) [8] Others studies have reported that some Italian strains of Psa3 also isolated from symptomatic plants show negative HR [13]. All these evidences suggest a coexistence of different strains and the existence of a gene exchange among different Psa populations from the same source. An analysis of 80 sequenced Psa genomes revealed a very high diversity even within the single clade from which the global pandemic arose [9] .However a robust core genome can be recognized with minimal impact of within-clade recombination. It was observed in the core genome the lack of genes for N-acyl homoserine lactone (AHL) or the typical complete *luxI*/R QS (quorum sensing) system that is absent in all studied strains [4].

Biovar 4 was created for low virulence strains since the affected kiwi fruit plants did not show trunk cankers the leaf symptomatology was minor and decreasing production and death of plants were not observed. Recently through a comparative analysis at the phenotypic genetic and phylogenetic level strains of biovar 4 had been reclassified and renamed as a new pathovar *P. syringae* pv. *actinidifoliorum* (Psaf) [14]. Until now the strains of pathovar *actinidifoliorum* do not have a significant economic impact on kiwifruit production in the countries where it has been reported that are France. [15] Spain. [16] and Japan [17] Psaf lineages had been reported suggesting that a high variability exists in this group and that each lineage has its own specific characteristics [14] and [19]. Additionally new strains that present similar profiles (phenotypic and molecular) to Psaf but are not identicals to the French strains were isolated in Spain. These new strains have been called “Psaf look alike” they produce a symptomatology similar to Psaf when inoculated in leaves of kiwi cultivar ‘Hayward’ [20] The current data also suggest a genetic diversity among them.

Two new biovars, 5 and 6 (Psa5 and Psa6), have been recently reported in Japan [21]. Psa5 was isolated from. ‘Hort16’ in Saga Prefecture and Psa6 was isolated from *A. deliciosa cv.* ‘Hayward’ in Nagano Prefecture. The fact that Psa5 and Psa6 had been detected in limited areas suggests that these new biovars may be endemic and rather related to Psa2. Indeed a recent study suggests that Psa5 appears to share an ancestor with the Korean Psa-2 strains [9].

Another *P. syringae* pathovar that affects kiwi fruit but to a lesser extent in comparison to Psa is *P. syringae* pv. *syringae* (Pss) [22] a causal agent of floral buds necrosis that can cause remarkable economic damages in different countries when conditions are favourable for this pathovar. The symptomatology includes canker on twigs necrotic spots on leaves browning and rots of flowers and buds [23]. Biodiversity of kiwi strains of this pathovar has not been studied in depth but there is information about populations with INA-positive and negative strains in Italy [24]. Consequently there is a high genetic and phenotypic diversity of *P. syringae* strains that affect kiwi fruit crops. Biodiversity was also reported in other hosts and is typical of this species complex divided into 50 pathovar variants and distributed into at least seven distinct phylogroups based on the nucleotide sequence divergence of a small set of housekeeping genes [25, 26, 27].

The *P. syringae* species complex possesses a remarkably broad host range although individual lineages often show a high degree of host-specificity and with some exceptions as pv. *syringae* re generally constricted to a small number of potential host species. It is also important to remark that inside this complex several processes of gene dynamics and *phytoadaptation* exist which is a consequence of molecular interactions (by recombination and/or mutations) between different populations. An example of these processes is the mechanism that allow many *P. syringae* pathovars to evade the Effector-Triggered Immunity (ETI) response [28]. In the case of *P. syringae* strains from kiwi the study of its variability would make it possible to know the role of interactions within the Psa populations and with the native microbiota which would ultimately help in developing preventive and curative control strategies to efficiently manage the bacterial diseases that affect kiwi fruit.

Acknowledgement

The authors thank Monterde A, Penyalver J, González A and Landeras E the excellent technical support and F. X. Silva-Hernandez for the English edition. The IVIA group acknowledge the grants from INIA for RTA 2013-00072-C03-03 and the support of Spanish Ministry of Agriculture to the National Reference of Laboratory for Phytopathogenic Bacteria.

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