

Abstract

Fire blight is considered the most serious disease affecting pome fruit and ornamental and wild rosaceae, due to its difficult chemical control, its easy dissemination and to affect plant species of great economic importance, such as apple, pear, loquat and quince. The causal agent of this disease is the bacterium *Erwinia amylovora*, belonging to the family *Erwiniaceae* and considered a quarantine organism in the European Union.

This species has been extensively studied, but at the genomic level there is still much to know, as there are currently only two genomes published completely sequenced and annotated and another thirteen were assembled in scaffolds of strains of *E. amylovora* from different geographical origins and guests. Therefore its pangenoma is considered open, although with a core or conserved genome with a high sequence identity in these strains. With these data we can say that there is very little intraspecific variability, which is manifested in a low genotypic diversity, being noticed that the plasmids are the major source of genetic variability. This could explain the differences in virulence in strains that harbour plasmids, as well as their better adaptation to the different environmental conditions.

This thesis consists in a general introduction chapter, a specific and published bibliographical revision, based in the plasmids of the species of *Erwinia* pathogenic and epiphytes associated to pepita fruit trees and ornamental rosaceae, and other four experimental works of *E. amylovora* and *E. piriflorinigrans*.

On one hand, the origin of this work arose when observing that the plasmid pEA29 described in the majority of the strains of *E. amylovora*, and with a quantitative effect in virulence, was not found in some Spanish isolates of the

bacterium. The study of these strains without plasmid gave way to the discovery of another plasmid of about 70 Kb called pEI70, which is present in strains of *E. amylovora* of several European countries. Therefore, after pEA29, the plasmid pEI70 is the one with the highest presence in the strains of this species. The function, distribution and genetic content of this plasmid, as well as the effect of pEA29 and pEI70 on the expression of the chromosomal genes in strain bearing them, after infection in immature fruit, have been studied. The inoculation experiments on fruit with the strains to which the plasmid pEI70 or pEA29 had been introduced compared to that same strain without plasmids showed an increase in virulence, which was manifested in a reduction in the time of the emergence of symptoms and in which they appeared more aggressively.

Taking into account these results, an experiment was carried out using a microarray, in order to study if the presence of each one of these plasmids could affect the expression on certain chromosomal genes that would explain that variation in virulence of the carrier strain. To have this information a differential gene expression experiment was performed using a microarray with probes from the complete genome of the bacterium as well as the plasmids pEA29 and pEI70. The results obtained demonstrated the role of both plasmids to affect gene expression, between 120 and 180 chromosomal genes according to the plasmid carrying the strain, in each case enriching different functional categories, although 28 of them were coincident in the two cases.

On the other hand, *E. piriflorinigrens* is a newly described pathogenic species that produces necrosis only in pear blossoms but does not appear to affect other organs. In addition, both species share phenotypic and molecular characteristics, making their distinction difficult. Its detection and correct identification was a challenge because the symptoms it causes in flowers are practically indistinguishable from those caused by *E. amylovora*. In this work the genetic content of the new plasmid pEPIR37 found in this new species was studied also. This plasmid is present in all analyzed strains from *E. piriflorinigrens* and it has also been evaluated. When this plasmid was introduced into strains of the species

E. amylovora cured of plasmids, and therefore with reduced virulence, they showed an increase in virulence comparable to that observed in the strains carrying the plasmid pEA29, suggesting that pEPIR37 plasmid produces a similar effect. Therefore, two specific and sensitive real-time and conventional PCR protocols have also been developed to identify, detect and differentiate *E. piriflorinigrans* from *E. amylovora* and other species of this genus using primers designed from specific sequences, annotated in this same work, from plasmid pEPIR37. This has allowed to identify this new species in other hosts as *Pyracantha* sp., besides pear tree and in other regions where previously it had not been detected.

Likewise, these results have allowed to know biological and epidemiological aspects of *E. piriflorinigrans* that contribute to have new key scientific information to establish strategies for its control in pome fruit trees.

The study of the plasmids and their functions in these two phylogenetically related species and their role in the adaptation to the environment in which these species live, as well as in the virulence of the strains that carry them, could give new clues about the origin of both pathogens, their evolution, their biological cycle and interaction with the host plants.