UNIVERSITY OF AMSTERDAM

Host Specificity in Fusarium Oxysporum.

P. van Dam

Summary

Fusarium oxysporum is a fungus that occurs frequently in soils worldwide, usually without negative consequences for other organisms. In some cases, however, it can cause wilt disease and root rot in various plant species. Because of this, it is listed in the top 10 most important plant-pathogenic fungi. An interesting aspect of the biology of *F. oxysporum* is the fact that every individual strain is restricted to causing disease in only one or a few plant species. When it is confronted with another plant, it will try to start the colonization process, but usually it cannot proceed further than the outer cell layers of the roots. The central question addressed in this thesis targets this host specificity: what determines the fact that one strain is capable of infecting tomato plants, whereas another strain specifically causes disease in melon plants. The answer is not only fundamentally interesting, but is also relevant for growers and plant breeders. The understanding of what makes it so the fungus can or cannot infect particular plants will help us in developing plant resistance against *Fusarium*.

To anwer this question, we looked at the DNA of many different fungal strains in order to compare them to each other. The DNA contains the information that determined how living organisms will look. It is the biological blueprint that is stored in the nucleus of each and every cell, including the cells of fungi. This information is arranged in small packages on the DNA: the genes. Each gene results in a protein; a product or building block that has a specific function for the organism.

From earlier research, it is known that the fungus produces small proteins that are secreted during plant infection and can act as a 'key' in this process. These proteins (called 'effectors') can deregulate the plant's defence responses, allowing the fungus to penetrate the root tissue, enter the vasculature of the plant and further colonize it. By carefully examining the DNA regions on which these previously described effectors are located, we found a way of predicting novel effectors.

In **chapter 2** we search the DNA of 59 individual *F. oxysporum* strains, most of which cause disease in cucumber, melon, watermelon and tomato, for the presence of new effector genes. The most important finding described in this chapter is that the strains affecting the same host plants also share a similar set of effectors. Moreover, it turned out that the genes encoding these effectors typically also share an identical DNA sequence between strains that cause disease in the same plant. This is surprising because normally spontaneous mutations in the DNA occur over time. These mutations are found back in the other genes of these fungi, which is an indication that the effector genes have probably been 'horizontally transmitted'. What this means will be explained in the next section.

In 2010, a strain causing tomato wilt disease, *F. oxysporum* f. sp. *lycopersici* (Fol) 4287, was found to have 15 chromosomes. The second smallest chromosome contains all of the effector genes of this strain. Moreover, under lab conditions it could be shown that this chromosome could be horizontally 'moved' (without the interference of sex) to a strain that previously

did not cause any disease in tomato. Upon transfer, the recipient strain turned out to have gained that capability of infecting tomato plants. The hypothesis was thus that this process of horizontal chromosome transfer may also occur frequently in the 'wild', since the recipient strain gains a huge evolutionary advantage.

In **chapter 3** we describe the chromosome composition of an extraordinary strain of *F. oxysporum:* Forc016, that is capable of infecting cucumber, melon *and* watermelon. It turned out that this strain, too, has a single chromosome that resembles the 'pathogenicity'-chromosome of Fol4287. By growing this strain in the presence of the non-pathogenic strain Fo47, we succeeded in transferring the chromosome to Fo47. The new strains turned out to be capable of the same as the Forc parent strain: all three tested plant species became diseased. In another experiment, we induced the loss of this chromosome in Forc016, resulting in strains that were no longer capable of causing disease.

We sequenced the Forc016 DNA with a relatively new sequencing technique ('PacBio'), that produces long stretches of DNA sequence. This allowed us to examine this chromosome as a whole in stead of in small parts, as is often the case with other sequencing methods. We compared the pathogenicity chromosome of Forc016 with a highly similar chromosome, that of Fom001 (only pathogenic towards melon plants). Large parts of the chromosome proved to be 100% identical to each other. However, we also found a region that was highly distinct between both strains. In this particular region, we identified almost all of the predicted effector genes. We therefore believe that this is an important region determining that one strain is restricted to only infecting melon plants while the other can additionally cause disease in cucumber and watermelon.

The fact that *Fusarium oxysporum* can so 'easily' pass on entire chromosomes to others explains why strains affecting the same plant species also have similar (identical) effectors. This knowledge has been applied in **chapter 4** by designing molecular markers based on these effector genes. At the moment, diagnostics is still often performed through time consuming disease assays to identify whether a newly found fungal strain can cause disease in the suspected target plant. With molecular (q)PCR markers, that can determine whether a particular DNA sequence is present in the found strain, this process can be performed many times faster. One of the conclusions drawn from the results presented in this chapter is that the chromosomes that allow pathogenic infection of cucumber, melon, watermelon and several other cucurbits probably have a shared evolutionary ancestry.

This chapter illustrates the step towards application in practice using results obtained in fundamental scientific research. For companies that e.g. produce cucumber seeds, it can be of imporant to have a quick and reliable detection method that allows them to check if their seed batches are not contaminated with pathogens towards cucumber plants.

Finally, in **chapter 5**, we examine three strains that were initially thought to also be *F. oxysporum,* but later turned out to belong to other *Fusarium* species. In the genomes of these strains, belonging to the species *Fusarium proliferatum* and *Fusarium hostae,* we find

indications of horizontal transfer of genetic material (maybe even parts of chromosomes) between *F. oxysporum* and other *Fusarium* species. Two exciting findings support this hypothesis: the presence of : i) so-called '*mimps*' (transposable elements) and ii) effector genes in the identified strains. The fact that these *mimps* and effectors were sometimes identical to DNA fragments of *F. oxysporum* was convincing for the hypothesis of horizontal transfer. These three strains were isolated from diseased lily and hyacinth flower bulbs. Thus, horizontal transfer of (part of) a chromosome potentially occurred in flower bulb fields.

All in all, the results in this thesis have brought us a little bit closer to understanding how host-specific pathogenic interactions work in *Fusarium oxysporum* and the evolutionary mechanisms behind them.