Identification of pathogenicity factors in *Penicillium digitatum*

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INTRODUCTION

Penicillium digitatum is the main postharvest pathogen of citrus fruit all over the world, causing green mold on harvested fruit. It is a specialized necrotroph pathogen that only infects citrus fruit through wounds inflicted during fruit harvesting and handling. Despite the high economic impact of this fungus for the fresh fruit industry there is almost a complete lack of knowledge on its pathogenicity / virulence factors.

The recently sequenced genome of *P. digitatum* (1) offers a first glimpse into the vast array of genes putatively involved in pathogenicity. Previously the group has generated a collection of T-DNA tagged mutants using *Agrobacterium tumefaciens*-mediated transformation (ATMT). The pathogenicity of 1920 transformants was analysed by inoculation of citrus fruits and 19 mutants showed a clear reduction in virulence.

GENERAL OBJECTIVE

- Identify pathogenesis related genes in *P.digitatum*.

SPECIFIC OBJECTIVES

- Identify the best *P. digitatum* transformants for reduction of infection in citrus fruits.
- Sequence the genome of selected transformants and identify T-DNA insertion site.
- Direct deletion of 3 genes probably related with pathogenesis in *P. digitatum*.

DEVELOPMENT & RESULTS

The pathogenicity of 19 transformants was analysed by inoculation of citrus fruits. Fifteen fruits with four wounds per fruit were inoculated with 10 µl of a spore suspension (ca. 1.0 x 104 con/ml). After three independent assays 6 mutants that showed a clear reduction in virulence towards citrus fruits were selected. (Fig. 1).

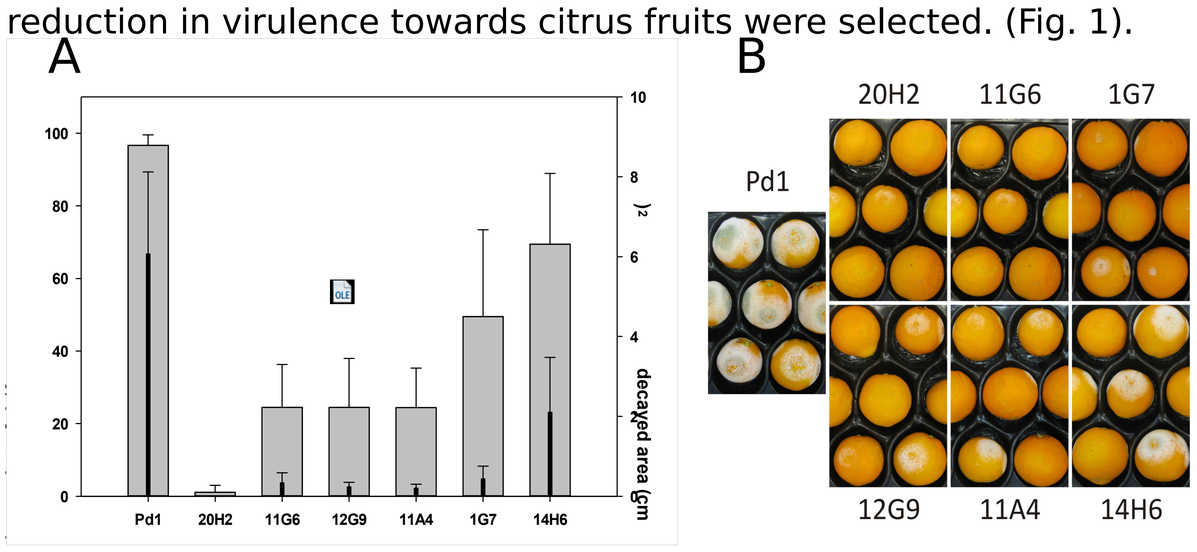

Fig. 1. Pathogenicity analysis of selected transformants. A) Incidence (% of infected wounds, grey bras) and severity (decayed area, black bars) were determined 5 days post-inoculation in citrus fruits infected with the selected transformants. Results show the average ± SD of three independent experiments, each consisting of 15 fruits with four wounds per fruit. B) Visual aspect of infected fruits 7 days post-inoculation.

Genomic DNAs from selected transformants were pooled and sequenced (Illumina HiSeq 2000, 2 X 100 paired end. The trimmed reads (ca 350 million) were mapped to the LB and RB of the T-DNA. Broken paired reads were then mapped against the genome of *P. digitatum* Pd1. This allowed us to identify the integration sites of the T-DNA. An example is shown in **Fig. 3**. Final assignment between T-DNA integration sites and transformants was conducted by PCR (not s

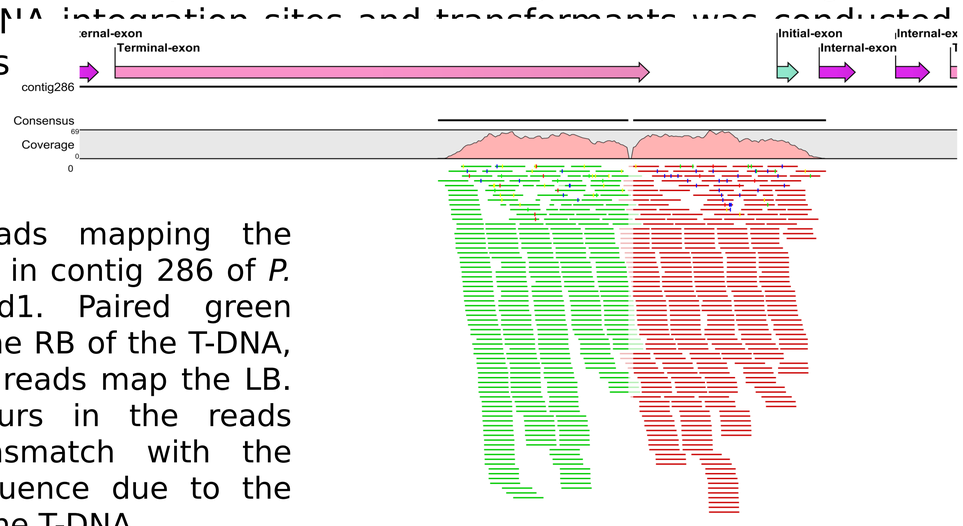

Fig. 3. Reads mapping the insertion site in contig 286 of *P. digitatum* Pd1. Paired green reads map the RB of the T-DNA, whereas red reads map the LB. Lighter colours in the reads denote mimsmatch with the genome sequence due to the insertion of the T-DNA.

Table 1 shows a summary of the six selected transformants with the location of T-DNA insertion sites. In four transformants the insertion took place within a gene, whereas in two transformants the T-DNA was integrated in intergenic regions. To the best of our knowledge none of the targeted genes has previously been implicated in virulence in any fungal pathogen. Targeted gene disruption of these genes will allow us to confirm their role in

Mutant	Contig	Inside a gen?	bp deleted in: genome	LB	RB	Putative protein function
20H2	308	Yes	1661	774	21	Protein containing 2 TRP domains (pfam06011): transient receptor potential (TRP) ion channels
11G6	263	188 bp upstream	10	11	21	Zinc finger protein
12G9	286	Yes	17	77	21	Protein containing 1 foie-grass_1 (pfam11817) and 2 Gryzun domains (pfam07919, trafficking through Golgi)
11A4	418	359 bp upstream	9	17	21	60s ribosomal protein l24a
1G7	447	Yes	0	8	22	Leucine rich protein
14H6	102	Yes	7	0	22	Protein involved in the movement of nuclear encoded pre-proteins into mitochondria

Table 1. Location of the T-DNA insertion site in selected transformants. Deletions in both the *P. digitatum* genome and the T_DNA left (LB) and right (RB) borders are shown.

All the transformants, except mutant 20H2, grew and sporulated as well as the parental Pd1 strain in PDA plates (**Fig. 2**). All the transformants contained a single T-DNA integration as confirmed by qPCR (2)(data not shown).

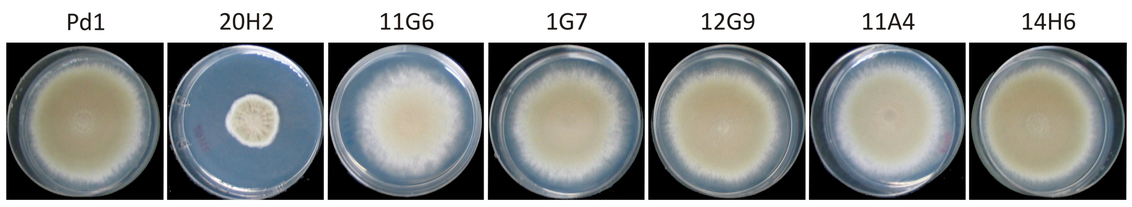

Fig. 2. Growth and sporulation of selected transformants in PDA. Plates were incubated for 7 days at 24 ºC.

FUTURE & POSSIBLE USES

We are currently working in direct deletion of 3 selected genes (located in contigs 308, 263 and 286). Once they are ready we will analyze their virulence, so we could confirm their role in pathogenesis. This aproach will allow a better understanding of the mechanisms leading to citrus infection by *P. digitatum*.

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