

Identification of accessory chromosome regions involved in the pathogenicity in the banana wilt pathogen, *Fusarium oxysporum* f. sp. *ubense* race 1

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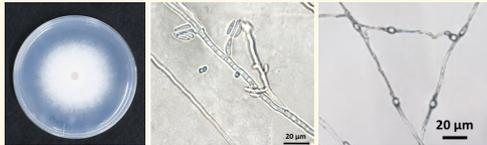
Abstract

Fusarium oxysporum f. sp. *ubense* (*Foc*) is a soilborne fungal pathogen causing the banana wilt (or, Panama disease) on banana (*Musa* spp.). The whole genome information of *Foc* race 1 160527 (*Foc1*) isolated in Okinawa, Japan (Asai et al. 2019) and its comparison with other *F. oxysporum* isolates suggested that the contig 2 and contig 12 in *Foc1* are accessory chromosomes. We generated accessory region-loss mutants in *Foc1* by treatment with 15 µg/ml benomyl, a cell-division inhibitor. Both of Δ ctg2#3 and Δ ctg2#2 mutants losing the entire contig 2 and the 2.5 Mb-region of contig 2, respectively, lost their pathogenicity on banana cv. Shima-banana, whereas the mutant Δ ctg12#1 losing a part of the contig 12 showed no difference in pathogenicity compared to *Foc1* wild type. The growth of Δ ctg2#2 and Δ ctg2#3 on PDA medium was not significantly different with that of *Foc1* wild type. These suggested that the 2.5 Mb-region of the contig 2 of *Foc1* is involved in pathogenicity on banana. The 2.5 Mb-region of the contig 2 contains putative effector genes such as *SIX1s*, *SIX6s*, *SIX9* and *SIX13s*.

Background

Fusarium oxysporum

- Plant-pathogenic ascomycetes



- Sub-classified by host plant into *formae speciales* (ff. spp.) and races.
- The combination of secreted effectors, which are secreted from the fungus into the plant tissues and crucial for pathogenicity, determines the host specificity.
- Carries about 12–16 chromosomes.
- Carries core chromosomes, essential for survival.
- Carries accessory chromosomes, dispensable and not essential for survival. Accessory chromosomes often have genes encoding effectors.



f. sp. *lycopersici* (*Fo*)



f. sp. *melonis*

Fusarium wilt of Banana

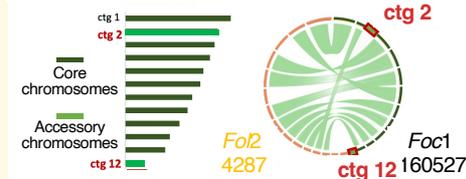


With symptoms of yellowing, wilt, vascular browning and death. Caused by *F. oxysporum* f. sp. *ubense* (*Foc*).



Accessory chromosomes in *Foc1*

F. oxysporum f. sp. *ubense* race 1 (*Foc1*) 160527



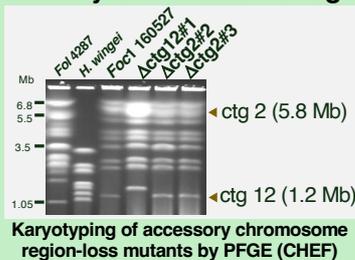
In *Foc1* 160527 **ctg 2** and **ctg 12** are accessory chromosomes

Objectives

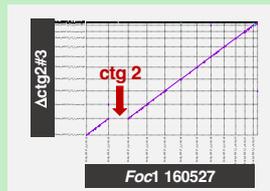
Clarifying the relationship between accessory chromosome regions and pathogenicity in *Foc1*

Results 1

Three accessory chromosome region-loss mutants were generated by 15 µg/ml benomyl-treatment of *Foc1*

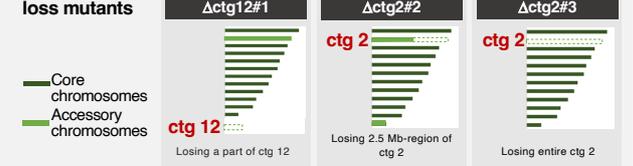


Karyotyping of accessory chromosome region-loss mutants by PFGE (CHEF)



Δ ctg2#3 loses entire ctg 2

Chromosome-loss mutants



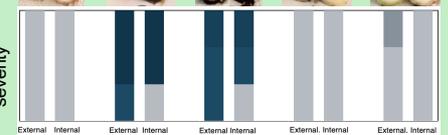
Results 2

Two ctg 2 deletion mutants (Δ ctg2#2 and Δ ctg2#3) lost pathogenicity to banana

- The growth of the three mutants was not significantly different with that of *Foc1* 160527.
- Banana (cv. Shima-banana) plants were inoculated with each mutant by pouring the spore suspension (2×10^7 /ml, 5 ml) to soil, and ca. 40 days later the disease severity of each plant was evaluated.
- ctg 12-deletion mutant (Δ ctg12#1) did not present any phenotypic difference between *Foc1*.
- Both of the ctg 2-deletion mutants (Δ ctg2#2 losing 2.5 Mb-region and Δ ctg2#3 losing entire ctg 2) lost pathogenicity.
- These reveal that the 2.5 Mb-region of ctg 2 is involved in pathogenicity in *Foc1*.

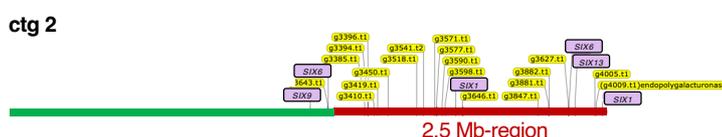


Percentage of individuals showing disease severity



Results 3

Putative effector genes located on ctg 2



- Around the 2.5 Mb-region of ctg 2 (which is lost in Δ ctg2#2), putative effector genes including *SIXs* (secreted-in-xylem) exist.
- Genes *SIX6* and *SIX9* exist outside of the 2.5 Mb-region and seem not involved in pathogenicity.
- Gene targeting for *SIX13* did not present reduced pathogenicity (data not shown).
- We are now targeting *SIX1s* which are unique in entire *Foc1* genome.