DNA methylation in Verticillium dahliae requires only one of three putative DNA methyltransferases, yet is dispensable for growth, development and virulence

Martin Kramer
Wageningen UR: Wageningen University & Research
David Cook
Kansas State University
Grardy van den Berg
Wageningen UR: Wageningen University & Research
Michael Seidl
Wageningen University & Research
Bart Thomma (bart.thomma@wur.nl)
Wageningen University https://orcid.org/0000-0003-4125-4181

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Abstract

Background: DNA methylation is an important epigenetic control mechanism that in many fungi is restricted to genomic regions containing transposons. Two DNA methyltransferases, Dim2 and Dnmt5, are known to perform methylation at cytosines in fungi. While most ascomycete fungi encode both Dim2 and Dnmt5, only few functional studies have been performed in species containing both.

Methods: In this study, we report functional analysis of both Dim2 and Dnmt5 in the plant pathogenic fungus Verticillium dahliae.

Results: Our results show that Dim2, but not Dnmt5 or the putative sexual-cycle related DNA methyltransferase Rid, is responsible for nearly all DNA methylation. Single or double DNA methyltransferase mutants did not show altered development, virulence, or transcription of genes or transposons. In contrast, Hp1 and Dim5 mutants that are impacted in chromatin-associated processes upstream of DNA methylation are severely affected in development and virulence and display extensive transcriptional reprogramming in specific hypervariable genomic regions (so-called lineage-specific (LS) regions) that contain genes associated with host colonization. As these LS regions are largely devoid of DNA methylation and of Hp1- and Dim5-associated heterochromatin, the differential transcription is likely caused by pleiotropic effects rather than by differential DNA methylation.

Conclusion: Overall, our study suggests that Dim2 is the main DNA methyltransferase in V. dahliae and, in conjunction with work on other fungi, is likely the main active DNMT in ascomycetes, irrespective of Dnmt5 presence. We speculate that Dnmt5 acts under specific, presently enigmatic, conditions or, alternatively, acts in DNA-associated processes other than DNA methylation.

Full Text

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Figures

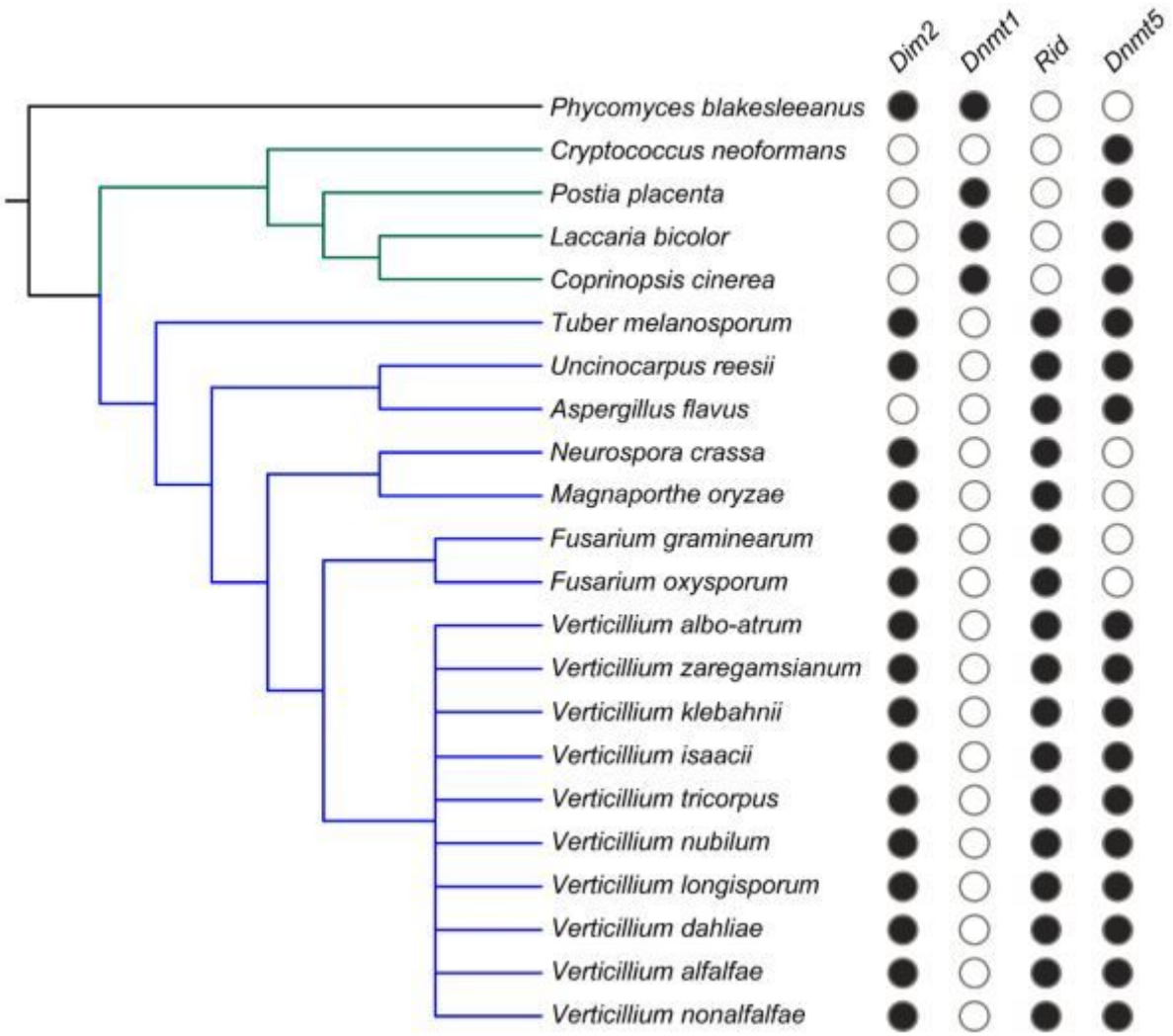

Figure 1

resence of putative 5mC DNA methyltransferases in various fungi. Phylogenetic tree showing a phycomycete (black line), basidiomycetes (green lines) and ascomycetes (blue lines). Filled circles indicate presence of the corresponding DNA methyltransferase as indentified in Figure S1.

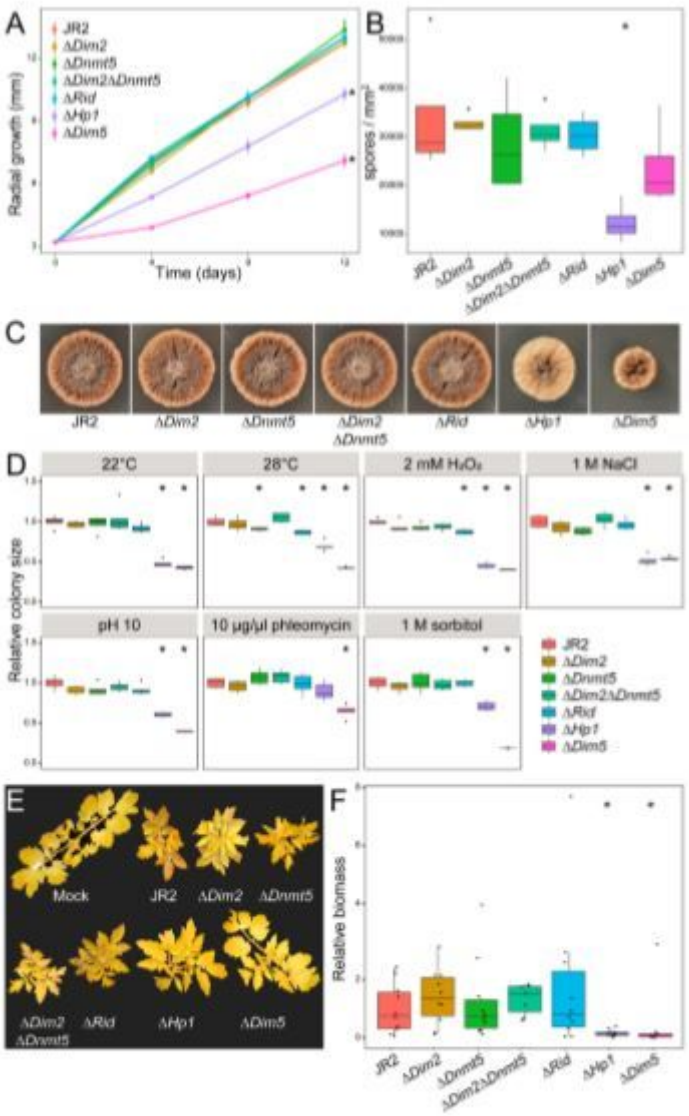

Figure 2

DNMT mutants of Verticillium dahliae do not show altered growth under axenic conditions, stress, or host colinization. A) Radial growth of wild-type and mutants over 12 days, with B) number of spores produced per mm2 of colony and C) pictures showing representative colony morphology after 12 days of growth. D) Colony area of wild-type and mutants subjected to various stress agents, relative to average colony area of wild-type. E) Representative pictures of infected tomato plants at 21 days after inoculation, with F) biomass of wild-type and mutants, relative to wild-type infection. Statistically significant differences from wild-type (Wilcoxon Signed Rank, $p < 0.01$) are indicated with asterisks. For (A), statistical tests were only performed on colony diameter at 12 dpi.

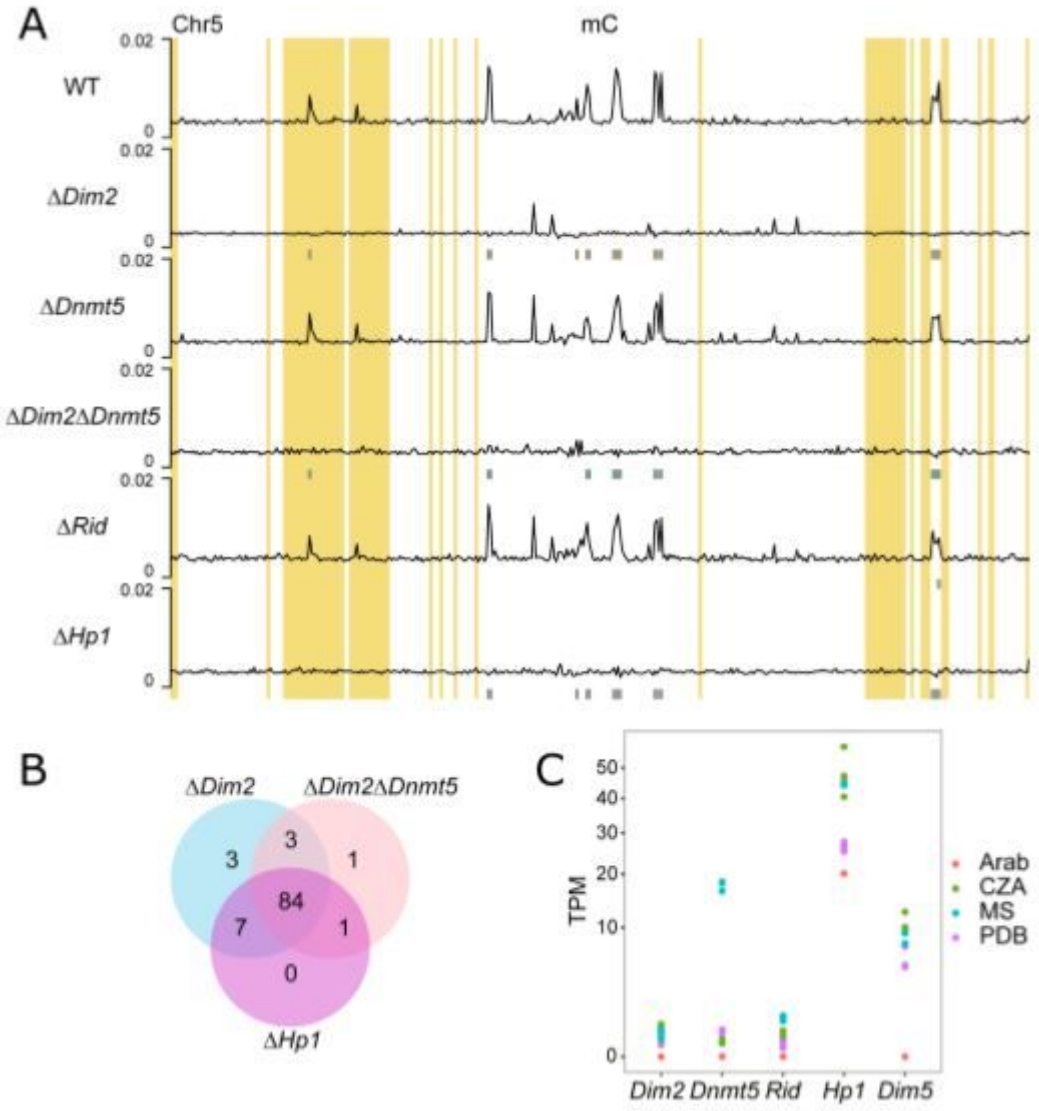

Figure 3

im2 is the main DNA methyltransferase in V. dahliae. A) Whole-chromosome plot displaying the fraction of methylated cytosines for non-overlapping 10 kb windows for wild-type, and DNMT and Hp1 deletion mutants with chromosome 5 as an example. Grey boxes, displayed below the DNA methylation tracks, indicate the hypomethylated windows compared to the wild-type strain in CG and CHG context from Table 1. Previously defined LS regions (Cook et al., 2020) are highlighted in yellow. B) Overlap of hypomethylated windows in mutant strains showing severe loss of methylation. C) Expression (TPM values) of DNA methyltransferase genes Dim2, Dnmt5 and Rid, as well as Hp1 and Dim5 of V. dahliae strain JR2 cultured in Czapec-Dox medium (CZA), half strength Murashige-Skoog medium (MS) and potato dextrose broth (PDB), and during Arabidopsis infection at 21 days post inoculation (Arab), in triplicates.

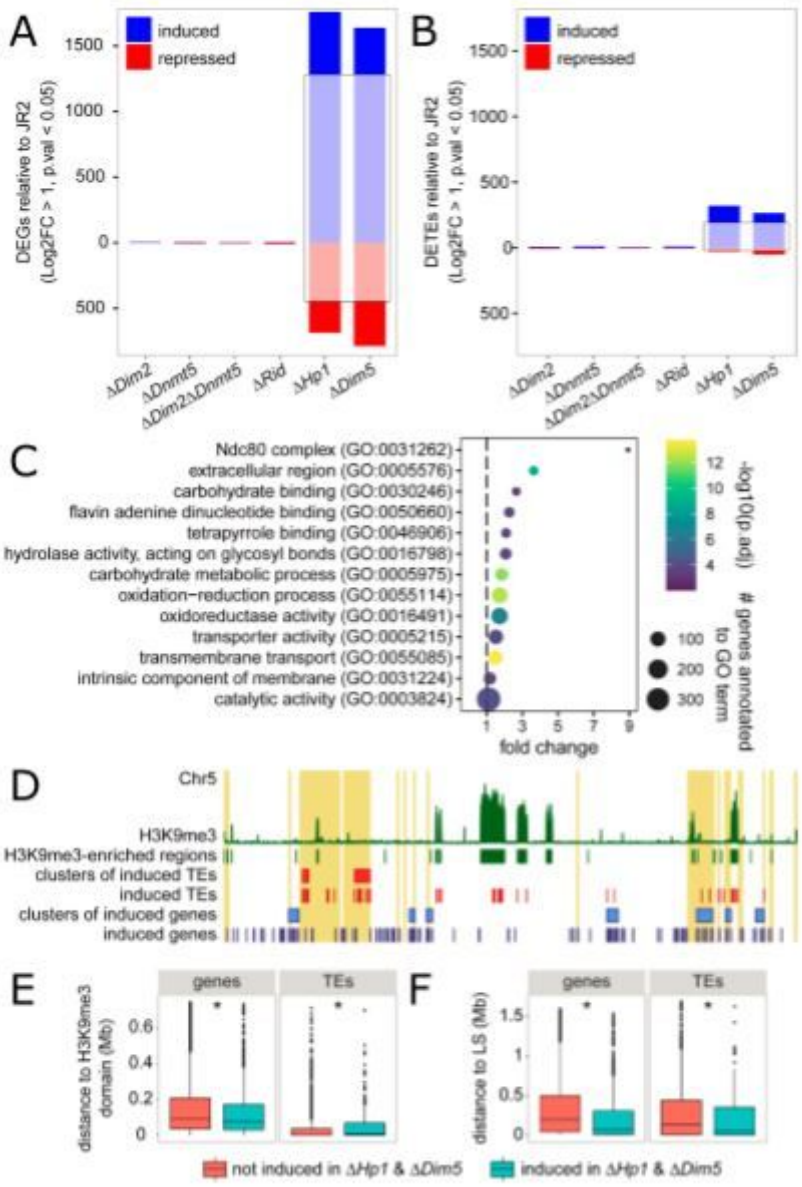

Figure 4

Genes and transposons that are induced in the Verticillium dahliae Hp1 and Dim5 mutants do 312 not associate with H3K9me3-marked chromatin. Differentially expressed genes (A) and transposons (B) in the mutants relative to wild-type. Induced genes and transposons are indicated in blue, repressed genes and transposons in red. The number of genes and transposons that are induced and repressed in both the Hp1 and Dim5 mutants are indicated by opaque coloring in black rectangles. The amounts of genes (C) and transposons (D) that are induced in both the Hp1 and Dim5 mutants, and occur clustered in the genome (blue line) compared to a distribution of 1000 random equally sized sets of genes and transposons. (E) Whole-chromosome plot displaying the location of induced genes (in blue) and transposons (in red) on chromosome 5 as an example. Clusters of induced genes and transposons are indicated as blue and red rectangles, respectively. H3K9me3-ChIP signal along the chromosome is indicated in green in the upper track. LS regions (Cook et al., 2020) are highlighted in yellow. The minimal distance of genes and transposons to H3K9me3-enriched genomic regions (F) and to LS regions (G). 323

Asterisks indicate statistical differences (Wilcoxon signed rank test, $p<0.01$) between genes and TEs induced in both the ΔHp1 and ΔDim5 mutants and those that are not induced in the mutants.

Supplementary Files

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- SUPPLEMENTALFIGURES.docx