

The *sfp*-type 4'-phosphopantetheinyl transferase Ppt1 of *Cochliobolus miyabeanus* controls development and pathogenicity

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Abstract

In filamentous fungi, the *sfp*-type 4'-phosphopantetheinyl transferase Ppt1 is required for activation of some of the enzymes for lysine biosynthesis, and peptide/polyketide secondary metabolites. *Cochliobolus miyabeanus* is a pathogen of brown spot disease on rice. In this study, *C. miyabeanus* *PPT1* mutants were derived from the wild-type (WT) strain WK1C (ATCC 56640, MAT1-2). The *PPT1* gene was deleted using the split marker method. The 5' and 3' flanking sequences of the predicted open reading frame of each target gene were amplified from WT using two specific primer sets (CmPPT1UF1, CmPPT1UR1, CmPPT1DF1 and CmPPT1DR1) that matched the upstream and downstream *PPT1* gene flanking sequences. Transformation of *C. miyabeanus* was carried out as previously described (Inderbitzin *et al.*, 2010; Oide *et al.*, 2006), with a slight modification. Deletion of the *PPT1* in the rice pathogen, *C. miyabeanus* was verified by Polymerase Chain Reaction (PCR) amplification using three diagnostic primer sets. Three independent *C. miyabeanus* mutants (*Cmppt1*-1, *Cmppt1*-4 and *Cmppt1*-8) were generated. Growth of all *Cmppt1* mutants was reduced to 50.5% compared to WT and their colony surfaces were hydrophilic. In addition, *ppt1* mutants of *C. miyabeanus* were also reduced in tolerance to oxidative stress and iron depletion. As expected, mutants were albino since they cannot produce the polyketide melanin and were auxotrophic for lysine because they lack an α -amino adipate reductase. Interestingly, the yielded strains were significantly reduced in virulence to rice. Because *C. miyabeanus* *ppt1* mutants make no or very few conidia, virulence was tested by inoculating a mycelial suspension of the mutants with supplemental lysine into rice leaves. At 10-day post inoculation, WT had developed large dark brown necrotic areas (6.10 ± 1.41 mm) surrounded by chlorotic halos. *Cmppt1* mutants caused much less damage and appeared as light brown patches without obvious chlorosis (3.69 ± 0.86 mm), in most cases.

Keywords: phosphopantetheinyl transferase, *Cochliobolus miyabeanus*, brown spot, rice, transformation.

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