Differentiation of pathogenic races of the lentil anthracnose pathogen C.lentis using effectors

The genus *Colletotrichum* has been under revision for the last decade. It contains species of high economic importance as pathogens of major crops, but also includes species that are model organisms to study host-pathogen interactions. The hemibiotroph *Colletotrichum lentis*, causative agent of anthracnose on *Lens culinaris* (lentil) was recently described as a new species. In the host cell, *C. lentis* initially generates thick, biotrophic primary hyphae restricted to one epidermal cell before switching to the necrotrophic phase by developing thin filamentous secondary hyphae that invade other host cells.

During its interaction with the host plant, *C. lentis* likely secretes numerous effector proteins including toxins to manipulate the plant's innate immunity thereby gaining access to the host tissues for nutrition. *In silico* analysis of expressed sequence tags generated from *C. lentis*-infected lentil leaflet tissues identified 15 candidate effectors. No sign of positive selection pressure was observed at the intraspecific level, suggesting that *C. lentis* effectors are under stabilizing selection. *In planta* infection stage-specific gene expression waves among candidate effectors were revealed for the appressorial penetration phase, biotrophic phase and necrotrophic phase through RT-qPCR. Homologs of 10 out of 15 *C. lentis* candidate effectors were identified in several other species of *Colletotrichum* with more than 65% peptide sequence identities across species. Similarly, a toxin protein ClToxB encoded by a single copy gene with four characteristic cysteine residues and further characterized as a potential a host-specific toxin through heterologous *in planta* expression, had homologs of extensive similarity in five species including three *Colletotrichum* spp., suggesting that effectors of different, but related species are not as unique as previously thought. *In silico* analysis of *ClToxB* sequence and comparative genomics revealed that *ToxB* is unlikely an alien gene in the *C. lentis* genome. Congruency between established species relationships and that established based on *ToxB* sequence data confirmed it arose through evolution from a common ancestor, whereas the bacterial gene *Arg* identified in ESTs of *C. lentis* and encoding argininosuccinate lyase was horizontally transferred from bacteria as previously shown for other *Colletotrichum* species. The knock-down of *ClToxB* transcripts by RNAi resulted in reduced virulence, suggesting that *ClToxB* is a virulence factor.

Two pathogenic races (virulent race 0 and less virulent race 1) were described in the Canadian population of *C. lentis*. A silent single nucleotide polymorphisms in the open reading frame of candidate effector *ClCE6* was used to develop Kompetitive Allele Specific PCR marker, which differentiated perfectly between race 0 isolates and race 1 isolates, suggesting that it may be co-segregating with the virulence governing locus/loci, hence can be used to determine the race identity of *C. lentis* isolates. Race indexing of *C. lentis* isolates is important not only for monitoring the population dynamics of the pathogen, but also for screening germplasm under field conditions.

Publication

Candidate effectors contribute to race differentiation and virulence of the lentil anthracnose pathogen Colletotrichum lentis.
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