

Molecular systematics and comparative draft genome analysis of strawberry leaf blight fungus

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The leaf blight fungus, formerly known as “*Phomopsis*” *obscurans* originally described from the United States, is a severe pathogen of strawberry. Morphological characters are often inadequate for identification of this species. Although this fungus is currently classified as a *Phomopsis* sp. (= *Diaporthe*), preliminary analyses of this study indicated it is not congeneric with *Diaporthe*. Phylogenetic analyses with representative species in the order Diaporthales were performed using five nuclear loci (28S, 18S, internal transcribed spacer regions with 5.8S of rDNA, translation elongation factor and DNA directed RNA polymerase II) to infer its evolutionary relationships. Genomic libraries of leaf blight fungal DNA were prepared for next generation whole genome sequencing. DNA and RNA were extracted and paired-end libraries were generated with Nextera library preparation kits (Illumina) and sequenced on Illumina Miseq instrument. Whole genome and transcriptome sequences obtained were initially analyzed with CLC Genomic Workbench and comparative analyses were performed using methods similar to the US-DOE Joint Genome Institute annotation pipeline. Results of phylogenetic analyses determined that “*P.*” *obscurans* represents a unique evolutionary lineage and possibly an undescribed genus within a new family. The whole genome assembly consisted of 5638 contigs (≥500 bp) with an estimated genome size of 48 Mbp. The N₅₀ contig length is 13853 bp and the G+C content is 52%. The output resulting from MAKERP, predicted a total of 12431 genes and was modeled with *Cryphonectria parasitica* EP155 genome. The estimated mitochondrial genome size is 98658 bp which is comparatively larger than the average sized mitogenomes of fungi. Analysis with the CAZy database (dbCAN) identified in total 778 putative carbohydrate active enzyme encoding genes, including 341 glycoside hydrolases (GHs), 112 glycosyl transferases, 25 polysaccharide lyases, 138 carbohydrate esterases, 51 carbohydrate-binding modules, and 111 auxiliary activities. Therefore, “*P.*” *obscurans* is equipped with all necessary enzymes to breach plant cell wall, penetrate, successfully infect and cause severe plant disease. Among GHs, majority (i.e. 20 from each) belong to GH3, GH5, GH16, and GH28 families that possess xylanase, cellulase, chitinase, polygalacturanases which are also potentially involved in maceration and soft-rot of plant tissues including fruits.

Keywords: CAZymes, Diaporthales, Genome sequencing, Phytopathogens

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