Draft Genome Sequences of *Elsinoë fawcettii* and *Elsinoë australis* Causing Scab Diseases on Citrus

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Abstract

Elsinoë fawcettii and *E. australis* (phylum Ascomycota) are phytopathogenic fungi causing scab diseases on citrus plants. We report here the high-quality draft genome sequences and ab initio gene predictions of two *E. fawcettii* strains and one *E. australis* strain, which differ in their host range. This genome sequence information will provide valuable resources to underpin genomic attributes for determining host range through comparative genomic analyses of citrus scab fungi.

Keywords

genome, citrus scab, *Elsinoë*

Citrus scab disease is one of the most prevalent fungal diseases in many citrus-producing areas (Kunta et al. 2013). The disease affects not only the leaves and twigs but also fruit of susceptible cultivars causing fruit blemishes, which greatly reduce the value of fruit for the fresh market (Chung 2011). Two species of fungi are generally recognized as causal agents of scab diseases: *Elsinoë fawcettii* and *E. australis*, causing citrus scab (sour orange scab or common scab) (Gopal et al. 2014) and sweet orange scab (Chung 2011), respectively. Both fungal species belong to the phylum Ascomycota, class Dothideomycetes, order Myriangiales, and family Elsinoaceae. The symptoms of citrus scab vary depending on citrus cultivars and timing of infection but they often appear as warty pustules that are raised on leaves and fruit. There are mainly six pathotypes of *E. fawcettii*: Florida broad host range (FBHR), Florida narrow host range, Tryon's, Lemon, Jingeul, and SRGC; and two pathotypes in *E. australis*: sweet orange and natsudaikai (Hyun et al. 2009). Both *E. fawcettii* and *E. australis* are known to produce elsinochrome, a nonhost-selective perylenequinone-containing phytotoxin, a prerequisite for full fungal virulence and lesion formation (Chung 2011).

E. fawcettii SM16-1 was isolated from satsuma mandarin (SM) in Jeju Island, Korea and *E. fawcettii* DAR70024 from *Citrus indica* in New South Wales, Australia. *E. australis* Ea-1 was obtained from Argentina. *E. fawcettii* SM16-1 and DAR70024 and *E. australis* Ea-1 were classified into different pathotypes according to their ability to infect differential cultivars (*E. fawcettii* SM16-1, FBHR; *E. fawcettii* DAR70024, Tryon's; and *E. australis* Ea-1, sweet orange) (Hyun et al. 2009). Based on the phylogenetic tree obtained from the alignment of internal transcribed spacer and translation elongation factor sequences, isolates were identified as *E. fawcettii* and *E. australis*.

The genome sequencing of three isolates was performed with Illumina technology using paired-end and mate-pair libraries (GreenGene BioTech, Yong-In, Korea). In total, 403,757,008 paired-end and mate-pair raw reads of 101 bp were generated for *E. fawcettii* SM16-1, while

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399,958,543 reads for *E. fawcettii* DAR70024 and 379,895,328 reads for *E. australis* Ea-1 were generated. FASTQC was used to check the quality of the reads. The low-quality bases with Phred score less than Q33 and adapter sequence contamination were trimmed by using TrimGalore-0.5.0 and Cutadapt-2.1 to obtain 385,060,456 reads, which corresponds to approximately 128× sequence depth. De novo assemblies of all three genomes reported in this study was performed by using SPAdes genome assembler 3.9.0 (Bankevich et al. 2012) with default settings. The de novo assembly of *E. fawcettii* SM16-1 reads resulted in a genome size of 26,655,928 bp, G+C content of 52.79%, and 1,276 contigs with a scaffold length at which 50% of the total assembly length is covered (N_{50}) value of 952.195 bp and the number of contigs whose summed length is N_{50} (L_{50}) of 6. SSPACE-3.0 (Boetzer et al. 2011) was used for scaffolding to obtain the final version, with 72 total scaffolds and a total genome size of 26.65 Mb. The de novo assembly of two other isolates, *E. fawcettii* DAR70024 (26.32 Mb) and *E. australis* Ea-1 (23.79 Mb), produced 1,650 contigs with N_{50} value of 843,756 bp and 1,750 contigs with N_{50} value of 620,754 bp, respectively. In comparison, the genome of *E. australis* NL1, available in the NCBI public database (accession NHZQ000000000), is 23.22 Mb with 52.7% GC content. *E. australis* NL1 genome consists of 453 scaffolds with N_{50} value of 897,805, and L_{50} of 9; in total, 9,223 genes were reported on this genome (Table 1).

The ab initio gene prediction using Augustus (Keller et al. 2011) (-species = *fusarium_graminearum* -strand = both -gene model = complete) for the assembled genome of *E. fawcettii* SM16-1 resulted in 10,340 (exons = 27,304 and introns = 17,063) protein-coding genes, while 9,930 (exons = 26,910 and introns = 16,991) and 9,002 (exons = 24,384 and introns = 15,387) protein-coding genes were predicted on *E. fawcettii* DAR70024 and *E. australis* Ea-1, respectively. The genome assembly completeness for *E. fawcettii* SM16-1 (82%), *E. fawcettii* DAR70024 (92%), and *E. australis* Ea-1 (91%) was determined by BUSCO v3 (Waterhouse et al. 2018), using the fungi dataset.

The functional annotation of genes was done by searching homology of predicted genes with Ascomycetes protein sequences from SwissProt (Bairoch and Apweiler 2000) using BLASTP (Altschul et al. 1990). The functional annotation of *E. fawcettii* SM16-1 (10,340 genes), *E. fawcettii* DAR70024 (9,930 genes), and *E. australis* Ea-1 (9,002 genes) revealed that 83.87% (8,673), 82.81% (8,224), and 86.54% (7,791) of genes were annotated, respectively. The pathogenicity genes were predicted by using BLASTP against the Pathogen–Host Interaction (PHI) database (Winnenburg et al. 2006), which identified 981 (9.48%), 963 (9.69%), and 873 (9.69%) putative PHI genes in *E. fawcettii* SM16-1, *E. fawcettii* DAR70024, and *E. australis* Ea-1, respectively. Secreted proteins were predicted by first identifying proteins harboring signal peptides using SignalP 3.0 (Nielsen 2017) and then removing the proteins with mitochondria and chloroplast targeting signals using TargetP (Emanuelsson et al. 2007). This revealed that 382 (3.69%), 367 (3.69%), and 328 (3.64%) proteins are putative secreted proteins in *E. fawcettii* SM16-1, *E. fawcettii* DAR70024, and *E. australis* Ea-1, respectively.

Carbohydrate-active enzymes (CAZymes) that play a vital role in breakdown of cell wall components of the host to establish a successful infection process were predicted by using CAT and dbCAN servers (Yin et al. 2012). The CAZymes analysis revealed that 3,205 (30.99%), 2,979 (30%), and 2,790 (30.99%) proteins from *E. fawcettii* SM16-1, *E. fawcettii*

Table 1. Genome assembly statistics of assembled *Elsinoë fawcettii* strains and *E. australis*

	<i>E. fawcettii</i>		<i>E. australis</i> Ea-1
	SM16-1	DAR70024	
Pathotype	FBHR ^a	Sweet orange	Tryon's
NCBI accession	VAAB000000000	SWCR000000000	SWCS000000000
Genome size (Mb)	26.65	26.32	23.79
Contigs	1,276	1,650	1,750
N_{50} (bp)	952,195	843,756	620,754
GC content (%)	52.79	52.29	52.64
Number of genes	10,340	9,930	9,002
Average gene length (bp)	1,621	1,693	1,718
Number of annotated genes	8,673	8,224	7,791
Number of unannotated genes	1,667	1,706	1,211

^a FBHR = Florida broad host range.

DAR70024, and *E. australis* Ea-1, respectively, are CAZymes. Further classification of CAZymes based on their catalytic activity showed that glycosyl hydrolases (1,162 [36.25%], 1,027 [34.47%], and 1,046 [37.49%]) are high in number, followed by glycoside transferases (1,057 [32.88%], 978 [32.82%], and 981 [35.16%]), carbohydrate esterases (387 [12.07%], 406 [13.62%], and 307 [11%]), carbohydrate-binding modules (271 [8.45%], 257 [8.62%], and 194 [6.95%]), auxiliary activities (317 [9.89%], 292 [9.80%], and 251 [8.99%]), and polysaccharide lyases (14 [0.42%], 19 [0.63%], and 11 [0.39%]) in *E. fawcettii* SM16-1, *E. fawcettii* DAR70024, and *E. australis* Ea-1, respectively.

Secondary metabolites are essential for fungal growth and development, providing protection against various environmental stresses (Calvo et al. 2002). The search for secondary metabolite clusters on the *E. fawcettii* SM16-1, *E. fawcettii* DAR70024, and *E. australis* Ea-1 genome using the anti-SMASH fungal version (Blin et al. 2017) revealed the presence of 23 clusters (8 NRPS-like, 6 T1PKS, 5 NRPS, 3 terpene, and 1 lanthipeptide), 21 clusters (8 NRPS-like, 6 T1PKS, 4 NRPS, and 3 terpene), and 20 clusters (7 NRPS-like, 5 NRPS, 4 T1PKS, and 4 terpene), respectively. Elsinochrome, a photosensitizing compound, is reported to be highly toxic to citrus by causing peroxidation of cell membranes and inducing rapid electrolyte leakage from tissues (Chung 2011). The secondary metabolite cluster analysis showed the presence of T1PKS encoding elsinochrome A (*E. fawcettii* SM16-1) and elsinochrome B/C (*E. fawcettii* DAR70024 and *E. australis* Ea-1) cluster.

The draft genomes of *E. fawcettii* SM16-1, *E. fawcettii* DAR70024, and *E. australis* Ea-1 reported in this study are of high-quality genome assemblies, which can serve as reference genomes for other species or strains within the family Elsinoaceae. The genomes of *E. fawcettii* SM16-1, *E. fawcettii* DAR70024, and *E. australis* Ea-1 reported here have been deposited at GenBank under the accession numbers VAAB000000000 (BioProject number PRJNA534191), SWCR000000000 (BioProject number PRJNA534185), and SWCS000000000 (BioProject number PRJNA534188), respectively.

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