Draft Genome Sequence of Cumin Blight Pathogen *Alternaria burnsii*

Zhonghong Feng,¹ Tom Hsiang,² Xiaofei Liang,¹ Rong Zhang,^{1,†} and Guangyu Sun^{1,†}

¹ State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi Province 712100, China

² School of Environmental Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Abstract

The fungal genus *Alternaria* consists of highly diverse species. They can be isolated readily from soil, water, and many plants, and even from animals and humans. *Alternaria burnsii* is a small-spored species of section *Alternaria*. It has been reported as a pathogen, an endophyte, and a saprophyte, and can also be found in indoor air. It causes cumin blight, a destructive disease on cumin (*Cuminum cyminum*), and also causes other serious diseases, such as pumpkin seed rot, date palm leaf spot, wheat leaf spot, and gray spot of *Notopterygium incisum*. In this study, we sequenced and assembled the first genome of *A. burnsii* isolate CBS 107.38. The draft genome can be used as a reference for the further study of related pathogens and comparative genomics of *Alternaria* species.

Alternaria burnsii, *Alternaria* section *Alternaria*, is a small-spored species. It is widely distributed in Asia, Europe, America, and Africa, and has been found as a pathogen, a saprophyte, or an endophyte. Cumin blight caused by *A. burnsii* was first reported in India (Uppal et al. 1938). Since then, the disease has been observed in many cumin (*Cuminum cyminum* L.) growing areas, such as Rajasthan state of India (Mathur 1949), Turkey (Kocaturk 1988; Özer and Bayraktar 2015), Pakistan (Shakir et al. 1995), and Iran (Kamkar et al. 2011). In severe disease years, the yield losses can be up to 70% (Holliday 1980). In addition, *A. burnsii* can cause gray leaf spot of the traditional Chinese medicine plant Qianghuo (*Notopterygium incisum* Ting ex H.T. Chang) (Wang et al. 2009), pumpkin seed rot on *Cucurbita maxima* Duch. ex Lam. (Paul et al. 2015), leaf spot of date palm (*Phoenix dactylifera* L.) and wheat (*Triticum aestivum* L.) (Al-Nadabi et al. 2018), and a leaf disease on common bean (*Phaseolus vulgaris* L.) (Sendi et al. 2018). *A. burnsii* was isolated from asymptomatic leaves of Pandanaceae in southern Thailand as an endophyte (Tibpromma et al. 2018). It was also discovered in the indoor air in the United States and considered a saprophyte (Woudenberg et al. 2015a). Genome sequence resources provide fundamental information to explore the molecular mechanisms of species evolution and adaptability. In recent years, genomes of some other *Alternaria* species have been sequenced (Dang et al. 2015; Hou et al. 2016; Nguyen et al. 2016; Rajarammohan et al. 2019; Wolters et al. 2018; Woudenberg et al. 2015b).

Here, *A. burnsii* Uppal, Patel & Kamat CBS 107.38, the type strain isolated from India causing leaf blight on cumin, was selected for whole genome sequencing. The strain was grown on potato dextrose agar (PDA) at 25°C in darkness for 7 days, and the colony was used for extraction of total genomic DNA using a modified CTAB procedure (Chen et al. 1993). The genome of *A. burnsii* was sequenced using the Illumina HiSeqX10 platform (Biomarker Technologies, Beijing, China) with paired-end 150 bp reads (insert size of 270 bp and

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Keywords

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[†]Corresponding authors: G. Y. Sun; sgy@nwsuaf.edu.cn, R. Zhang; rongzh@nwsuaf.edu.cn

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Table 1. Genome features of *Alternaria burnsii* CBS 107.38 and comparisons with two other strains

Variables	<i>A. burnsii</i>	<i>A. alternata</i> ^a	<i>A. brassicae</i> ^b
Sequencing methods	Illumina	Illumina	Nanopore
Sequence coverage	146x	not available	not available
Assembly size (Mb)	32.97	33.12	33.77
Number of scaffolds	72	552	14
Scaffold N50 (Kbp)	1,806	229	3,090
GC content (%)	50.90	50.90	50.98
Number of predicted genes	11,475	11,704	11,495
Repeat rate (%)	0.91	not available	2.43
BUSCO genome completeness (%) ^c	97	97	96 ^d
Predicted proteome completeness (%) ^c	97	97 ^d	98

^a Bihon et al. (2016).^b Rajarammohan et al. (2019).^c Completeness = total BUSCOs – fragmented BUSCOs – missing BUSCOs / total BUSCOs × 100^d These results were analyzed in this study.

approximate coverage of 146x). In total, there were 16,519,548 clean reads (around 5 Gbp), Q30 was up to 94.46%, and GC content was 50.66%. All the clean data was used for de novo assembly using the ABySS assembler v2.0.2 (Simpson et al. 2009). The results showed that the draft genome assembly of *A. burnsii* comprised 72 scaffolds with a total genome size of 32.97 Mbp (N50 = 1.81 Mbp) (Table 1). The number of scaffolds of *A. burnsii* was much lower, and the N50 was higher than those reported for draft genomes generated using second generation sequencing platforms (Dang et al. 2015; Hou et al. 2016) where scaffold numbers ranged from 393 to 31,070, and N50 length range from 3 to 757 Kbp. However, the number of scaffolds was higher than those of assemblies of *Alternaria* spp. derived from third generation sequencing (Nguyen et al. 2016; Rajarammohan et al. 2019; Wolters et al. 2018). Repetitive sequences across this genome were identified by RepeatMasker v4.0.5 (<http://www.repeatmasker.org>), with a repeat rate of 0.91%, which was lower than that identified for other *Alternaria* species, such as *Alternaria* spp. section *Alternaria* (1.4–2.7%), *A. solani* (1.5%), *A. infectoria* (5.3%), *A. brassicicola* (7.1%), *A. avenicola* (11.9%), *A. papaveraceae* (5.3%), and *A. alternantherae* (16.5%) (Woudenberg et al. 2015b). For genome annotation, GeneMark-ES (Ter-Hovhannisyan et al. 2008) was first used for de novo gene structure prediction. The genome assembly and predicted genes of *A. tagetica* was selected as the optimal gene model (according to sensitivity and specificity of gene and exon level) among 24 species of section *Alternaria* (Dang et al. 2015) trained by Augustus v. 3.1 (Stanke and Morgenstern 2005). Then, MAKER2 v2.31.8 (Holt and Yandell 2011) was used to predict coding genes by integrating gene models predicted from GeneMark-ES and Augustus and protein sequences of the 24 species of section *Alternaria* (Dang et al. 2015). In total, the *A. burnsii* genome had 11,907 predicted protein-coding genes.

The software BUSCO v1.2 was used to assess the completeness of the gene predictions with comparison with the universal fungal library fungi_odb9 containing 1,438 single-copy orthologs (Simão et al. 2015) and revealed 97% completeness with 1,330 single-copy orthologs and 78 duplicated BUSCOs. Only 30 BUSCO orthologs were missing or fragmented. This result was similar to the genomic completeness of *A. alternata* PPRI 21032 (97%) (Bihon et al. 2016) or *A. brassicae* J3 (96%) (Rajarammohan et al. 2019) by BUSCO analysis. This draft genome of *A. burnsii* CBS 107.38 will be useful in the future studies of *Alternaria* species and is significant for the classification of *Alternaria* spp. at the whole genomic level.

This draft genome assembly, version 1, has been deposited at GenBank under the accession number JAAABM000000000 (BioProject: PRJNA600135; BioSample: SAMN13783390).

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