

IMA Genome-F 1: *Ceratocystis fimbriata*Draft nuclear genome sequence for the plant pathogen, *Ceratocystis fimbriata*P. Markus Wilken¹, Emma T. Steenkamp², Michael J. Wingfield¹, Z. Wilhelm de Beer², and Brenda D. Wingfield¹¹Department of Genetics, ²Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria; corresponding author e-mail: markus.wilken@fabi.up.ac.za

Abstract: The draft nuclear genome of *Ceratocystis fimbriata*, the type species of *Ceratocystis*, is comprised of 29 410 862 bp. *De novo* gene prediction produced 7 266 genes, which is low for an ascomycete fungus. The availability of the genome provides opportunities to study aspects of the biology of this and other *Ceratocystis* species.

Key words:

Ceratocystis fimbriata
genome
Microascales

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INTRODUCTION

The fungal genus *Ceratocystis* (*Microascales*, *Sordariomycetes*, *Ascomycota*) includes numerous important plant pathogens, some of considerable economic importance. Species in the *C. fimbriata* complex include *C. platani* that causes a serious wilt of *Platanus* trees in Europe (Ocasio-Morales *et al.* 2007), *C. manginecans*, causal agent of Mango wilt disease (van Wyk *et al.* 2007), and *C. fimbriata sensu stricto*, a pathogen of sweet potato (Baker *et al.* 2003). The genus also encompasses several other species complexes that include economically important species (e.g. the thielaviopsis morph, Punja & Sun 1999), agents of blue stain in timber (e.g. *C. polonica*, Christiansen 1985) and saprophytes. These fungi all have intriguing and little-understood associations with insects (Seifert *et al.* 2013).

Recent studies on *Ceratocystis* species have focused on species delimitation (van Wyk *et al.* 2010), reproductive strategies (Harrington & McNew 1997, Witthuhn *et al.* 2000) and links between pathogenicity and host range (Ferreira *et al.* 2011). Although genome sequence information represents an invaluable resource for such studies, whole genome sequences have not yet been determined for *Ceratocystis* species or other members of the *Microascales*. In this study, we report the availability of the nuclear genome sequence for an isolate of *C. fimbriata*. This *Ceratocystis* species was chosen for sequencing because it is the type species of the genus (Seifert *et al.* 2013).

SEQUENCED STRAIN

USA: North Carolina: isol. ex *Ipomoea batatas* (sweet potato), December 1998, D. McNew (CBS 114723, CMW

14799). Dried culture also preserved in the CBS fungarium, CBS H-21516.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The Whole Genome Shotgun project of the *Ceratocystis fimbriata* genome has been deposited in DDBJ/EMBL/GenBank under the accession APWK000000000. The version described in this paper is the first version, CFim_1.0.

METHODS

DNA was extracted and subjected to 454 pyrosequencing (Roche Diagnostics, Mannheim, Germany) at Inqaba Biotechnology (Pretoria, South Africa). The resulting reads were assembled into a draft genome consisting of 3 668 contigs by using the Newbler v. 2.3 genome assembler. The “create detailed mapping report” command of the CLC Genomics workbench package v. 5.0.1 (CLC bio, Aarhus, Denmark) was used to produce statistics for the draft sequence.

RESULTS AND DISCUSSION

The draft genome had an estimated size of 29 410 862 bp (as calculated by summation of all the contig sizes), 20× average coverage, N50 contig size of 42 879 bases and an estimated GC content of 48.06 %. All contigs with a length of > 199 bp were submitted to NCBI’s genome database. To assess the completeness of the genome, contigs of size ≥ 500 bp (2641 contigs) were analysed with the CEGMA pipeline (Parra *et al.*

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2007), which produced a 96.77 % indication of completeness. Although we did not produce a complete annotation for the *C. fimbriata* genome, analysis with AUGUSTUS (Stanke et al. 2006) identified 7 266 putative ORFs at a gene density of 246 ORFs/Mb. Of the putative protein coding genes, the majority (97 %) had 100 or more amino acids.

The *C. fimbriata* genome is relatively small (29.4 Mb) and harbours fewer genes than other fungal species such as *Fusarium graminearum* (36.1 Mb, 11 640 genes) (Cuomo et al. 2007) and *Neurospora crassa* (39.9 Mb, 10 082 genes) (Galagan et al. 2003). Whether this difference is linked to the different lifestyles of these fungi requires further research. The availability of this *Ceratocystis* genome sequence will contribute to our understanding of the molecular and cellular mechanisms underlying the biology of these and other fungi.

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REFERENCES

- Baker CJ, Harrington TC, Krauss U, Alfenas AC (2003) Genetic variability and host specialization in the Latin American clade of *Ceratocystis fimbriata*. *Phytopathology* **93**: 1274–1284.
- Christiansen E (1985) *Ceratocystis polonica* inoculated in Norway spruce: blue-staining in relation to inoculum density, resinosis and tree growth. *European Journal of Forest Pathology* **15**: 160–167.
- Cuomo CA, Güldener U, Xu J-R, Trail F, Turgeon BG, Di Pietro A, Walton JD, Ma L-J, Baker SE, Rep M, Adam G, Antoniw J, Baldwin T, Calvo S, Chang Y-L, DeCaprio D, Gale LR, Gnerre S, Goswami RS, Hammond-Kosack K, Harris LJ, Hilburn K, Kennell JC, Kroken S, Magnuson JK, Mannhaupt G, Mauceli E, Mewes H-W, Mitterbauer R, Muehlbauer G, Münsterkötter M, Nelson D, O'Donnell K, Ouellet T, Qi W, Quesneville H, Roncero MIG, Seong K-Y, Tetko IV, Urban M, Waalwijk C, Ward TJ, Yao J, Birren BW, Kistler HC (2007) The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. *Science* **317**: 1400–1402.
- Ferreira MA, Harrington TC, Alfenas AC, Mizubuti ESG (2011) Movement of genotypes of *Ceratocystis fimbriata* within and among *Eucalyptus* plantations in Brazil. *Phytopathology* **101**: 1005–1012.
- Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma L-J, Smirnov S, Purcell S, Rehman B, Elkins T, Engels R, Wang S, Nielsen CB, Butler J, Endrizzi M, Qui D, Ianakiev P, Bell-Pedersen D, Nelson MA, Werner-Washburne M, Selitrennikoff CP, Kinsey JA, Braun EL, Zelter A, Schulte U, Kothe GO, Jedd G, Mewes W, Staben C, Marcotte E, Greenberg D, Roy A, Foley K, Naylor J, Stange-Thomann N, Barrett R, Gnerre S, Kamal M, Kamvysselis M, Mauceli E, Bielke C, Rudd S, Frishman D, Krystofova S, Rasmussen C, Metznerberg RL, Perkins DD, Kroken S, Cogoni C, Macino G, Catcheside D, Li W, Pratt RJ, Osmani SA, DeSouza CPC, Glass L, Orbach MJ, Berglund JA, Voelker R, Yarden O, Plamann M, Seiler S, Dunlap J, Radford A, Aramayo R, Natvig DO, Alex LA, Mannhaupt G, Ebbole DJ, Freitag M, Paulsen I, Sachs MS, Lander ES, Nusbaum C, Birren B (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature* **422**: 859–868.
- Harrington TC, McNew DL (1997) Self-fertility and uni-directional mating-type switching in *Ceratocystis coerulea*, a filamentous ascomycete. *Current Genetics* **32**: 52–59.
- Ocasio-Morales RG, Tsopelas P, Harrington TC (2007) Origin of *Ceratocystis platani* on native *Platanus orientalis* in Greece and its impact on natural forests. *Plant Disease* **91**: 901–904.
- Parra G, Bradnam K, Korf I (2007) CEGMA: a pipeline to accurately annotate core gene in eukaryotic genomes. *Bioinformatics* **23**: 1061–1067.
- Punja ZK, Sun LJ (1999) Morphological and molecular characterization of *Chalara elegans* (*Thielaviopsis basicola*), cause of black root rot on diverse plant species. *Canadian Journal of Botany* **77**: 1801–1812.
- Seifert KA, De Beer ZW, Wingfield MJ (2013) *The Ophiostomatoidei Fungi: Expanding Frontiers*. CBS Biodiversity Series No. 12. CBS-KNAW Fungal Biodiversity Centre, CBS, Utrecht, The Netherlands.
- Stanke M, Tzvetkova A, Morgenstern B (2006) AUGUSTUS at EGASP: using EST, protein and genomic alignments for improved gene prediction in the human genome. *Genome Biology* **7** Suppl 1: S11.1–S11.8.
- Van Wyk M, Adawi AOA, Khan QA, Deadman ML, Al Jahwari AA, Wingfield BD, Ploetz R, Wingfield MJ (2007) *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. *Fungal Diversity* **27**: 213–230.
- Van Wyk M, Wingfield BD, Marin M, Wingfield MJ (2010) New *Ceratocystis* species infecting coffee, cacao, citrus and native trees in Colombia. *Fungal Diversity* **40**: 103–117.
- Witthuhn RC, Harrington TC, Wingfield BD, Steimel JP, Wingfield MJ (2000) Deletion of the *MAT-2* mating-type gene during uni-directional mating-type switching in *Ceratocystis*. *Current Genetics* **38**: 48–52.