### IMA Genome-F 1: Ceratocystis fimbriata

# Draft nuclear genome sequence for the plant pathogen, Ceratocystis fimbriata

P. Markus Wilken<sup>1</sup>, Emma T. Steenkamp<sup>2</sup>, Michael J. Wingfield<sup>1</sup>, Z. Wilhelm de Beer<sup>2</sup>, and Brenda D. Wingfield<sup>1</sup>

<sup>1</sup>Department of Genetics, <sup>2</sup>Department 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

Article info: Submitted: 10 November 2013; Accepted: 2 December 2013; Published: 6 December 2013.

### INTRODUCTION

fungal genus Ceratocystis (Microascales, includes Sordariomycetes, Ascomycota) 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 APWK00000000. 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),  $20\times$  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  $\geq$  500 bp (2641 contigs) were analysed with the CEGMA pipeline (Parra *et al.* 

Attribution: You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).

Non-commercial: You may not use this work for commercial purposes. No derivative works: You may not alter, transform, or build upon this work.

For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.

VOLUME 4 · NO. 2 357

<sup>© 2013</sup> International Mycological Association

You are free to share - to copy, distribute and transmit the work, under the following conditions:

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.

#### **ACKNOWLEDGEMENTS**

This research was funded by The University of Pretoria, the National Research Foundation (NRF) and the DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB), South Africa. This work is based on the research supported in part by a number of grants from the National Research Foundation of South Africa (includes Grant specific unique reference number (UID) 83924). The Grant holders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the author(s), and that the NRF accepts no liability whatsoever in this regard.

## **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, Metzenberg 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 coerulescens*, 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 Ophiostomatoid 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.

358 IMA FUNGUS