Genome Resource for *Neocamarosporium betae* (syn. *Pleospora betae*), the Cause of Phoma Leaf Spot and Root Rot on *Beta vulgaris*

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Abstract

Neocamarosporium betae (syn. *Phoma betae*, *Pleospora betae*) is the cause of Phoma leaf spot and root decay on *Beta vulgaris* worldwide. Despite the economic importance of the pathogen, many aspects of its life cycle and population biology remain unknown. The first genome assembly of *N. betae* was constructed to facilitate identification of mating-type loci and development of microsatellite markers for population genetics studies. The de novo assembled genome is provided as a resource for future genetic studies to understand the genetic mechanisms underlying disease development and host-pathogen interactions.

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Keywords

Phoma betae, beetroot, table beet

Genome Announcement

Phoma leaf spot is a seedborne and soilborne disease of table beet, sugar beet (*Beta vulgaris* var. *vulgaris*), and Swiss chard (*B. vulgaris* var. *cicla*) worldwide, causing significant yield losses through reduced seed germination and seedling emergence, foliar lesions, root decay, and storage rot (Harveson et al. 2009; Pethybridge et al. 2018). The causal agent was previously described as *Phoma betae* (syn. *Pleospora betae*) but was recently reclassified as *Neocamarosporium betae* within *Neocamarosporiaceae* (Ariyawansa et al. 2015; Valenzuela-Lopez et al. 2018; Wanasinghe et al. 2017). Despite the economic importance of this pathogen, the mechanisms underlying disease development and the relative contribution of inoculum sources to epidemics remain largely unknown (Pethybridge et al. 2018). Here, we present the first de novo genome assembly of *N. betae*, to facilitate future genetic and genomic studies.

Strain ICMP 22602 (= Pb1), collected from a table beet field in New York in 2014, was used to generate the genome assembly. DNA was extracted as described by Vaghefi et al. (2016). To confirm the identity of the strain as *N. betae*, the large subunit (LSU) of nuclear ribosomal DNA (nrDNA) (Vilgalys and Hester 1990), internal transcribed spacers and the intervening 5.8S region (ITS) of the nrDNA (de Hoog Gerrits van den Ende 1998; White et al. 1990), RNA polymerase II second largest subunit (*rpb2*) (Liu et al. 1999; Sung et al. 2007),

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Table 1. Multilocus sequence data used to confirm the identity of ICMP 22602 as *Neocamarosporium betae*^a

Species	Strain ^b	GenBank accession no. ^c							
		LSU	SSU	ITS	<i>rpb2</i>	<i>tef1-α</i>	<i>tub2</i>	<i>act</i>	<i>cmdA</i>
<i>N. betae</i>	CBS 523.66	EU754179 (0)	EU754080 (0)	FJ426981(0)	KT389670(1)	MK255062 (0)	KT389842 (0)	JF740118 (1)	MK255061 (1)
	CBS 109410	MK249662 (0)	MK249665 (0)	MK249659 (0)	MK250855 (3)	-	MK255063 (1)	JF740119 (2)	MK255064 (2)
	ICMP 22602;	MK249660	MK249663	MK249657	MK250853	MK250859	MK250856	MK250857	MK250858
	Pb1								
<i>N. goegapense</i>	ICMP 10945	MK249661 (0)	MK249664 (0)	MK249658 (0)	MK250854 (0)	-	-	-	-
	CBS 138008;	KJ869220 (2)	-	KJ869163 (40)	-	-	-	-	-
	CPC 23676 ^T								

^a Accession numbers produced in this study are indicated in bold. The numbers in parentheses indicate the number of nucleotide differences in reference strains compared with the corresponding sequence of ICMP 22602.

^b CBS = Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, CPC = Personal collection of Pedro Crous, Utrecht, The Netherlands, ICMP = International Collection of Microorganisms from Plants, Auckland, New Zealand. Pb1 is from the culture collection of S. J. Pethybridge at Cornell University, Geneva, NY, U.S.A.

^c LSU = partial 28S nuclear ribosomal DNA (nrDNA); SSU = partial 18S nrDNA; ITS = internal transcribed spacers and the intervening 5.8 region of the nrDNA; *rpb2* = RNA polymerase II second largest subunit; *tef1-α* = translation elongation factor 1-α, *tub2* = β-tubulin, and *cmdA* = calmodulin.

translation elongation factor 1-α (*tef1-α*) (Carbone and Kohn 1999), beta tubulin (*tub2*) (Woudenberg et al. 2009), actin (*act*) (Carbone and Kohn 1999), and calmodulin (*cmdA*) (Carbone and Kohn 1999) regions were sequenced (Table 1). The LSU and ITS sequences of ICMP 22602 showed only 99.8 and 92.6% similarity to those of the type species of the genus *Neocamarosporium* (*N. goegapense*; ex-Holotype CBS 138008), however, they were identical to the LSU and ITS sequences of *N. betae* reference strains CBS 109410, CBS 523.66, and ICMP 10945 (Table 1).

A total of 3.9 μg of genomic DNA of strain ICMP 22602 was submitted to the Cornell University Institute of Biotechnology Genomics Facility, Ithaca, New York, for PCR-free library preparation with an average insert of approximately 550 bp. The PCR-free library was generated using Illumina TruSeq Nano DNA LT sample preparation kits, following the manufacturer instructions. Sequencing of the library, using the Illumina paired-end (2× 300 bp) MiSeq platform, yielded approximately 4,488,573 paired reads totaling approximately 2.6 Gb, with mean base quality of 28.6 estimated using FastQC v.0.11.2 in the GALAXY portal (Afgan et al. 2016). The genome size was estimated to be about 48 Mb, based on processing the raw Illumina sequences data in the Kmer counting software Jellyfish v.2.2.3 (Marçais and Kingsford 2011). A genome coverage of approximately 54× was estimated.

The raw sequence data were assembled in DISCOVAR de novo v.52488 (Weisenfeld et al. 2014). The draft genome assembly had a total assembly size of approximately 45.30 Mbp, with a scaffold N50 value of 623,529 Mbp. The completeness of the final genome assembly was assessed by BUSCO v.2.0.1 (Simão et al. 2015), using sets of Benchmarking universal single-copy orthologs (BUSCO) for ascomycetes (Ascomycota_odb9). This estimated a genome completeness of 99%; 1,302 complete BUSCOs, four fragmented BUSCOs, and nine missing BUSCOs in a total of 1,315 BUSCO groups searched. Gene prediction using the annotation pipeline Maker v.2.31.9 (Cantarel et al. 2008) predicted a total of 11,513 genes. This was done through a preliminary annotation using the ab initio gene predictor SNAP (Korf 2004), the result of which was used to produce a hidden-Markov-model profile for *N. betae* that was further refined with a second stage of SNAP training and used for the final annotation.

The genome of *N. betae* has been used for microsatellite marker development and identification of a mating-type locus in *N. betae* populations in the states of New York and Washington (Koenick et al. 2017, 2018a and b). The draft assembly of *N. betae* ICMP 22602 is published here with the GenBank accession number PEBR00000000 to facilitate future studies on pathogenicity factors and genetic mechanisms underlying disease development and host-pathogen interactions.

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