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Short Notes

Draft genome sequence of *Phyllosticta ampelicida*, the cause of grapevine black rot

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Summary. *Phyllosticta ampelicida* causes grapevine black rot, a potentially damaging disease for grape production. This paper reports the draft genome sequence of *P. ampelicida* PA1 Galicia CBS 148563, which is 30.55 Mb and encodes 10,691 predicted protein-coding genes. This is the first sequence genome assembly of *P. ampelicida*, and this information is a valuable resource to support genomic attributes for determining pathogenic behaviour and comparative genomic analyses of grapevine black rot fungi.

Keywords. Grapevine disease, microbe-plant interaction, *Vitis vinifera* L.

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Phyllosticta ampelicida (Engelm.) Aa (syn. *Guignardia bidwellii*, following the recommendation of the International Commission on the Taxonomy of Fungi, Rossman *et al*., 2015) is the causal agent of black rot of grapevine. *Phyllosticta ampelicida* (*Ascomycota*, *Dothideomycetes*, *Botryosphaeriales*, *Phyllostictaceae*) causes Black rot, which is an economically important disease, especially in grape producing regions characterised by humid growing seasons (Ramsdell and Milholland, 1988). In epidemic years, black rot can cause crop losses between 5 and 80% (Ramsdell and Milholland, 1988), and in severely affected vineyards virtually complete crop loss if not effectively managed (Rinaldi *et al.,* 2013). All *Vitis vinifera* cultivars are highly susceptible to black rot (Wilcox and Hoffman, 2019). Chemical treatments against downy and powdery mildews are sufficient to prevent black rot, although in recent years, especially because of the adoption of downy mildew *V. vinifera* resistant varieties and the increased use of active ingredients specific against Oomycetes, black rot is of increasing importance (Pertot *et al*., 2017).

All herbaceous tissues of grapevine plants are susceptible to infection by the pathogen, including leaves, shoots, tendrils, petioles and berries, with young leaves and fruit being extremely susceptible (Vezzulli *et al.,* 2022)

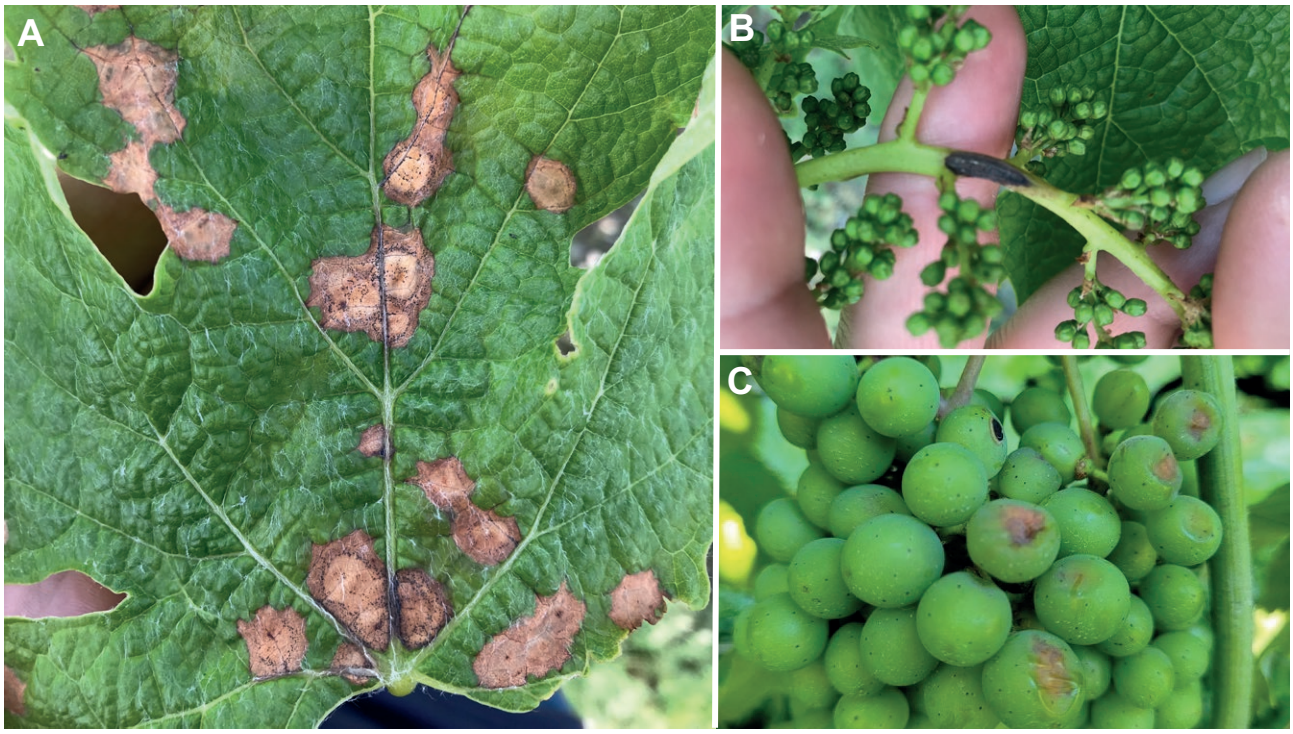

Figure 1. Symptoms on grapevine tissues caused by *Phyllosticta ampelicida*. Reddish-brown circular lesions on infected leaves, with pycnidia in the lesion centres (A), necrotic spots on the rachis (B), and brown to black spots on berries (C).

(Figure 1). Black rot is a polycyclic disease with repeated cycles of primary and secondary infections. Three *formae speciales* (f. sp.) of "*G. bidwellii*", with different host specificities, have been described (Luttrell, 1946; Luttrell, 1948). These are: "*G. bidwellii*" f. sp. *euvitis*, which is pathogenic to *V. vinifera* and to the American bunch grape species of the section *Vitis*; "*G. bidwellii*" f. sp. *muscadinii*, which is pathogenic to *V. rotundifolia* and *V. vinifera*; and "*G. bidwellii*" f. sp. *parthenocissi*, which is pathogenic to *Parthenocissus* spp. High genetic variability has been found among *P. ampelicida* isolates collected from different geographic areas (Narduzzi-Wicht *et al*., 2014; Rinaldi *et al*., 2017). Describing the *P. ampelicida* genome sequence is an important step toward enhancing understanding of the grapevine and *P. ampelicida* interaction, and will provide a basis for pathogenicity mechanism studies and development of disease management strategies.

We report the genome sequencing and assembly of the *P. ampelicida* PA1 Galicia CBS 148563, which was isolated from diseased leaves of 25-year-old *V. vinifera* 'Mencia', in Leiro-Ourense (Galicia, Spain). The strain was purified by single-spore isolation and maintained on potato dextrose agar (PDA) medium at 25°C in the darkness. DNA was extracted with NucleoSpin Tissue (Macherey-Nagel), following the manufacturer's protocol. Firstly, the ITS regions, including the 5.8S gene, were amplified with ITS1/ITS4 (White *et al*., 1990), the amplicon was then sequenced according to Eichmeier *et al*. (2010), and the sequence was submitted to GenBank (Accession No. MZ914563).

The same DNA was used for genome library construction using the Nextera XT DNA Library Preparation Kit (Illumina Inc.). The library was sequenced using MiniSeq High Output Reagent Kit (300-cycles) (Illumina Inc.) with 2 × 150PE read option. A total of 14,796,001 high-quality reads passed the filter. The sequence quality was checked using the FastQC-0.10.1 program (Andrews, 2010). A FASTX-Toolkit Clipper (http://hannonlab.cshl.edu/fastx_toolkit/), specifying the Q33 parameter, was used to remove the adaptors, and low-quality reads were discarded. Contigs of individual reads were assembled *de novo* using the SPAdes genome assembler v. 3.15.2 (Prjibelski *et al*., 2020) with default settings. *De novo* assembly of *P. ampelicida* PA1 Galicia CBS 148563 resulted in a genome size of 30,547,631 bp, G+C content of 54.49%, and 6,675 contigs, with a scaffold length at which 50% of the total assembly length is covered (N50) value of 20,626 bp and the number of contigs whose summed length is N50 (L50) of 428.

The *ab initio* gene prediction using Augustus (Keller *et al*., 2011) (-species = botrytis_cinerea -strand = both -gene model = complete) for the assembled genome of *P. ampelicida* PA1 Galicia resulted in 31,876 exons and 10,691 predicted coding sequences. Using BUSCO 5.2.2 (Manni *et al*., 2021), 745 complete single-copy proteins were identified with known functions (Supplementary Data).

Carbohydrate-active enzymes (CAZymes) that play vital roles in breakdown of host cell wall components establish successful infections were predicted, using CAT and dbCAN servers (Yin *et al*., 2012). Fifty-eight signal peptides were detected by HMMER (Zhang and Wood, 2003) using dbCAN (Supplementary data). Signal peptides act as zip codes marking the protein secretion pathway as well as the protein target location. In addition to protein targeting, a number of critical functions with or without regard to the passenger proteins have been attributed to signal peptides (Owji *et al*., 2018). A total of 43,636 translated amino acid sequences was predicted by FragGeneScan (Rho *et al*., 2010). Using Hotpep analysis (Busk *et al*., 2017), 4,914 hits of CAZyme sequences were detected. The most represented CAZymes belonged to two groups (GT41 and GT48) of glycosyl transferases. Fungal glycosyl transferases may facilitate pathogenesis of plants by enabling hyphal growth on solid surfaces, a phenomenon previously reported by King *et al*. (2017). Further classification of CAZymes based on their catalytic activity showed a high proportion of glycosyl hydrolases (39.4%), followed by glycoside transferases (31.2%), auxiliary activities (12.8%), carbohydrate-binding modules (12.8%), carbohydrate esterases (2.1%) and polysaccharide lyases (1.7%). Using MicroStation Reader BioTek ELx808BLG (Biolog Inc.) and carbon sources (CS) in FF MicroPlate (Biolog Inc. USA), consumption was detected of 72 CS by *P. ampelicida* PA1 Galicia CBS 148563 (Supplementary Data). This fungus is not included in any database of Biolog Inc.

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 using antiSMASH fungal version (Blin *et al*., 2017) revealed the presence of 17 clusters (10 NRPS and NRPS-like, 3 T1PKS, 3 terpene and 1 betalactone).

The draft genome of *P. ampelicida* PA1 Galicia CBS 148563 reported here are of high-quality genome assemblies, which can serve as reference genomes for other species or strains within the family *Phyllostictaceae*. The genome of *P. ampelicida* PA1 Galicia CBS 148563 reported here has been deposited in GenBank under Acc. No. JAIFKG000000000.1 (BioProject No. PRJNA753299).

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