



New Draft Genome Sequence of the Ergot Disease Fungus *Claviceps paspali*

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ABSTRACT Here, we report a new draft genome sequence of an isolate of the ascomycete *Claviceps paspali* that is responsible for ergot disease in grasses of the *Paspalum* genus. This new draft genome sequence will provide useful data for evaluating intraspecies and interspecies genome variation in *C. paspali* and other *Claviceps* genus members.

Claviceps paspali (Sordariomycetes, Ascomycota) is a phytopathogenic causal agent of ergot disease in *Paspalum* spp. This disease is important for dairy and beef production because it affects highly productive, drought-resistant forage grasses, such as *Paspalum dilatatum* (1, 2). Ergot results in seed losses by seed replacement with the sclerotia of the fungus and also the production of tremorgenic toxins by the fungus, which are toxic to feeding animals (3, 4).

C. paspali ILB432 was isolated from sclerotia obtained from *Paspalum urvillei*-infected inflorescences collected near Portezuelo, Maldonado, Uruguay (global positioning system coordinates 34.888591S, 55.030316W), using a previously reported *C. paspali* isolation procedure (5). ILB432 was cultured at 26°C on *Claviceps* medium containing 36 g/liter potato dextrose agar, 2 g/liter yeast extract, 10 g/liter malt extract, 10 g/liter sucrose, and 5 g/liter agar (6). Phylogenetic analysis based on the partial gene coding for the second largest subunit of RNA polymerase subunit II (*RPB2*) showed that ILB432, like reference isolate RRC-1481, belongs to the most frequent lineage of *C. paspali* (5).

DNA was isolated from vegetative mycelia using the Quick-DNA fungal/bacterial kit (Zymo Research) following the manufacturer's instructions and was sent to Macrogen, Inc. (Seoul, South Korea), for sequencing. DNA libraries of 500-bp inserts were generated with the TruSeq Nano DNA kit (Illumina), and 150-bp paired ends (PEs) were sequenced with the Illumina HiSeq 2500 platform.

Raw reads were trimmed to remove the adapter and low-quality sequences with Trimmomatic v0.36 (7). A total of 63,447,991 PE filtered reads were used for the *de novo* genome assembly using SPAdes v3.13.1 (8) with multiple k-mer sizes (21, 33, 45, 57, 69, 81, 93, 105, and 117) and the “-careful” option. The resulting assembly was analyzed using QUAST v4.6.1 (9). Species-specific repeats were inferred using the program RepeatModeler v2.0.1 (10), and Repeatmasker v4.1.0 (11) was employed to mask resulting repeats. Genome completeness was assessed using BUSCO v4.0.6 (12) (genome mode) against the Ascomycota_odb10 database. The newly assembled genome sequence contained 1,678 (98.4%) complete (single-copy and duplicated) BUSCO orthologs of the 1,706 present in the Ascomycota_odb10 database. Genome size and completeness results were similar to those of reference strain RRC-1481, which contains 1,653 complete BUSCO orthologs (Table 1).

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TABLE 1 Detailed results from assembly of the two genomes of *C. paspali* isolates

Characteristics by category	Data for <i>C. paspali</i> isolate ^a :	
	ILB432	RRC-1481 ^b
Assembly		
Estimated genome size (Mb)	28.9	28.9
Genome coverage (×)	653	54.4
GC content (%)	47.8	48.0
No. of contigs	1,030	2,152
<i>N</i> ₅₀ of contigs (bp)	66,931	26,979
No. of scaffolds with >200 bp	352	NA
<i>N</i> ₅₀ of scaffolds (bp)	146,886	NA
Longest scaffold (bp)	616,281	NA
No. of Ns per 100 kb	23.0	NA
Repeat sequences (%)	22.2	17.5
GenBank accession no.	JABAJK000000000	GCA_000223175.2
Completeness		
BUSCO (%)	98.4 (S), 0.0 (D), 0.5 (F), 1.1 (M)	96.9 (S), 0.0 (D), 1.2 (F), 1.9 (M)

^aS, complete single copy; D, complete duplicated; F, fragmented; M, missing; NA, data not available.

^bAssembly stats based on reference 13. BUSCO results based on this work analysis.

This improvement in sequencing methodology is reflected in the lower number of contigs and higher *N*₅₀ values of this new genome sequence compared to those of the previous reference genome (Table 1). This study provides highly useful data for evaluating genome variation within *C. paspali* (7) and the *Claviceps* genus.

Data availability. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the BioProject number [PRJNA625338](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA625338). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [JABAJK000000000](https://www.ncbi.nlm.nih.gov/nuccore/JABAJK000000000). The version described in this paper is version [JABAJK010000000](https://www.ncbi.nlm.nih.gov/nuccore/JABAJK010000000). Raw reads are available under SRA accession number [SRR11565825](https://www.ncbi.nlm.nih.gov/sra/SRR11565825). The *RPB2* sequence is available under accession number [MT348393](https://www.ncbi.nlm.nih.gov/nuccore/MT348393). The pure isolate of *C. paspali* ILB432 is stored at the INIA Las Brujas fungal collection (ILB); for samples of the isolate, contact Eduardo Abreo at eabreo@inia.org.uy.

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REFERENCES

- Luttrell ES. 1977. The disease cycle and fungus-host relationships in dallis-grass ergot. *Phytopathology* 77:1461–1468. <https://doi.org/10.1094/Phyto-67-1461>.
- Schrauf GE, Blanco M, Cornaglia PS, Deregiibus V, Madia M, G Pacheco M, Padilla J, M Garcia A, Quarin C, M Garcia A, Quarin C. 2003. Ergot resistance in plants of *Paspalum dilatatum* incorporated by hybridisation with *Paspalum urvillei*. *Trop Grasslands* 37:182–186.
- Cole RJ, Dörner JW, Lansden JA, Cox RH, Pape C, Cunfer B, Nicholson SS, Bedell DM. 1977. *Paspalum* staggers: isolation and identification of tremorgenic metabolites from sclerotia of *Claviceps paspali*. *J Agric Food Chem* 25:1197–1201. <https://doi.org/10.1021/jf60213a061>.
- Uhlig S, Botha CJ, Vrålstad T, Rolén E, Miles CO. 2009. Indole-diterpenes and ergot alkaloids in *Cynodon dactylon* (Bermuda grass) infected with *Claviceps cynodontis* from an outbreak of tremors in cattle. *J Agric Food Chem* 57:11112–11119. <https://doi.org/10.1021/jf902208w>.
- Oberti H, Dalla-Rizza M, Reyno R, Murchio S, Altier N, Abreo E. 2020. Diversity of *Claviceps paspali* reveals unknown lineages and unique alkaloid genotypes. *Mycologia* 112:230–243. <https://doi.org/10.1080/00275514.2019.1694827>.
- Gilmore BS, Alderman SC, Knaus BJ, Bassil NV, Martin RC, Dombrowski JE, Dung J. 2016. Simple sequence repeat markers that identify *Claviceps* species and strains. *Fungal Biol Biotechnol* 3:1. <https://doi.org/10.1186/s40694-016-0019-5>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUASt: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Smit A, Hubley R. 2015. RepeatModeler Open-1.0, 2008–2015. <http://www.repeatmasker.org>.
- Smit A, Hubley R, Green P. 2015. Repeatmasker Open-4.0, 2013–2015. <http://repeatmasker.org>.
- Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.

13. Schardl CL, Young CA, Hesse U, Amyotte SG, Andreeva K, Calie PJ, Fleetwood DJ, Haws DC, Moore N, Oeser B, Panaccione DG, Schweri KK, Voisey CR, Farman ML, Jaromczyk JW, Roe BA, O'Sullivan DM, Scott B, Tudzynski P, An Z, Arnaoudova EG, Bullock CT, Charlton ND, Chen L, Cox M, Dinkins RD, Florea S, Glenn AE, Gordon A, Guldener U, Harris DR, Hollin W, Jaromczyk J, Johnson RD, Khan AK, Leistner E, Leuchtmann A,

Li C, Liu JGe, Liu J, Liu M, Mace W, Machado C, Nagabhyru P, Pan J, Schmid J, Sugawara K, Steiner U, Takach JE, Tanaka E, Webb JS, Wilson EV, Wiseman JL, Yoshida R, Zeng Z. 2013. Plant-symbiotic fungi as chemical engineers: multi-genome analysis of the Clavicipitaceae reveals dynamics of alkaloid loci. *PLoS Genet* 9:e1003323. <https://doi.org/10.1371/journal.pgen.1003323>.