The Genome Sequence of Five Genotypes of *Fusarium oxysporum* f. sp. *vasinfectum*: A Resource for Studies on Fusarium Wilt of Cotton

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Abstract

Fusarium oxysporum f. sp. *vasinfectum* is an important plant pathogen responsible for vascular wilt disease on cotton. Members of this group are known to carry supernumerary chromosomes that encode virulence factors. We sequenced the genomes of five *F. oxysporum* f. sp. *vasinfectum* isolates, including the genome of a representative of the highly virulent genotype race 4, at a high coverage to assemble reference-quality genomes. These genomes provide a necessary resource for comparative genomic analyses to identify genes or genome features that are involved in pathogenicity on cotton and may ultimately be used to identify improved management strategies.

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Keywords

genomics, metabolomics, proteomics

Genome Announcement

Members of the *Fusarium oxysporum* species complex (FOSC) are an economically important group of fungal pathogens, causing vascular wilt diseases in a broad range of plant species (Michielse and Rep 2009). Previous experimental and genomic analyses of members of the FOSC have revealed that these isolates carry supernumerary chromosomes that harbor host-specific virulence traits (Coleman et al. 2011; Ma et al. 2010; van Dam et al. 2017; Williams et al. 2016). Importantly, these chromosomes have been experimentally shown to be able to be laterally transferred to other isolates of the FOSC, expanding host range (Ma et al. 2010; Vlaardingerbroek et al. 2016; van Dam et al. 2017). *F. oxysporum* f. sp. *vasinfectum* is an agriculturally important pathogen on cotton (*Gossypium* spp.), causing Fusarium wilt in all cotton-growing areas worldwide (Davis et al. 2006). *F. oxysporum* f. sp. *vasinfectum* may exist in a disease complex with nematodes, resulting in increased disease severity, where infected plants can display symptoms of stunting, wilting, chlorosis, necrosis of the vascular tissue and leaves, and, ultimately, plant death (Davis et al. 2006). *F. oxysporum* f. sp. *vasinfectum* has been divided into several different genotypes, classified by DNA sequence analysis and pathogenicity assays (Cianchetta et al. 2015; Davis et al. 2006; Skovgaard et al. 2001). Isolates of the race 4 genotype are the most destructive, able to cause disease in the absence of nematodes, and has been a significant problem in California the past 20 years and has since been isolated in Texas (Halpern et al. 2018; Kim et al. 2005). Other emerging genotypes have resulted in significant crop losses in different cotton cultivars (Bell et al. 2019). These isolates are able to persist in infected soils for several years (Smith and Snyder 1975) and, therefore, the most practical measure for managing the

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disease is breeding for increased resistance. Understanding of the evolution of virulence of these pathogens for cotton could aid in an effective breeding strategy for cotton cultivars with increased resistance. Here, we report the genome assemblies of five different genotypes of *F. oxysporum* f. sp. *vasinfectum* isolates: races 1 and 4, MDS-12, LA108, and LA127/140.

Five isolates were collected from infected cotton presenting with symptoms indicative of Fusarium wilt and a pure culture was grown on potato dextrose agar plates. DNA was extracted following a cetyltrimethylammonium bromide and Qiagen genomic-tip procedure (Kohler et al. 2015). *F. oxysporum* f. sp. *vasinfectum* isolates TF1 (race 1), 89-1A (race 4), LA1E (MDS-12), LA3B (LA108), and 14-004 (LA 127/140) were sequenced to 163x, 178x, 184x, 238x, and 179x coverage, respectively, using the PacBio RS II System at the Duke Center for Genomic and Computational Biology. De novo assemblies of raw reads were performed using Canu v1.4 with the default settings, except the expected size was set for the race 4 genome at 65 Mb, the MDS-12 genome at 55 Mb, and the remaining *F. oxysporum* f. sp. *vasinfectum* genomes at 50 Mb (Koren et al. 2017). The quality of the assemblies was validated via QUAST v 4.6.3 and BUSCO v 3.0.1 programs using the dikarya ortholog gene lineage dataset and the -sp parameter, fusarium_graminearum (Gurevich et al. 2013; Simão et al. 2015). The draft genomes of isolates TF1, LA3B, and 14-004 were approximately 50 Mb in size and the LA1E isolate had a slightly larger genome size of 53 Mb (Table 1). The race 4 isolate had distinctive genome characteristics when compared with the other *F. oxysporum* f. sp. *vasinfectum* isolates; in particular, a larger genome size, 63 Mb, which was distributed in 94 scaffolds (Table 1). Despite the differences, the percent GC content was almost the same at approximately 47% for all the genomes. The BUSCO results indicated that the assembled genomes were more than 98% complete.

The five genome assemblies were annotated using Funannotate v 1.5.0 pipeline (Palmer 2017), with raw transcriptomic data sets generated by gossypol-treated race 4 *F. oxysporum* f. sp. *vasinfectum* 89-1A isolate samples at two different time points. Total RNA was extracted as previously described (Coleman et al. 2009), and the quality and integrity of the RNA was verified by Bioanalyzer before sequencing by Illumina with 50-bp, paired-end sequencing at the Hudson Alpha Institute of Biotechnology. Prior to Funannotate, the repeated regions in the assembled genomes were softmasked by RepeatMasker. The masked genome was used for ab initio gene model prediction training by Augustus and GeneMark-ES gene prediction tools, and functional annotation was processed to protein-coding gene models. Overall, the race 4 genome encoded a larger number of genes ($n = 20,222$) as well as higher proportion of repetitive sequences (18.44%) compared with the other sequenced *F. oxysporum* f. sp. *vasinfectum* genomes, where there were approximately 18,000 protein-coding genes, and less than 10% repetitive regions in their genomes. The genome data has been deposited in the National Center for Biotechnology Information GenBank database, BioProject number PRJNA552091, with the accession numbers and BioSamples in Table 1.

Table 1. Genome assembly statistics of five *Fusarium oxysporum* f. sp. *vasinfectum* isolates

Statistics	Genotype				
	Race 1	Race 4	MDS-12	LA108	LA127/140
Isolate	TF1	89-1A	LA1E	LA3B	14-004
Coverage	163x	178x	184x	238x	179x
Number of scaffolds	17	94	26	17	18
Genome size (bp)	50,043,501	63,323,888	53,532,301	50,437,808	51,686,574
GC content (%)	47.40	47.65	47.44	47.48	47.35
N ₅₀ (bp)	4,223,171	3,569,865	3,332,013	4,733,764	4,700,795
L ₅₀	5	7	7	5	5
Longest contig (bp)	6,421,299	6,635,028	5,403,155	6,382,753	6,464,399
Contigs > 25 kb	17	86	25	17	17
BUSCO completeness	98.80%	98.70%	98.20%	98.80%	98.50%
Number of predicted genes	17,975	20,222	19,054	18,132	18,195
Repetitive sequence (bp)	3,125,885	11,679,362	4,607,798	3,585,278	3,914,995
Repetitive sequence	6.25%	18.44%	8.61%	7.11%	7.57%
NCBI accession	VINL00000000	VINP00000000	VINN00000000	VINM00000000	VINO00000000
BioSample	SAMN12175309	SAMN12175305	SAMN12175307	SAMN12175308	SAMN12175306

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Literature Cited

- Bell, A. A., Gu, A., Olvey, J., Wagner, T. A., Tashpulatov, J. J., Prom, S., Quintana, J., Nichols, R. L., and Liu, J. 2019. Detection and characterization of *Fusarium oxysporum* f. sp. *vasinfectum* VCG0114 (race 4) isolates of diverse geographic origins. *Plant Dis.* 103:1998-2009.
- Cianchetta, A. N., Allen, T. W., Huttmacher, R. B., Kemerait, R. C., Kirkpatrick, T. L., Lawrence, G. W., Lawrence, K. S., Mueller, J. D., Nichols, R. L., Olsen, M. W., Overstreet, C., Woodward, J. E., Davis, R. M., Allen, T. W., and Mueller, D. 2015. Survey of *Fusarium oxysporum* f. sp. *vasinfectum* in the United States. *J. Cotton Sci.* 19:328-336.
- Coleman, J. J., Rounsley, S. D., Rodriguez-Carres, M., Kuo, A., Wasmann, C. C., Grimwood, J., Schmutz, J., Taga, M., White, G. J., Zhou, S., Schwartz, D. C., Freitag, M., Ma, L. J., Danchin, E. G. J., Henrissat, B., Coutinho, P. M., Nelson, D. R., Straney, D., Napoli, C. A., Barker, B. M., Gribskov, M., Rep, M., Kroken, S., Molnár, I., Rensing, C., Kennell, J. C., Zamora, J., Farman, M. L., Selker, E. U., Salamov, A., Shapiro, H., Pangilinan, J., Lindquist, E., Lamers, C., Grigoriev, I. V., Geiser, D. M., Covert, S. F., Temporini, E., and Vanetten, H. D. 2009. The genome of *Nectria haematococca*: Contribution of supernumerary chromosomes to gene expansion. *PLoS Genet.* 5:e1000618.
- Coleman, J. J., Wasmann, C. C., Usami, T., White, G. J., Temporini, E. D., McCluskey, K., and VanEtten, H. D. 2011. Characterization of the gene encoding pisatin demethylase (FoPDA1) in *Fusarium oxysporum*. *Mol. Plant-Microbe Interact.* 24:1482-1491.
- Davis, R. M., Colyer, P. D., Rothrock, C. S., and Kochman, J. K. 2006. *Fusarium* wilt of cotton: Population diversity and implications for management. *Plant Dis.* 90:692-703.
- Gurevich, A., Saveliev, V., Vyahhi, N., and Tesler, G. 2013. QUAST: Quality assessment tool for genome assemblies. *Bioinformatics* 29:1072-1075.
- Halpern, H. C., Bell, A. A., Wagner, T. A., Liu, J., Nichols, R. L., Olvey, J., Woodward, J. E., Sanogo, S., Jones, C. A., Chan, C. T., and Brewer, M. T. 2018. First report of *Fusarium* wilt of cotton caused by *Fusarium oxysporum* f. sp. *vasinfectum* race 4 in Texas, U.S.A. *Plant Dis.* 102:446.
- Kim, Y., Huttmacher, R. B., and Davis, R. M. 2005. Characterization of California isolates of *Fusarium oxysporum* f. sp. *vasinfectum*. *Plant Dis.* 89:366-372.
- Kohler, A., Kuo, A., Nagy, L. G., Morin, E., Barry, K. W., Buscot, F., Canbäck, B., Choi, C., Cichocki, N., Clum, A., Colpaert, J., Copeland, A., Costa, M. D., Doré, J., Floudas, D., Gay, G., Girlanda, M., Henrissat, B., Herrmann, S., Hess, J., Högberg, N., Johansson, T., Khouja, H.-R., LaButti, K., Lahrmann, U., Levasseur, A., Lindquist, E. A., Lipzen, A., Marmeisse, R., Martino, E., Murat, C., Ngan, C. Y., Nehls, U., Plett, J. M., Pringle, A., Ohm, R. A., Perotto, S., Peter, M., Riley, R., Rineau, F., Ruytinx, J., Salamov, A., Shah, F., Sun, H., Tarkka, M., Tritt, A., Veneault-Fourrey, C., Zuccaro, A., Mycorrhizal Genomics Initiative Consortium, Tunlid, A., Grigoriev, I. V., Hibbett, D. S., and Martin, F. 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nat. Genet.* 47:410-415.
- Koren, S., Walenz, B. P., Berlin, K., Miller, J. R., Bergman, N. H., and Phillippy, A. M. 2017. Canu: Scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. *Genome Res.* 27:722-736.
- Ma, L.-J., van der Does, H. C., Borkovich, K. A., Coleman, J. J., Daboussi, M.-J., Di Pietro, A., Dufresne, M., Freitag, M., Grabherr, M., Henrissat, B., Houterman, P. M., Kang, S., Shim, W.-B., Woloshuk, C., Xie, X., Xu, J.-R., Antoniw, J., Baker, S. E., Bluhm, B. H., Breakspear, A., Brown, D. W., Butchko, R. A. E., Chapman, S., Coulson, R., Coutinho, P. M., Danchin, E. G. J., Diener, A., Gale, L. R., Gardiner, D. M., Goff, S., Hammond-Kosack, K. E., Hilburn, K., Hua-Van, A., Jonkers, W., Kazan, K., Kodira, C. D., Koehrsen, M., Kumar, L., Lee, Y.-H., Li, L., Manners, J. M., Miranda-Saavedra, D., Mukherjee, M., Park, G., Park, J., Park, S.-Y., Proctor, R. H., Regev, A., Ruiz-Roldan, M. C., Sain, D., Sakthikumar, S., Sykes, S., Schwartz, D. C., Turgeon, B. G., Wapinski, I., Yoder, O., Young, S., Zeng, Q., Zhou, S., Galagan, J., Cuomo, C. A., Kistler, H. C., and Rep, M. 2010. Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* 464:367-373.
- Michielse, C. B., and Rep, M. 2009. Pathogen profile update: *Fusarium oxysporum*. *Mol. Plant Pathol.* 10:311-324.
- Palmer, J. 2017. Funannotate: Eukaryotic genome annotation pipeline. <https://funannotate.readthedocs.io/en/latest/>
- Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., and Zdobnov, E. M. 2015. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210-3212.
- Skovgaard, K., Nirenberg, H. I., O'Donnell, K., and Rosendahl, S. 2001. Evolution of *Fusarium oxysporum* f. sp. *vasinfectum* races inferred from multigene genealogies. *Phytopathology* 91:1231-1237.
- Smith, S. N., and Snyder, W. C. 1975. Persistence of *Fusarium oxysporum* f. sp. *vasinfectum* in fields in the absence of cotton. *Phytopathology* 65:190-196.
- van Dam, P., Fokkens, L., Ayukawa, Y., van der Gragt, M., Ter Horst, A., Brankovics, B., Houterman, P. M., Arie, T., and Rep, M. 2017. A mobile pathogenicity chromosome in *Fusarium oxysporum* for infection of multiple cucurbit species. *Sci. Rep.* 7:9042.
- Vlaardingerbroek, I., Beerens, B., Rose, L., Fokkens, L., Cornelissen, B. J. C., and Rep, M. 2016. Exchange of core chromosomes and horizontal transfer of lineage-specific chromosomes in *Fusarium oxysporum*. *Environ. Microbiol.* 18:3702-3713.
- Williams, A. H., Sharma, M., Thatcher, L. F., Azam, S., Hane, J. K., Sperschneider, J., Kidd, B. N., Anderson, J. P., Ghosh, R., Garg, G., Lichtenzweig, J., Kistler, H. C., Shea, T., Young, S., Buck, S. A. G., Kamphuis, L. G., Saxena, R., Pande, S., Ma, L. J., Varshney, R. K., and Singh, K. B. 2016. Comparative genomics and prediction of conditionally dispensable sequences in legume-infecting *Fusarium oxysporum* formae speciales facilitates identification of candidate effectors. *BMC Genomics* 17:191.