



# Draft Genome Sequence of *Fusarium equiseti* K3, a Fungal Species Isolated from Hexachlorocyclohexane-Contaminated Soil

Nelson Khan,<sup>a</sup> Rodolfo Brizola Toscan,<sup>d</sup> Accadius Lunayo,<sup>c</sup> Benson Wamalwa,<sup>b</sup> Edward Muge,<sup>a</sup> Francis J. Mula,<sup>a</sup>  René Kallies,<sup>d</sup> Hauke Harms,<sup>d</sup> Lukas Y. Wick,<sup>d</sup>  Ulisses Nunes da Rocha<sup>d</sup>

<sup>a</sup>Department of Biochemistry, University of Nairobi, Nairobi, Kenya

<sup>b</sup>Department of Chemistry, University of Nairobi, Nairobi, Kenya

<sup>c</sup>Center for Biotechnology and Bioinformatics, University of Nairobi, Nairobi, Kenya

<sup>d</sup>Department of Environmental Microbiology, Helmholtz Centre for Environmental Research—UFZ, Leipzig, Germany

**ABSTRACT** We present the draft genome sequence of *Fusarium equiseti* strain K3, a fungus isolated from a hexachlorocyclohexane (HCH)-contaminated soil (Kitengela, Kenya). The 37.88-Mb draft genome sequence consists of 206 contigs, 12,311 predicted protein-coding sequences, and 261 tRNA sequences. This genome sequence contributes to our understanding of fungal-bacterial interactions during hexachlorocyclohexane degradation.

The organochlorine pesticide hexachlorocyclohexane (HCH) was used for many years to control agricultural pests (1, 2). Despite a complete ban or severe restrictions on the use of HCH in many countries (3, 4), it continues to pose considerable environmental risks due to its toxicity, environmental persistence, and bioaccumulation in the food chain (2). HCH biodegradation as an effective bioremediation approach (5) has been studied extensively in bacteria (6) and white-rot fungi (7–9). At the same time, fewer data exist on degradation by non-white-rot fungi such as *Fusarium* species (2, 10).

Here, we present the genome sequence of *Fusarium equiseti* strain K3, isolated from HCH-contaminated Kenyan soil from a former storage site at Kitengela, Kenya (01.49 S, 37.048 E), highly contaminated by organochloride pesticide (11). We identified our strain as *F. equiseti* based on a phylogenetic tree constructed using internal transcribed spacer 1, the 5.8S rRNA gene, and internal transcribed spacer 2 (complete sequence) and the large subunit rRNA gene (partial sequence) (Fig. 1). The fungus was isolated on minimum salt medium (MSM) agar plates (12) supplemented with 100  $\mu$ g/ml  $\gamma$ -HCH in an inverted agar plate microcosm system described by Bravo et al. (13). An axenic fungal colony was obtained by subsequent repeated plating on 1:10 diluted potato dextrose agar supplemented with 100  $\mu$ g/ml  $\gamma$ -HCH. The fungus' low HCH degradation capacity was demonstrated in MSM medium as previously described by Sagar et al. (10).

Mycelium obtained from an agar plate overgrown with the fungus was used for DNA extraction using a Wizard genomic DNA purification kit (Promega, USA) and quantified applying a Qubit fluorometer (Thermo Fisher Scientific, USA). A NEBNext Ultra II FS DNA library prep kit (New England Biolabs, USA) was used to prepare a paired-end 300-bp library for genome sequencing on the Illumina MiSeq platform according to the manufacturer's instructions, generating a total of 2,602,796 paired-end reads. We used Sickle v1.33 (14), with a Phred quality score of >30, to control quality and trim the sequences. *De novo* sequence assembly was performed using SPAdes v3.15.2 (15), while QUAST v5.0.2 (16) and BUSCO v5.0.0 (17), with the fungi\_odb10 database, were used for a quality check and to provide completeness of the gene content within the assembly. Genome annotation was performed via the MAKER v2.31.11 pipeline (18) using AUGUSTUS v3.4.0 (19), and SNAP v2013\_11\_29

**Editor** Antonis Rokas, Vanderbilt University

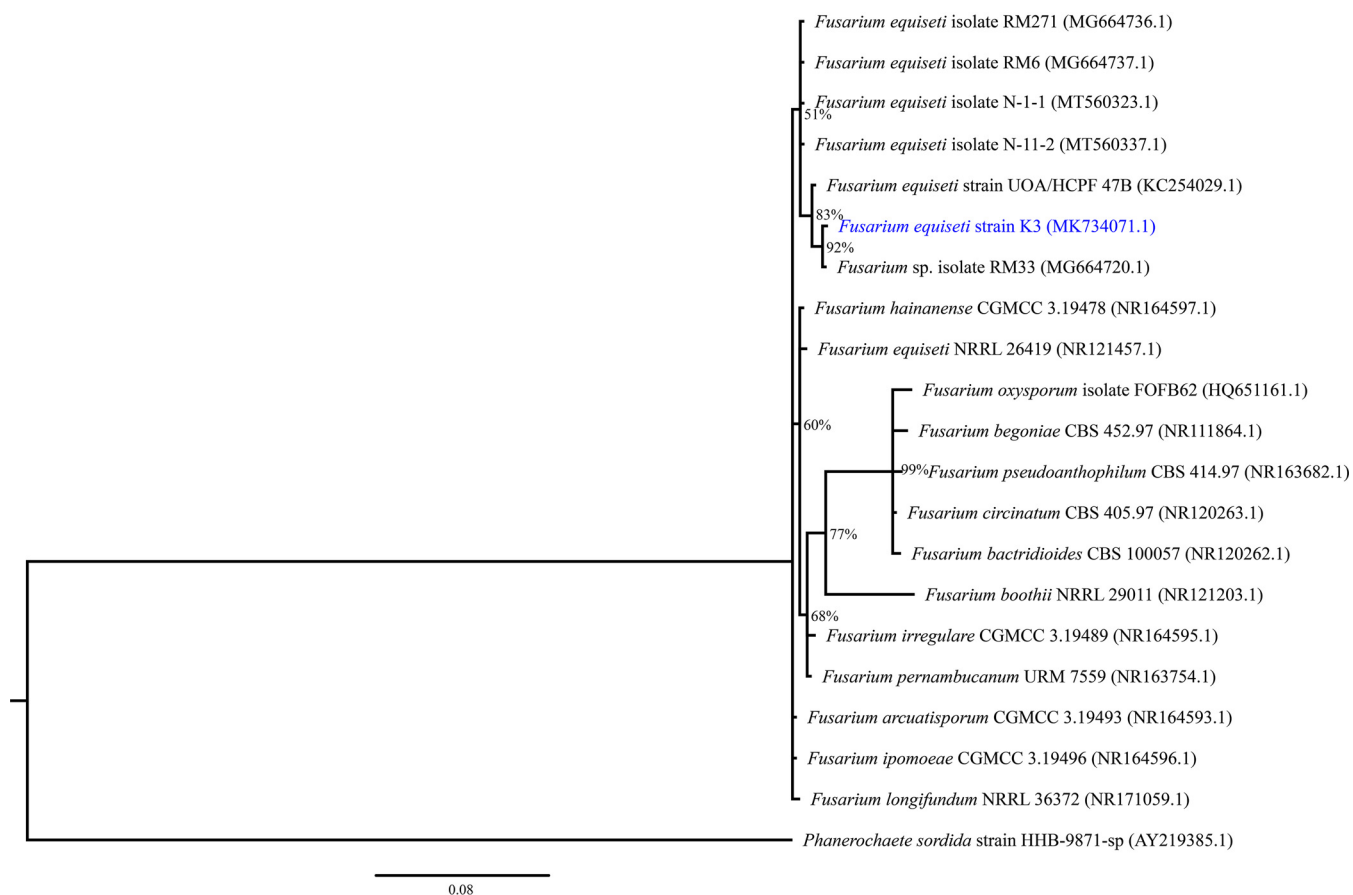
**Copyright** © 2021 Khan et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Ulisses Nunes da Rocha, [ulisses.rocha@ufz.de](mailto:ulisses.rocha@ufz.de).

**Received** 9 September 2021

**Accepted** 2 November 2021

**Published** 24 November 2021



**FIG 1** Phylogenetic tree showing the evolutionary relationship of *Fusarium equiseti* strain K3 (GenBank accession number [MK734071.1](#)) within the *Fusarium equiseti* clade, based on internal transcribed spacer 1 (ITS1), 5.8S rRNA gene sequences. The tree was constructed using MrBayes, a program for the Bayesian inference of phylogeny that is based on the Markov chain Monte Carlo (MCMC) method. Numbers at the nodes show percentages of posterior probabilities (derived from 1,000 samples), indicating the topological robustness of the tree. *Phanerochaete sordida* strain HHB-9871-sp was used as an outgroup to root the tree.

(20) was used for *ab initio* gene prediction, with *Fusarium graminearum* PH-1 (GenBank accession number [AACM0000000.2](#)) as the training species. Unless otherwise stated, default parameter settings were applied for all software used.

The genome assembly of *F. equiseti* strain K3 resulted in 206 contigs with a total length of 37,882,472 bp ( $N_{50}$  contig length, 601,073 bp; GC content, 48.03%). The draft genome consists of 12,311 predicted protein-coding sequences. Analysis of the predicted protein-coding sequences using BUSCO (17) and the fungi\_odb10 database (total of 758 genes) resulted in 749 (98.8%) complete single-copy genes, 3 (0.4%) complete duplicated genes, 3 (0.4%) fragmented genes, and 3 (0.4%) missing genes. A total of 261 tRNA genes were predicted using ARAGORN v1.2.36 (21). To gain insight into the *F. equiseti* strain K3 secondary metabolism, we used antiSMASH v6.0.0 (22) to predict the secondary metabolite biosynthetic gene clusters with the “fungi taxon” option. A total of 34 putative biosynthetic gene clusters previously reported for other *Fusarium* species were predicted. These included clusters likely to produce polyketides (23–25), terpenes (25, 26), and nonribosomal peptides (27–29).

The availability of the genome sequence of *F. equiseti* K3, together with ongoing efforts to understand its interactions with HCH-degrading bacteria, may provide invaluable insights into the use of fungal-bacterial cocultures for enhanced bioremediation of organic pollutants such as HCH (30).

**Data availability.** We deposited the *F. equiseti* K3 internal transcribed spacer 1, 5.8S rRNA gene, and internal transcribed spacer 2 (complete sequence) and large subunit rRNA gene (partial sequence) at ENA/DDBJ/GenBank under the accession number [MK734071.1](#). We deposited the *F. equiseti* K3 whole-genome shotgun project at ENA/DDBJ/GenBank

under the accession number [CAJSTJ0000000001](https://www.ncbi.nlm.nih.gov/trace/blast/blast.cgi?accession=CAJSTJ0000000001). The version described here is the first version. The raw data are available at the ENA Sequence Read Archive (SRA) under the BioProject accession number [PRJEB39686](https://www.ncbi.nlm.nih.gov/bioproject/PRJEB39686), BioSample accession number [SAMEA7112172](https://www.ncbi.nlm.nih.gov/biosample/SAMEA7112172), and SRA accession number [ERR4398881](https://www.ncbi.nlm.nih.gov/sra/ERR4398881).

## ACKNOWLEDGMENTS

The Helmholtz Association supported this project through the Helmholtz Young Investigator grant NG-1248 Micro “Big Data.” N. Khan was supported by funding from the German Academic Exchange Service (DAAD) and a grant from the International Federation of Science (IFS grant W/5798-1).

We thank Birgit Würz, Jana Reichenbach, and Rita Remer for skilled experimental help.

## REFERENCES

- Gvozdenac S, Indić D, Vuković S, Marković N, Takač A. 2014. Phyto-indicators in detection of lindane residues in water. *Commun Agric Appl Biol Sci* 79:545–551.
- de Guillén-Jiménez FM, Cristiani-Urbina E, Cancino-Díaz JC, Flores-Moreno JL, Barragán-Huerta BE. 2012. Lindane biodegradation by the *Fusarium verticillioides* AT-100 strain, isolated from Agave tequilana leaves: kinetic study and identification of metabolites. *Int Biodeterior Biodegradation* 74:36–47. <https://doi.org/10.1016/j.ibiod.2012.04.020>.
- Barriada-Pereira M, González-Castro MJ, Muniategui-Lorenzo S, López-Mahía P, Prada-Rodríguez D, Fernández-Fernández E. 2005. Organochlorine pesticides accumulation and degradation products in vegetation samples of a contaminated area in Galicia (NW Spain). *Chemosphere* 58: 1571–1578. <https://doi.org/10.1016/j.chemosphere.2004.10.016>.
- Mertens B, Boon N, Verstraete W. 2005. Stereospecific effect of hexachlorocyclohexane on activity and structure of soil methanotrophic communities. *Environ Microbiol* 7:660–669. <https://doi.org/10.1111/j.1462-2920.2005.00735.x>.
- Zhang W, Lin Z, Pang S, Bhatt P, Chen S. 2020. Insights into the biodegradation of lindane ( $\gamma$ -hexachlorocyclohexane) using a microbial system. *Front Microbiol* 11:522. <https://doi.org/10.3389/fmicb.2020.00522>.
- Lal R, Pandey G, Sharma P, Kumari K, Malhotra S, Pandey R, Raina V, Kohler H-PE, Holliger C, Jackson C, Oakeshott JG. 2010. Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. *Microbiol Mol Biol Rev* 74:58–80. <https://doi.org/10.1128/MMBR.00029-09>.
- Singh BK, Kuhad RC. 1999. Biodegradation of lindane ( $\gamma$ -hexachlorocyclohexane) by the white-rot fungus *Trametes hirsutus*. *Lett Appl Microbiol* 28: 238–241. <https://doi.org/10.1046/j.1365-2672.1999.00508.x>.
- Singh BK, Kuhad RC. 2000. Degradation of insecticide lindane ( $\gamma$ -HCH) by white-rot fungi *Cyathus bulleri* and *Phanerochaete sordida*. *Pest Manag Sci* 56:142–146. [https://doi.org/10.1002/1526-4998\(200002\)56:2<142::AID-PS104>3.0.CO;2-I](https://doi.org/10.1002/1526-4998(200002)56:2<142::AID-PS104>3.0.CO;2-I).
- Bumpus JA, Tien M, Wright D, Aust SD. 1985. Oxidation of persistent environmental pollutants by a white rot fungus. *Science* 228:1434–1436. <https://doi.org/10.1126/science.3925550>.
- Sagar V, Singh DP. 2011. Biodegradation of lindane pesticide by non-white-rots soil fungus *Fusarium* sp. *World J Microbiol Biotechnol* 27: 1747–1754. <https://doi.org/10.1007/s11274-010-0628-8>.
- Klánová J, Cupr P, Holoubek I, Borůvková J, Pribylová P, Kares R, Tomšej T, Ocelka T. 2009. Monitoring of persistent organic pollutants in Africa. Part 1: passive air sampling across the continent in 2008. *J Environ Monit* 11: 1952–1963. <https://doi.org/10.1039/b913415h>.
- Senoo K, Wada H. 1989. Isolation and identification of an aerobic  $\gamma$ -HCH-decomposing bacterium from soil. *Soil Sci Plant Nutr* 35:79–87. <https://doi.org/10.1080/00380768.1989.10434739>.
- Bravo D, Cailleau G, Bindschedler S, Simon A, Job D, Verrecchia E, Junier P. 2013. Isolation of oxalotrophic bacteria able to disperse on fungal mycelium. *FEMS Microbiol Lett* 348:157–166. <https://doi.org/10.1111/1574-6968.12287>.
- Joshi N, Fass JN. 2011. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files, version 1.33. <https://github.com/najoshi/sickle>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski 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>.
- Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.
- Cantarel BL, Korf I, Robb SMC, Parra G, Ross E, Moore B, Holt C, Sánchez Alvarado A, Yandell M. 2008. MAKER: an easy-to-use annotation pipeline designed for emerging model organism genomes. *Genome Res* 18: 188–196. <https://doi.org/10.1101/gr.6743907>.
- Stanke M, Keller O, Gunduz I, Hayes A, Waack S, Morgenstern B. 2006. AUGUSTUS: ab initio prediction of alternative transcripts. *Nucleic Acids Res* 34:W435–W439. <https://doi.org/10.1093/nar/gkl200>.
- Korf I. 2004. Gene finding in novel genomes. *BMC Bioinformatics* 5:59. <https://doi.org/10.1186/1471-2105-5-59>.
- Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res* 32:11–16. <https://doi.org/10.1093/nar/gkh152>.
- Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res* 47:W81–W87. <https://doi.org/10.1093/nar/gkz310>.
- Janevska S, Arndt B, Niehaus E-M, Burkhardt I, Rösler SM, Brock NL, Humpf H-U, Dickschat JS, Tudzynski B. 2016. Gibepyrone biosynthesis in the rice pathogen *Fusarium fujikuroi* is facilitated by a small polyketide synthase gene cluster. *J Biol Chem* 291:27403–27420. <https://doi.org/10.1074/jbc.M116.753053>.
- Studt L, Wiemann P, Kleigrew K, Humpf H-U, Tudzynski B. 2012. Biosynthesis of fusarubins accounts for pigmentation of *Fusarium fujikuroi* perithecia. *Appl Environ Microbiol* 78:4468–4480. <https://doi.org/10.1128/AEM.00823-12>.
- Miyamoto Y, Masunaka A, Tsuge T, Yamamoto M, Ohtani K, Fukumoto T, Gomi K, Peever TL, Tada Y, Ichimura K, Akimitsu K. 2010. ACTS3 encoding a polyketide synthase is essential for the biosynthesis of ACT-toxin and pathogenicity in the tangerine pathotype of *Alternaria alternata*. *Mol Plant Microbe Interact* 23:406–414. <https://doi.org/10.1094/MPMI-23-4-0406>.
- Brock NL, Huss K, Tudzynski B, Dickschat JS. 2013. Genetic dissection of sesquiterpene biosynthesis by *Fusarium fujikuroi*. *Chembiochem* 14: 311–315. <https://doi.org/10.1002/cbic.201200695>.
- Bonsch B, Belt V, Bartel C, Duensing N, Koziol M, Lazarus CM, Bailey AM, Simpson TJ, Cox RJ. 2016. Identification of genes encoding squalenstatin S1 biosynthesis and in vitro production of new squalenstatin analogues. *Chem Commun (Camb)* 52:6777–6780. <https://doi.org/10.1039/c6cc02130a>.
- Wollenberg RD, Saei W, Westphal KR, Klitgaard CS, Nielsen KL, Lysøe E, Gardiner DM, Wimmer R, Sondergaard TE, Sørensen JL. 2017. Chrysogine biosynthesis is mediated by a two-module nonribosomal peptide synthetase. *J Nat Prod* 80:2131–2135. <https://doi.org/10.1021/acs.jnatprod.6b00822>.
- Sims JW, Fillmore JP, Warner DD, Schmidt EW. 2005. Equisetin biosynthesis in *Fusarium heterosporum*. *Chem Commun (Camb)*:186–188. <https://doi.org/10.1039/b413523g>.
- Espinosa-Ortiz EJ, Rene ER, Gerlach R. 2021. Potential use of fungal-bacterial co-cultures for the removal of organic pollutants. *Crit Rev Biotechnol*: 1–23. <https://doi.org/10.1080/07388551.2021.1940831>.