Draft Genome Sequence Resource for *Phyllachora maydis*—An Obligate Pathogen That Causes Tar Spot of Corn with Recent Economic Impacts in the United States

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Abstract

Phyllachora maydis is an important fungal pathogen that causes tar spot of corn and has led to significant yield loss in the United States and other countries. *P. maydis* is an obligate biotroph belonging to the Sordariomycetes class of Ascomycota. Due to the challenges posed by their obligate nature, there is no genome sequence available in the *Phyllachora* genus. *P. maydis* isolate PM01 was collected from a corn field in Indiana and the genome was determined by next-generation sequencing. The assembly size is 45.7 Mb, with 56.46% repetitive sequences. There are 5,992 protein-coding genes and 59 are predicted as effector proteins. This genome resource will increase our understanding of genomic features of *P. maydis* and will assist in studying the corn–*P. maydis* interaction and identifying potential resistant candidates for corn breeding programs.

Keywords

corn, effectors, genomics, *Phyllachora maydis* isolate PM01, tar spot

Genome Announcement

The genus *Phyllachora* comprises of over 1,500 species of fungi (Parbery 1967). Members of this genus are generally obligate biotrophs that produce shiny, black, raised, semicircular stromata on the leaves and stems of their hosts, referred to as “tar spots” (Hock et al. 1995; Maharachchikumbura et al. 2016; Parbery 1967). Species in the *Phyllachora* genus have been reported on a wide range of grass species and other plants and, generally, are host species-specific (Cannon 1991; Parbery 1967).

Phyllachora maydis is an obligate fungal pathogen of corn in several Central and South American countries and the United States (Bajet et al. 1994; Bissonnette 2015; Dalla Lana et al. 2019; Hock et al. 1989, 1992, 1995; Malvick et al. 2020; Maublanc 1904; McCoy et al. 2018; Ruhl et al. 2016). It causes tar spot of corn, a disease that reduces grain yield, stover, husks, and silage quality. Grain losses can range between 11 to 46% in affected fields (Bajet et al. 1994; Hock et al. 1989; Maublanc 1904). Since 1904, tar spot of corn was limited to Central and South America, but recently, *P. maydis* has been identified in the United States and has become a new and emerging pathogen threatening corn production in the Midwest corn belt (Bissonnette 2015; Ruhl et al. 2016). In 2015, *P. maydis* was first confirmed in the United States in Indiana and Illinois but was thought to be of no economic consequence (Bissonnette 2015; Ruhl et al. 2016). However, in 2018, the first epidemic of tar spot on corn

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Table 1. Core metrics of the draft genome sequence of *Phyllachora maydis*

Metric	Value
Assembly size	45,742,705 bp
Number of scaffolds (≥ 500 bp)	11,228
L ₅₀ ($\geq N_{50}$)	1,076
N ₇₅	3,780 bp
N ₅₀	9,038 bp
N ₂₅	24,495 bp
Maximum scaffold length	124,943 bp
GC content	47.2%
Repeat	25,827,613 bp (56.46%)
Number of transfer RNAs	86
Number of genes	7,055
Number of protein-coding genes	5,992
GC content	62.5%
Average length	1,485.1 bp
Secreted proteins	462
Effectors	59
BUSCOs	
Complete and single copy	84.1%
Complete and duplicated	0.0%
Fragmented	6.9%
Missing	9.0%

was reported in Illinois, Indiana, Iowa, Michigan, and Wisconsin and resulted in estimated corn yield loss of 4.5 million tons (Mueller et al. 2019).

Current management recommendations for tar spot of corn are limited for United States corn production, as research on *P. maydis* biology, ecology, and epidemiology is lacking due to its relatively recent introduction (Mottaleb et al. 2019; Telenko et al. 2019). Most of the understanding of tar spot management is based on epidemics in Central and South America, more specifically, Mexico. These disease management recommendations can be inefficient due to differences in the environments, hybrid genetics, fungal populations, and cropping systems (Mottaleb et al. 2019). Prior to 2018, no field screening of United States corn germplasms for resistance to tar spot had been completed, and screening hybrid susceptibility to tar spot is of utmost importance for disease management. Fungicides are available to manage the disease in the United States, conditions and timing of one or more fungicide applications for best protection and control is not fully established. Further, the obligate nature of *P. maydis* currently limits standard biological, epidemiological, and molecular studies, since it cannot grow in pure culture in vitro. In addition, in-depth genome assembly of obligate pathogens is challenging because of limited amounts of DNA, owing to unknown growth requirements, comparatively large genomes, and a high number of repeat regions (Lasken and McLean 2014). Therefore, a draft genome sequence of *P. maydis* will provide an initial resource for understanding host-plant interactions, pathogenicity, host evolution, and establishment in the United States, which will aid in increasing knowledge about this pathogen and in the development of effective disease management strategies.

Corn leaves infected by *P. maydis* were collected from a research field located at the Pinney Purdue Agricultural Center, Wanatah, Indiana, in 2018. The raised, black fungal structures of *P. maydis* were excised from an infected corn leaf and was named as *P. maydis* isolate PM01. Genomic DNA (gDNA) was extracted using a modified cetyltrimethylammonium bromide-based protocol (Healey et al. 2014). A subsample of DNA was first used for internal transcribed spacer sequencing analysis to confirm PM01 as a *P. maydis* isolate. The integrity, quality, and concentration of DNA was analyzed by agarose gel electrophoresis and NanoDrop (ThermoFisher Scientific, Waltham, MA, U.S.A.). To obtain a draft genome, a 2×150 bp paired-end library was constructed with an insert size of 550 bp (base pair), using the TrueSeq DNA Nano library construction kit (Illumina Inc., San Diego, CA, U.S.A.). The quality of the gDNA library was assessed with the QubitHs-dsDNA BR assay kit on a Qubit fluorometer (Invitrogen, Carlsbad, CA, U.S.A.) and was then used for paired-end sequencing (2×150 bp) on the NovaSeq 6000 platform at Purdue Genomic Facility, West Lafayette, Indiana.

A total of 19,767,990 paired-end reads were generated. After removing the low-quality reads with the NGS_QC_Toolkit v2.3.3 (Patel and Jain 2012), 19,383,365 high-quality paired reads were obtained. Since the DNA sample was isolated from infected plant tissues, there was a great possibility that corn gDNA was also included in the total extracted gDNA needed

for whole genome sequencing and that the total high-quality paired reads will contain corn reads. Hence, these reads were first mapped to the genome of *Zea mays* (Schnable et al. 2009) with bowtie2 (Langmead and Salzberg 2012) to identify corn sequences. After filtering out the reads mapped to the corn genome, a total of 18,547,886 clean paired reads were obtained and were used for de novo assembly of the *P. maydis* genome by SPAdes v3.13.1 (Bankevich et al. 2012), with a combination of k-mer size 21, 33, 55, and 77, and the assembled sequences were further filtered by the National Center for Biotechnology Information (NCBI) contamination screening pipeline. The assembly of *P. maydis* genome is 45.7 Mbp in size and comprises 11,228 scaffolds (Table 1). The maximum scaffold length is 124,943 bp, and the N₅₀ of assembly is 9,038 bp. The GC content is 47.2%.

Repeats were identified with RepeatModeler v1.0.11, and the genome sequence was masked with RepeatMasker v4.0. Notably, 56.46% of the genomic sequence is repetitive sequences. The masked genomic sequence was used for gene prediction. In total, 7,055 genes were called *ab initio* by GeneMark-ES v4.48, with fungal-specific parameters (Ter-Hovhannisyan et al. 2008). Among them, 5,992 are protein-coding genes that have an average length of 1,485.1 bp and 62.5% GC content. By analyzing with tRNAscan-SE 2.0 (Chan et al. 2019), 86 transfer RNA genes were identified.

Benchmarking universal single-copy ortholog (BUSCO [v3.0.2]) (Simão et al. 2015) analysis with fungal dataset suggests that 91.0% (84.1% of which are complete and single copy and 6.9% are fragmented [Table 1]) of the conserved fungal proteins are present in the draft genome of *P. maydis*. Compared with the genome of *Cochliobolus heterostrophus* (Ohm et al. 2012), another fungal pathogen of corn, 83.6% of the predicted protein-coding genes (5,011) are common between these two fungi. In addition, proteins of 32 species belonging to different fungal phyla were downloaded from Ensembl Fungi, Joint Genome Institute MycoCosm, and NCBI databases and were used for BLASTP (Camacho et al. 2009) searches with proteins encoded by predicted *P. maydis* genes (with the cut-off e-value <1e-5). Whereas 5,544 proteins are conserved in other fungal species, 448 proteins are unique to *P. maydis*. Some of them may be important for corn infection and tar spot symptom development.

P. maydis is an ascomycete, but unlike many other ascomycetes, it cannot be cultured. As an obligate fungal pathogen, secreted proteins, especially the effector proteins, may play important roles in the plant-microbe interaction. By analysis with SignalP v4.1 (Petersen et al. 2011), a total of 462 secreted proteins were identified. Among them, 59 proteins were identified as effector candidates by EffectorP v2.0 (Sperschneider et al. 2018). Although it is difficult to isolate high-quality DNA directly from an infected corn leaf, enough DNA for preliminary genome sequencing (Illumina sequencing) was accomplished. However, further study is required on the growth nature of the pathogen to resolve challenges for in-depth genome assembly. Because *P. maydis* has recently become a highly important pathogen, significantly reducing corn production in the United States, this draft genome is extremely important to help research to mitigate future losses. The draft genome presented here is the first available genome sequence of *Phyllachora maydis*. The genome assembly and annotation data have been deposited at the NCBI GenBank database under the accession number JAALGG000000000.

Author-Recommended Internet Resources

RepeatModeler v1.0.1: <http://www.repeatmasker.org>
RepeatMasker v4.0.7: <http://www.repeatmasker.org>

Literature Cited

- Bajet, N. B., Renfro, B. L., and Carrasco, J. M. V. 1994. Control of tar spot of maize and its effect on yield. *Int. J. Pest Manage.* 40:121-125.
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Pribelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., and Pevzner, P. A. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19:455-477.
- Bissonnette, S. 2015. Corn disease alert: New fungal leaf disease “tar spot” *Phyllachora maydis* identified in 3 northern Illinois counties. In: *The Bulletin: Pest Management Crop Development Information for Illinois*. Department of Crop Sciences, University of Illinois, Urbana, IL, U.S.A.
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T. L. 2009. BLAST+: Architecture and applications. *BMC Bioinformatics* 10:421.
- Cannon, P. F. 1991. A revision of *Phyllachora* and some similar genera on the host family Leguminosae. *Mycological Paper*, CABI, Wallingford, U.K.
- Chan, P. P., Lin, B. Y., Mak, A. J., and Lowe, T. M. 2019. tRNAscan-SE 2.0: Improved detection and functional classification of transfer RNA genes. *bioRxiv*. 10.1101/614032. Published online.

- Dalla Lana, F., Plewa, D. E., Philippi, E. S., Garzonio, D., Hesterman, R., Kleczewski, N. M., and Paul, P. A. 2019. First report of tar spot of maize (*Zea mays*), caused by *Phyllachora maydis*, in Ohio. *Plant Dis.* 103:1780.
- Healey, A., Furtado, A., Cooper, T., and Henry, R. J. 2014. Protocol: A simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. *Plant Methods* 10:21.
- Hock, J., Dittrich, U., Renfro, B. L., and Kranz, J. 1992. Sequential development of pathogens in the maize tar spot disease complex. *Mycopathologia* 117:157-161.
- Hock, J., Kranz, J., and Renfro, B. L. 1995. Studies on the epidemiology of the tar spot disease complex of maize in Mexico. *Plant Pathol.* 44:490-502.
- Hock, J., Kranz, J., and Renfro, B. 1989. El complejo "mancha de asfalto" de maíz: Su distribución geográfica, requisitos ambientales e importancia económica en México. *Rev. Mex. Fitopatol.* 7:129-135.
- Langmead, B., and Salzberg, S. L. 2012. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9:357-359.
- Lasken, R. S., and McLean, J. S. 2014. Recent advances in genomic DNA sequencing of microbial species from single cells. *Nat. Rev. Genet.* 15:577-584.
- Maharachchikumbura, S. S. N., and Hyde, K. D., et al. 2016. Families of Sordariomycetes. *Fungal Divers.* 79:1-317.
- Malvick, D. K., Plewa, D. E., Lara, D., Kleczewski, N. M., Floyd, C. M., Arenz, B. E. 2020. First Report of Tar Spot of Corn Caused by *Phyllachora maydis* in Minnesota. *Plant Dis.* Published online.
- Maublanc, A. 1904. Espèces nouvelles de champignons inférieurs. *Bull. Soc. Mycol. Fr.* 20:72-74.
- McCoy, A. G., Romberg, M. K., Zaworski, E. R., Robertson, A. E., Phibbs, A., Hudelson, B. D., Smith, D. L., Beiriger, R. L., Raid, R. N., Byrne, J. M., and Chilvers, M. I. 2018. First report of tar spot on corn (*Zea mays*) caused by *Phyllachora maydis* in Florida, Iowa, Michigan, and Wisconsin. *Plant Dis.* 102:1851.
- Mottaleb, K. A., Loladze, A., Sonder, K., Kruseman, G., and San Vicente, F. 2019. Threats of tar spot complex disease of maize in the United States of America and its global consequences. *Mitig. Adapt. Strategies Glob. Change* 24: 281-300.
- Mueller, D., Wise, K., and Sisson, A. 2019. Corn disease loss estimates from the United States and Ontario, Canada—2018. *Crop Protection Network.* Published online.
- Ohm, R. A., Feau, N., Henrissat, B., Schoch, C. L., Horwitz, B. A., Barry, K. W., Condon, B. J., Copeland, A. C., Dhillon, B., Glaser, F., Hesse, C. N., Kosti, I., LaButti, K., Lindquist, E. A., Lucas, S., Salamov, A. A., Bradshaw, R. E., Ciuffetti, L., Hamelin, R. C., Kema, G. H., Lawrence, C., Scott, J. A., Spatafora, J. W., Turgeon, B. G., de Wit, P. J., Zhong, S., Goodwin, S. B., and Grigoriev, I. V. 2012. Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi. *PLoS Pathog.* 8:e1003037.
- Parbery, D. G. 1967. Studies on *Graminicolous* species of *Phyllachora* Nke. in Fekl. V. A taxonomic monograph. *Aust. J. Bot.* 15:271-375.
- Patel, R. K., and Jain, M. 2012. NGS QC Toolkit: A toolkit for quality control of next generation sequencing data. *PLoS One* 7:e30619.
- Petersen, T. N., Brunak, S., von Heijne, G., and Nielsen, H. 2011. SignalP 4.0: Discriminating signal peptides from transmembrane regions. *Nat. Methods* 8: 785-786.
- Ruhl, G., Romberg, M. K., Bissonnette, S., Plewa, D., Creswell, T., and Wise, K. A. 2016. First report of tar spot on corn caused by *Phyllachora maydis* in the United States. *Plant Dis.* 100:1496.
- Schnable, P. S., Ware, D., Fulton, R. S., Stein, J. C., Wei, F., Pasternak, S., Liang, C., Zhang, J., Fulton, L., Graves, T. A., Minx, P., Reilly, A. D., Courtney, L., Kruchowski, S. S., Tomlinson, C., Strong, C., Delehaunty, K., Fronick, C., Courtney, B., Rock, S. M., Belter, E., Du, F., Kim, K., Abbott, R. M., Cotton, M., Levy, A., Marchetto, P., Ochoa, K., Jackson, S. M., Gillam, B., Chen, W., Yan, L., Higginbotham, J., Cardenas, M., Waligorski, J., Applebaum, E., Phelps, L., Falcone, J., Kanchi, K., Thane, T., Scimone, A., Thane, N., Henke, J., Wang, T., Ruppert, J., Shah, N., Rotter, K., Hodges, J., Ingenthron, E., Cordes, M., Kohlberg, S., Sgro, J., Delgado, B., Mead, K., Chinwalla, A., Leonard, S., Crouse, K., Collura, K., Kudrna, D., Currie, J., He, R., Angelova, A., Rajasekar, S., Mueller, T., Lomeli, R., Scara, G., Ko, A., Delaney, K., Wissotski, M., Lopez, G., Campos, D., Braidotti, M., Ashley, E., Golser, W., Kim, H., Lee, S., Lin, J., Dujmic, Z., Kim, W., Talag, J., Zuccolo, A., Fan, C., Sebastian, A., Kramer, M., Spiegel, L., Nascimento, L., Zutavern, T., Miller, B., Ambroise, C., Muller, S., Spooner, W., Narechania, A., Ren, L., Wei, S., Kumari, S., Faga, B., Levy, M. J., McMahan, L., Van Buren, P., Vaughn, M. W., Ying, K., Yeh, C. T., Emrich, S. J., Jia, Y., Kalyanaraman, A., Hsia, A. P., Barbazuk, W. B., Baucom, R. S., Brutnell, T. P., Carpita, N. C., Chaparro, C., Chia, J. M., Deragon, J. M., Estill, J. C., Fu, Y., Jeddell, J. A., Han, Y., Lee, H., Li, P., Lisch, D. R., Liu, S., Liu, Z., Nagel, D. H., McCann, M. C., SanMiguel, P., Myers, A. M., Nettleton, D., Nguyen, J., Penning, B. W., Ponnala, L., Schneider, K. L., Schwartz, D. C., Sharma, A., Soderlund, C., Springer, N. M., Sun, Q., Wang, H., Waterman, M., Westerman, R., Wolfgruber, T. K., Yang, L., Yu, Y., Zhang, L., Zhou, S., Zhu, Q., Bennetzen, J. L., Dawe, R. K., Jiang, J., Jiang, N., Presting, G. G., Wessler, S. R., Aluru, S., Martienssen, R. A., Clifton, S. W., McCombie, W. R., Wing, R. A., and Wilson, R. K. 2009. The B73 maize genome: Complexity, diversity, and dynamics. *Science* 326:1112-1115.
- 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.
- Sperschneider, J., Dodds, P. N., Gardiner, D. M., Singh, K. B., and Taylor, J. M. 2018. Improved prediction of fungal effector proteins from secretomes with EffectorP 2.0. *Mol. Plant Pathol.* 19:2094-2110.
- Telenko, D. E. P., Chilvers, M. I., Kleczewski, N. Smith, D. L., Byrne, A. M., Devillez, P., Diallo, T., Higgins, R., Joos, D., Kohn, K., Lauer, J., Mueller, B., Singh, M. P., Widdicombe, W. D., and Williams, L. A. 2019. How tar spot of corn impacted hybrid yields during the 2018 Midwest epidemic. *Crop Protection Network.* Published online.
- Ter-Hovhannisyan, V., Lomsadze, A., Chernoff, Y. O., and Borodovsky, M. 2008. Gene prediction in novel fungal genomes using an *ab initio* algorithm with unsupervised training. *Genome Res.* 18:1979-1990.