















Draft Genome Sequence of *Xylaria grammica* EL000614, a Strain Producing Grammicin, a Potent Nematicidal Compound

Sook-Young Park^{a*} , Jongbum Jeon^{b*} , Jung A Kim^{c*} , Mi Jin Jeon^d , Nan Hee Yu^e , Seulbi Kim^e , Ae Ran Park^e , Jin-Cheol Kim^e , Yerim Lee^a , Youngmin Kim^a , Eu Ddeum Choi^a , Min-Hye Jeong^a , Yong-Hwan Lee^b  and Soonok Kim^d 

^aDepartment of Plant Medicine, Sunchon National University, Suncheon, Korea; ^bDepartment of Agricultural Biotechnology, Interdisciplinary Program in Agricultural Genomics, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul, Korea; ^cAnimal Resources Division, National Institute of Biological Resources, Incheon, Korea; ^dMicroorganism Resources Division, National Institute of Biological Resources, Incheon, Korea; ^eDepartment of Agricultural Chemistry, Institute of Environmentally Friendly Agriculture, Chonnam National University, Gwangju, Korea

ABSTRACT

An endolichenic fungus, *Xylaria grammica* strain EL000614, showed strong nematicidal effects against plant pathogenic nematode, *Meloidogyne incognita* by producing grammicin. We report genome assembly of *X. grammica* EL000614 comprised of 25 scaffolds with a total length of 54.73 Mb, N50 of 4.60 Mb, and 99.8% of BUSCO completeness. GC contents of this genome were 44.02%. Gene families associated with biosynthesis of secondary metabolites or regulatory proteins were identified out of 13,730 gene models predicted.

ARTICLE HISTORY

Received 1 February 2021
Revised 2 April 2021
Accepted 5 April 2021

KEYWORDS

Endolichenic fungi; grammicin; secondary metabolites; whole genome sequence; *Xylaria grammica*

Lichens provide habitats for many microorganisms, including endolichenic fungi. The endolichenic fungi have tremendous potential to produce bioactive metabolites. Compounds produced by endolichenic fungi have been shown to exhibit antitumor, antibacterial, antifungal, and nematicidal activities [1,2]. One endolichenic fungus, *Xylaria grammica* EL000614, showed strong nematicidal activity against deleterious plant pathogenic nematode, *Meloidogyne incognita*, causing root-knot disease on a wide range of host plants [2]. The active compound was purified and identified as grammicin [2]. Grammicin is an isomer of patulin, a notorious mycotoxin showing antibacterial and cytotoxic activity [3]. Unlike patulin, however, grammicin did not show antibacterial activities against various plant pathogenic bacteria, nor cytotoxic activity toward the human first-trimester trophoblast cell line SW.71 [2]. As a first step to reveal the grammicin biosynthetic pathway, the genome of EL000614 was sequenced.

Colonies of *X. grammica* strain EL000614 were yellowish-white on the front (Figure 1(A)) and dark red on the reverse side (Figure 1(B)) when grown for two weeks on potato dextrose agar medium. This dark red pigment was accumulated only in the

dark (Figure 1(C)), but not under constant light condition (Figure 1(D)). Black pigment was accumulated in illuminated plates. The fungus was grown in potato dextrose broth for 2 days at 23 °C, and genomic DNA was extracted from young mycelium using the DNeasy mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

Whole-genome sequencing was performed by the Theragen Etx Bio Institute (Suwon, Korea) using a combination of HiSeq 2000 (Illumina, CA, USA) and PacBio RS II (Pacific Biosciences, CA, USA). A short-insert (fragment size of 400 bp) paired-end (PE) library was constructed using the TruSeq DNA sample prep kit (Illumina), and two long-insert mate-pair (MP) libraries (insert sizes of 5 kb and 10 kb, respectively) were generated using a Nextera mate-pair library prep kit (Illumina). Eight PacBio RS II single-molecule real-time (SMRT) cells (total of 943,964 reads with an average read size of 7,891 bp) were obtained according to the PacBio standard library preparation protocol using the PacBio DNA Template Prep kit with the average fragment size of 20 kb. The genome was *de novo* assembled using the overlap-layout-consensus (OLC) algorithm in CANU v1.8 [4,5]. The

CONTACT Yong-Hwan Lee  yonglee@snu.ac.kr; Soonok Kim  sokim90@korea.kr

*These authors contributed equally to this work.

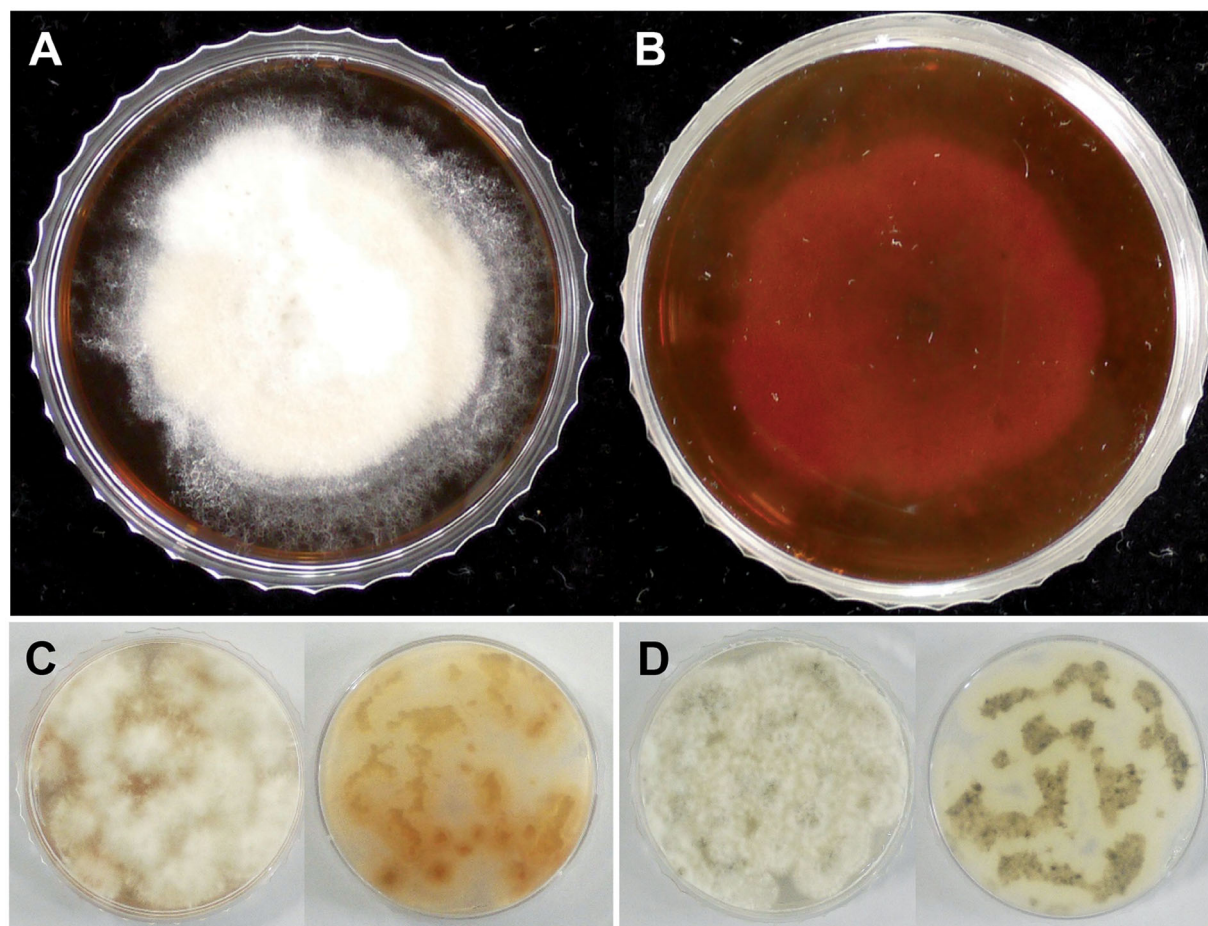


Figure 1. Morphological characteristics of *Xylaria grammica* strain EL000614. (A) Two weeks-old colony on the upper side of PDA. (B) Dark red pigmentation on the reverse side of PDA. Four-day-old colony on the upper side (left) and the reverse side (right) of PDA under constant dark condition (C) and under constant light condition (D).

assembled genome of long-reads was polished based on Illumina paired-end library (70,717,638 reads) using Pilon v 1.22 [6]. The final genome consists of 25 contigs and one mitochondrial genome with a total length of 54,730,220 bp and 196,363 bp, respectively. The assembly has N50 being 4.60 Mbp, and the GC content of the genome was 44.02%. Genome completeness was assessed using BUSCO v. 5.0.0 against the “fungi_odb10” database, showing 99.8% of 758 fungal gene sets were complete (Table 1) [7].

Gene prediction was performed by BRAKER2 [8] using constructed model from RNA-seq and protein models of five close species in the NCBI nonredundant (nr) database. A total of 13,730 gene models were predicted, of which 12,900 genes (93.96%) had hits in UniProt, nr, or InterPro database. Ninety-eight Biosynthetic gene clusters (BGCs), including 45 polyketide synthase (PKS) and 42 non-ribosomal protein synthetase (NRPS), 21 terpene synthase, were identified through antiSMASH v5.0 [9]. Among these were two orthologs encoding PatK, a PKS backbone gene in patulin biosynthesis [10]. Further, 476 genes encoding transcription factor and 222 genes encoding cytochrome P450 were

Table 1. Genome assembly and annotation statistics of *Xylaria grammica* EL000614.

Statistics	<i>X. grammica</i> EL000614
NCBI accession	NGZP00000000
Sequence Reads Archive Coverage (X)	SRR14073447–SRR14073457 513.77
Number of scaffolds	25
Genome size (bp)	54,730,220
N50 (bp)	3,878,448
GC content (%)	44.02
BUSCO completeness (%)	99.8
Number of predicted gene models	13,730
Number of functional annotation	12,900
Repetitive sequence (%)	16.36
Number of ncRNA	531

cataloged through Fungal Transcription Factor Database (FTFD) v1.0 [11], and Fungal Cytochrome P450 Database v1.0 [12], respectively. Genome was composed of 16.36% of repetitive sequences such as retrotransposons, DNA transposons, simple sequence repeats, and unclassified repeats through the combining of RebBase [13,14] search and *de novo* identification using RepeatModeler 1.0.8 and RepeatMasker 4.0.6 [15]. A total of 531 non-coding RNAs, including 43 rRNA from BLASTn search against NCBI, 225 tRNA through tRNAscan-SE [16], 121 microRNA and 142 small nucleolar RNA

through Infernal search against Rfam 11.0 [17], were also identified. This draft genome of *X. grammica* EL000614 will help further characterize the genes involved in grammicin biosynthesis and their regulation.

The sequence data and RNA-seq data of *X. grammica* EL000614 were deposited at Sequence Read Archive (SRA) under the accession number SRR14073447 to SRR14073457. The assembly data also deposited GenBank under the accession no. NGZP00000000. The version described in this article is NGZP02000000 (<https://www.ncbi.nlm.nih.gov/nucleotide/NGZP00000000.2>). The associated Bioproject number is PRJNA368785

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Institute of Biological Resources, funded by the Ministry of Environment of the Republic of Korea (projects NIBR201921101 and NIBR202021101).

ORCID

Sook-Young Park  <http://orcid.org/0000-0003-1267-1111>

Yong-Hwan Lee  <http://orcid.org/0000-0003-2462-1250>

Soonok Kim  <http://orcid.org/0000-0003-1654-3643>

References

- [1] Kellogg J, Raja HA. Endolichenic fungi: a new source of rich bioactive secondary metabolites on the horizon. *Phytochem Rev.* 2017;16(2):271–293.
- [2] Kim TY, Jang JY, Yu NH, et al. Nematicidal activity of grammicin produced by *Xylaria grammica* KCTC 13121BP against *Meloidogyne incognita*. *Pest Manag Sci.* 2018;74(2):384–391.
- [3] Edwards RL, Maitland DJ, Pittayakhajonwut P, et al. Metabolites of the higher fungi. Part 33. Grammicin, a novel bicyclic C₇H₆O₄ furanopyranol from the fungus *Xylaria grammica* (Mont.) Fr. *Perkin Trans.* 2001;1(11):1296–1299.
- [4] Wang A, Wang Z, Li Z, et al. BAUM: improving genome assembly by adaptive unique mapping and local overlap-layout-consensus approach. *Bioinformatics.* 2018;34(12):2019–2028.
- [5] Koren S, Walenz BP, Berlin K, et al. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res.* 2017;27(5):722–736.
- [6] Walker BJ, Abeel T, Shea T, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE.* 2014;9(11):e112963.
- [7] Waterhouse RM, Seppely M, Simao FA, et al. BUSCO applications from quality assessments to gene prediction and phylogenomics. *Mol Biol Evol.* 2018;35(3):543–548.
- [8] Brůna T, Hoff KJ, Lomsadze A, et al. BRAKER2: automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database. *NAR Genom Bioinform.* 2021;3:108.
- [9] Blin K, Shaw S, Steinke K, et al. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res.* 2019;47(W1):W81–W87.
- [10] Artigot MP, Loiseau N, Laffitte J, et al. Molecular cloning and functional characterization of two CYP619 cytochrome P450s involved in biosynthesis of patulin in *Aspergillus clavatus*. *Microbiology.* 2009;155(Pt 5):1738–1747.
- [11] Park J, Park J, Jang S, et al. FTFD: an informatics pipeline supporting phylogenomic analysis of fungal transcription factors. *Bioinformatics.* 2008;24(7):1024–1025.
- [12] Park J, Lee S, Choi J, et al. Fungal cytochrome P450 database. *BMC Genomics.* 2008;9:402.
- [13] Bao W, Kojima KK, Kohany O. Repbase Update, a database of repetitive elements in eukaryotic genomes. *Mob Dna.* 2015;6:11.
- [14] Kapitonov VV, Jurka J. A universal classification of eukaryotic transposable elements implemented in Repbase. *Nat Rev Genet.* 2008;9(5):411–412.
- [15] Smit AFA, Hubley R, Green P. RepeatMasker. 2015. Available from: <http://repeatmasker.org>.
- [16] Chan PP, Lowe TM. tRNAscan-SE: searching for tRNA genes in genomic sequences. *Methods Mol Biol.* 2019;1962:1–14.
- [17] Burge SW, Daub J, Eberhardt R, et al. Rfam 11.0: 10 years of RNA families. *Nucleic Acids Res.* 2013;41(Database issue):D226–D232.