




Draft Genome Sequence of *Aspergillus terreus* High-Itaconic-Acid-Productivity Mutant TN-484

Shin Kanamasa,^{a,b} Tomoyuki Minami,^a Mitsuyasu Okabe,^c Enoch Y. Park,^{d,e,f} Tsukasa Fujimoto,^g Anna Takahashi,^h Masataka Murase,ⁱ Shuichi Fukuyoshi,^j Akifumi Oda,^k Kenji Satou,^l  Hiro Takahashi^{g,i,m}

^aGraduate School of Bioscience and Biotechnology, Chubu University, Kasugai, Japan

^bCollege of Bioscience and Biotechnology, Chubu University, Kasugai, Japan

^cAureo Co., Ltd., Tokyo, Japan

^dResearch Institute of Green Science and Technology, Shizuoka University, Shizuoka, Japan

^eGraduate School of Integrated Science and Technology, Shizuoka University, Shizuoka, Japan

^fGraduate School of Science and Technology, Shizuoka University, Shizuoka, Japan

^gGraduate School of Horticulture, Chiba University, Matsudo, Japan

^hFaculty of Information Technologies and Control, Belarusian State University of Informatics and Radio Electronics, Minsk, Belarus

ⁱGraduate School of Medical Sciences, Kanazawa University, Kanazawa, Japan

^jInstitute of Medical, Pharmaceutical, and Health Sciences, Kanazawa University, Kanazawa, Japan

^kFaculty of Pharmacy, Meijo University, Nagoya, Japan

^lFaculty of Biological Science and Technology, Institute of Science and Engineering, Kanazawa University, Kanazawa, Japan

^mFundamental Innovative Oncology Core Center, National Cancer Center, Tokyo, Japan

ABSTRACT Itaconic acid is an important organic acid used in the chemical industry. *Aspergillus terreus* strain TN-484 is a high-itaconic-acid-productivity mutant derived from strain IFO6365. Here, we report the draft genome sequence of strain TN-484, advancing the understanding of the biosynthesis of itaconic acid in filamentous fungi.

Itaconic acid is an unsaturated dibasic acid that is widely used as a raw material, food additive, and industrial product additive in synthetic resins and printing inks. Itaconic acid is known to be produced by filamentous fungi such as *Aspergillus itaconicus* and yeasts such as *Candida tsukubaensis*. *Aspergillus terreus* is used for industrial production of itaconic acid due to its high compound production (1). Itaconic acid is a relatively expensive organic acid, and further application expansion can occur with cost decreases (2). To more efficiently produce itaconic acid, we developed an itaconic acid-resistant strain, TN-484, by treating *A. terreus* IFO6365 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) and screened for an itaconic acid-resistant and high-itaconic-acid-productivity mutant (3). In addition, we cloned and analyzed the CAD1 gene encoding *cis*-aconitic acid decarboxylase (CAD). We showed that *A. terreus* CAD produced itaconic acid using *cis*-aconitic acid as the substrate (4). We previously showed that itaconic acid productivity was affected by medium pH and metal ion concentration in *A. terreus* (reviewed in reference 5). However, many itaconic acid biological functions remain unclear, and elucidating its underlying biosynthetic control will help clarify its functions.

TN-484 cells cultured in potato dextrose broth were homogenized using a bead crusher, and genomic DNA was isolated using a NucleoSpin plant II kit (TaKaRa Bio, Inc.). TN-484 genomic DNA was isolated from a liquid-submerged culture grown in potato dextrose broth at 30°C. Using the NEBNext Ultra II FS DNA library prep kit for Illumina (New England BioLabs [NEB], MA, USA), a 500-ng DNA aliquot was fragmented using DNA Fragmentase to generate DNA fragments (average, 600 bp) for library preparation. The DNA was end repaired and A tailed, adaptors were ligated, PCR was performed, and library purification was done according to the manufacturer's instructions. Sequencing

Citation Kanamasa S, Minami T, Okabe M, Park EY, Fujimoto T, Takahashi A, Murase M, Fukuyoshi S, Oda A, Satou K, Takahashi H. 2019. Draft genome sequence of *Aspergillus terreus* high-itaconic-acid-productivity mutant TN-484. *Microbiol Resour Announc* 8:e01170-19. <https://doi.org/10.1128/MRA.01170-19>.

Editor Jason E. Stajich, University of California, Riverside

Copyright © 2019 Kanamasa 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 Shin Kanamasa, kanamasa@isc.chubu.ac.jp, or Hiro Takahashi, takahasi@p.kanazawa-u.ac.jp.

S.K. and H.T. contributed equally to this work.

Received 25 September 2019

Accepted 6 November 2019

Published 5 December 2019

of the TruSeq DNA library (paired-end 2×300 -bp reads) generated 18,647,110 reads. Removal of the sequencing primers and low-quality read trimming were conducted using CLC Genomics Workbench version 12.0.2 (Qiagen, Valencia, CA, USA), with default parameters. *De novo* assembly was performed using CLC Genomics Workbench, with default parameters. After *de novo* assembly, the reference-guided scaffolding of draft genomes was conducted using RaGOO version 1.1 (6), with default parameters, and *A. terreus* NIH2624 genome sequences (GenBank accession number [GCA_000149615](https://doi.org/10.1093/nar/gkt418)). The resulting genome assembly had a length of 29,733,777 bp divided into 24 contigs. The N_{50} contig length was 1,952,528 bp, the GC content was 52.5%, and the genome coverage was $174.9\times$. The coding regions of the chromosomes were predicted using AUGUSTUS version 3.2.2 (7) and implemented in OmicsBox version 1.1.164 (Qiagen), with default parameters, using *A. terreus* as a gene model (the *A. terreus* model is contained in the AUGUSTUS package). The estimated number of genes in the draft genome was 10,301. This genomic information could provide insight into the genetic basis of itaconic acid production by this strain.

Data availability. The draft genome sequence for strain TN-484 has been deposited in GenBank/ENA/DBJ under the accession number [BKZM00000000](https://doi.org/10.1093/nar/gkt418) (BKZM02000001 to BKZM02000024). The SRA/DRA/ERA accession number is [DRA008975](https://doi.org/10.1093/nar/gkt418).

ACKNOWLEDGMENTS

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI (grants JP18H03330, JP18K06297, and JP19K22892), the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) KAKENHI (grants JP26114703 and JP17H05659), the Research Foundation of the Research Institute for Biological Functions, Chubu University, and the Research Foundation for the Electrotechnology of Chubu.

REFERENCES

1. Calam CT, Oxford AE, Raistrick H. 1939. Studies in the biochemistry of micro-organisms: itaconic acid, a metabolic product of a strain of *Aspergillus terreus* Thom. *Biochem J* 33:1488–1495. <https://doi.org/10.1042/bj0331488>.
2. Yahiro K, Shibata S, Jia S-R, Park Y, Okabe M. 1997. Efficient itaconic acid production from raw corn starch. *J Ferm Bioeng* 84:375–377. [https://doi.org/10.1016/S0922-338X\(97\)89265-3](https://doi.org/10.1016/S0922-338X(97)89265-3).
3. Yahiro K, Takahama T, Park Y, Okabe M. 1995. Breeding of *Aspergillus terreus* mutant TN-484 for an itaconic acid production with high yield. *J Ferm Bioeng* 79:506–508. [https://doi.org/10.1016/0922-338X\(95\)91272-7](https://doi.org/10.1016/0922-338X(95)91272-7).
4. Kanamasa S, Dwiarti L, Okabe M, Park EY. 2008. Cloning and functional characterization of the *cis*-aconitic acid decarboxylase (CAD) gene from *Aspergillus terreus*. *Appl Microbiol Biotechnol* 80:223–229. <https://doi.org/10.1007/s00253-008-1523-1>.
5. Okabe M, Lies D, Kanamasa S, Park EY. 2009. Biotechnological production of itaconic acid and its biosynthesis in *Aspergillus terreus*. *Appl Microbiol Biotechnol* 84:597–606. <https://doi.org/10.1007/s00253-009-2132-3>.
6. Alonge M, Soyk S, Ramakrishnan S, Wang X, Goodwin S, Sedlazeck FJ, Lippman ZB, Schatz MC. 2019. RaGOO: fast and accurate reference-guided scaffolding of draft genomes. *Genome Biol* 20:224. <https://doi.org/10.1186/s13059-019-1829-6>.
7. Hoff KJ, Stanke M. 2013. WebAUGUSTUS: a Web service for training AUGUSTUS and predicting genes in eukaryotes. *Nucleic Acids Res* 41:W123–W128. <https://doi.org/10.1093/nar/gkt418>.