

Genome Sequence of the White Koji Mold *Aspergillus kawachii* IFO 4308, Used for Brewing the Japanese Distilled Spirit Shochu

Taiki Futagami, Kazuki Mori, Ayaka Yamashita, Shotaro Wada, Yasuhiro Kajiwarra, Hideharu Takashita, Toshiro Omori, Kaoru Takegawa, Kosuke Tashiro, Satoru Kuhara and Masatoshi Goto
Eukaryotic Cell 2011, 10(11):1586. DOI:
10.1128/EC.05224-11.

Updated information and services can be found at:
<http://ec.asm.org/content/10/11/1586>

These include:

REFERENCES

This article cites 11 articles, 6 of which can be accessed free at:
<http://ec.asm.org/content/10/11/1586#ref-list-1>

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), [more»](#)

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

GENOME ANNOUNCEMENT

Genome Sequence of the White Koji Mold *Aspergillus kawachii* IFO 4308, Used for Brewing the Japanese Distilled Spirit Shochu

Taiki Futagami,¹ Kazuki Mori,¹ Ayaka Yamashita,¹ Shotaro Wada,² Yasuhiro Kajiwaru,²
Hideharu Takashita,² Toshiro Omori,² Kaoru Takegawa,¹ Kosuke Tashiro,¹
Satoru Kuhara,¹ and Masatoshi Goto^{1*}

Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan,¹ and
Sanwa Research Institute, Sanwa Shurui Co. Ltd., Usa 879-0495, Japan²

Received 30 August 2011/Accepted 30 August 2011

The filamentous fungus *Aspergillus kawachii* has traditionally been used for brewing the Japanese distilled spirit shochu. *A. kawachii* characteristically hyperproduces citric acid and a variety of polysaccharide glycoside hydrolases. Here the genome sequence of *A. kawachii* IFO 4308 was determined and annotated. Analysis of the sequence may provide insight into the properties of this fungus that make it superior for use in shochu production, leading to the further development of *A. kawachii* for industrial applications.

Several species of the filamentous fungus genus *Aspergillus* have traditionally been used as koji molds for brewing alcoholic beverages in Japan. Koji is rice or barley that has been polished, steamed, and covered with the hyphal growth of a fungus, whose secreted enzymes convert the starch present in the grains to sugars (1). Yellow koji mold, *Aspergillus oryzae*, has been used for brewing sake (5), while a black koji mold, *A. awamori*, and its albino mutant, the white koji mold *A. kawachii*, have been used for making the distilled spirit shochu. Since shochu is produced mainly in the southwest Japanese island of Kyushu, where the climate is relatively warmer than that in places more well known for sake brewing, citric acid-producing *A. awamori* and *A. kawachii* were selected for making shochu to prevent undesirable contamination from bacteria. Although these two species of koji mold are phylogenetically close to *A. niger*, they are distinctly separated from *A. niger* (12).

Here we present the genome sequence of *A. kawachii* IFO 4308. The genomic DNA of strain IFO 4308 was sequenced to 17-fold coverage by a whole-genome shotgun strategy. One shotgun and 0.5 pair-end runs were performed using a Roche 454 GS (FLX Titanium) pyrosequencer. All of the reads were assembled using the Newbler Assembler 2.5 software program (454 Life Science), which generated 1,687 large contigs (>500 bp) and 318 scaffolds with N_{50} sizes of 138 and 897 kb. The genome annotation of the obtained scaffolds was performed based on the AUGUSTUS v2.5 software program (11), which was trained for predicting genes in *A. fumigatus*, *A. nidulans*, *A. oryzae*, and *A. terreus* (4, 5, 7, 9), and on BLAST searches against a nonredundant protein sequence database.

The draft genome of *A. kawachii* IFO 4308 includes

36,575,290 bp and is comprised of 11,488 predicted coding sequences (CDSs) with a G+C content of 49.9%. The genome contains 267 tRNAs predicted by the tRNAscan-SE 1.21 server (10).

Several *A. niger* strains produce ochratoxin A (OTA), whose synthesis is thought to be mediated in part by polyketide synthase (An15g07920), encoded by the *pks* gene (8, 9). *A. niger* strain CBS 513.88 carries the *pks* gene (9), but *A. niger* ATCC 1015 has lost part of the *pks* gene (2). *A. kawachii* IFO 4308 does not produce OTA (12). Accordingly, genome sequencing revealed that this fungus has lost a 21-kb region in the region of An15g07920 in a manner similar to that for *A. niger* ATCC 1015 (2).

A. kawachii and *A. niger* characteristically produce large amounts of citric acid in culture. Our genomic analysis revealed that *A. kawachii* possesses a complete tricarboxylic acid cycle and that the genes involved in the synthesis and degradation of citric acid are conserved with those of *A. niger* (2, 9).

Aspergilli produce a variety of glycoside hydrolases (GHs) (6). The genes encoding GH in *A. kawachii* were identified and classified based on the CAZy database (3). In the *A. kawachii* genome, we identified 247 GH genes that could be classified into 53 families out of a total of 125 known GH families.

Nucleotide sequence accession numbers. The nucleotide sequence of the *A. kawachii* genome has been deposited in DDBJ/EMBL/GenBank under the accession numbers DF126447 to DF126592, BACL01000001 to BACL01001641, and AP012272.

We thank Akiko Enomoto and Tomoko Hidaka for technical support of 454 GS-FLX Titanium pyrosequencing.

REFERENCES

1. Akiyama, H. 2010. Sake: the essence of 2000 years of Japanese wisdom gained from brewing alcoholic beverages from rice. Translated by T. Inoue. Brewing Society of Japan, Tokyo, Japan.
2. Andersen, M. R., et al. 2011. Comparative genomics of citric-acid-producing

* Corresponding author. Mailing address: Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Fukuoka 812-8581, Japan. Phone and fax: 81-92-642-3959. E-mail: mgoto@brs.kyushu-u.ac.jp.

- Aspergillus niger* ATCC 1015 versus enzyme-producing CBS 513.88. *Genome Res.* **21**:885–897.
3. **Cantarel, B. L., et al.** 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res.* **37**:D233–D238.
 4. **Galagan, J. E., et al.** 2005. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. *Nature* **438**:1105–1115.
 5. **Machida, M., et al.** 2005. Genome sequencing and analysis of *Aspergillus oryzae*. *Nature* **438**:1157–1161.
 6. **Machida, M., O. Yamada, and K. Gomi.** 2008. Genomics of *Aspergillus oryzae*: learning from the history of Koji mold and exploration of its future. *DNA Res.* **15**:173–183.
 7. **Nierman, W. C., et al.** 2005. Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature* **438**:1151–1156.
 8. **O'Callaghan, J., M. X. Caddick, and A. D. Dobson.** 2003. A polyketide synthase gene required for ochratoxin A biosynthesis in *Aspergillus ochraceus*. *Microbiology* **149**:3485–3491.
 9. **Pel, H. J., et al.** 2007. Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. *Nat. Biotechnol.* **25**:221–231.
 10. **Schattner, P., A. N. Brooks, and T. M. Lowe.** 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. *Nucleic Acids Res.* **33**:W686–W689. doi:10.1093/nar/gki366.
 11. **Stanke, M., R. Steinkamp, S. Waack, and B. Morgenstern.** 2004. AUGUSTUS: a web server for gene finding in eukaryotes. *Nucleic Acids Res.* **32**:W309–W312. doi:10.1093/nar/gkh379.
 12. **Yamada, O., et al.** 2011. Molecular biological researches of Kuro-Koji molds, their classification and safety. *J. Biosci. Bioeng.* doi:10.1016/j.jbiosc.2011.05.005.