Genome sequence of the white Koji mold aspergillus kawachii IFO 4308, used for brewing the Japanese distilled spirit shochu

Futagami, Taiki
Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University

Mori, Kazuki
Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University

Yamashita, Ayaka
Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University

Wada, Shotaro
Sanwa Research Institute, Sanwa Shurui Co. Ltd.

他

https://hdl.handle.net/2324/25730

出版情報：Eukaryotic Cell. 10 (11), pp.1586-1587, 2011-11. American Society for Microbiology
バージョン：

August 30, 2011

Genome Announcement in Eukaryotic Cell

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 Kajiwara[2], Hideharu Takashita[2], Toshiro Omori[2], Kaoru Takegawa[1], Kosuke Tashiro[1], Satoru Kuhara[1], and Masatoshi Goto[1]*

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

*To whom correspondence should be addressed.
Department of Bioscience and Biotechnology,
Faculty of Agriculture, Kyushu University,
6-10-1 Hakozaki, Fukuoka 812-8581, Japan
Phone & Fax: +81-92-642-3959
E-mail: mgoto@brs.kyushu-u.ac.jp

Abstract

The filamentous fungus *Aspergillus kawachii* has traditionally been used for brewing the Japanese distilled spirit *Shochu*. *A. kawachii* characteristically hyper-produces 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 and lead to the further development of *A. kawachii* for industrial applications.

Several species of the filamentous fungal 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*. As *Shochu* is mainly produced 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 to make *Shochu* to prevent undesirable contamination of 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 Newbler Assembler 2.5 (454 Life Science), which generated 1,687 large contigs (>500 bp) and

318 scaffolds with N50 sizes of 138 and 897 kb. The genome annotation of the obtained scaffolds was performed based on AUGUSTUS v2.5 program (11) that 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 non-redundant 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 tRNAscan-SE 1.21 (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 a lost 21-kb region in the region of An15g07920 in a manner similar to *A. niger* ATCC 1015 (2).

A. kawachii, as well as *A. niger*, characteristically produces high 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-DF126592, BACL01000001-BACL01001641, and AP012272.

We thank Ms Akiko Enomoto and Ms 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. T. Inoue (translated). Brewing Society of Japan. Tokyo, Japan.

2. **Andersen, M. R., M. P. Salazar, P. J. Schaap, P. J. van de Vondervoort, D. Culley, J. Thykaer, J. C. Frisvad, K. F. Nielsen, R. Albang, K. Albermann, R. M. Berka, G. H. Braus, S. A. Braus-Stromeyer, L. M. Corrochano, Z. Dai, P. W. van Dijck, G. Hofmann, L. L. Lasure, J. K. Magnuson, H. Menke, M. Meijer, S. L. Meijer, J. B. Nielsen, M. L. Nielsen, A. J. van Ooyen, H. J. Pel, L. Poulsen, R. A. Samson, H. Stam, A. Tsang, J. M. van den Brink, A. Atkins, A. Aerts, H. Shapiro, J. Pangilinan, A. Salamov, Y. Lou, E. Lindquist, S. Lucas, J. Grimwood, I. V. Grigoriev, C. P. Kubicek, D. Martinez, N. N. van Peij, J. A. Roubos, J. Nielsen, and S. E. Baker.** 2011. Comparative genomics of citric-acid-producing Aspergillus niger ATCC 1015 versus enzyme-producing CBS 513.88. Genome Res. **21:**885-897. doi: 10.1101/gr.112169.110.

3. **Cantarel, B. L., P. M. Coutinho, C. Rancurel, T. Bernard, V. Lombard, and B. Henrissat.** 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res. **37:**D233-8. doi: 10.1093/nar/gkn663.

4. **Galagan, J. E., S. E. Calvo, C. Cuomo, L. J. Ma, J. R. Wortman, S. Batzoglou, S. I. Lee, M. Basturkmen, C. C. Spevak, J. Clutterbuck, V. Kapitonov, J. Jurka, C.**

Scazzocchio, M. Farman, J. Butler, S. Purcell, S. Harris, G. H. Braus, O. Draht, S. Busch, C. D'Enfert, C. Bouchier, G. H. Goldman, D. Bell-Pedersen, S. Griffiths-Jones, J. H. Doonan, J. Yu, K. Vienken, A. Pain, M. Freitag, E. U. Selker, D. B. Archer, M. A. Penalva, B. R. Oakley, M. Momany, T. Tanaka, T. Kumagai, K. Asai, M. Machida, W. C. Nierman, D. W. Denning, M. Caddick, M. Hynes, M. Paoletti, R. Fischer, B. Miller, P. Dyer, M. S. Sachs, S. A. Osmani, and B. W. Birren. 2005. Sequencing of Aspergillus nidulans and comparative analysis with A. fumigatus and A. oryzae. Nature. **438:**1105-1115. doi: 10.1038/nature04341.

5. **Machida, M., K. Asai, M. Sano, T. Tanaka, T. Kumagai, G. Terai, K. Kusumoto, T. Arima, O. Akita, Y. Kashiwagi, K. Abe, K. Gomi, H. Horiuchi, K. Kitamoto, T. Kobayashi, M. Takeuchi, D. W. Denning, J. E. Galagan, W. C. Nierman, J. Yu, D. B. Archer, J. W. Bennett, D. Bhatnagar, T. E. Cleveland, N. D. Fedorova, O. Gotoh, H. Horikawa, A. Hosoyama, M. Ichinomiya, R. Igarashi, K. Iwashita, P. R. Juvvadi, M. Kato, Y. Kato, T. Kin, A. Kokubun, H. Maeda, N. Maeyama, J. Maruyama, H. Nagasaki, T. Nakajima, K. Oda, K. Okada, I. Paulsen, K. Sakamoto, T. Sawano, M. Takahashi, K. Takase, Y. Terabayashi, J. R. Wortman, O. Yamada, Y. Yamagata, H. Anazawa, Y. Hata, Y. Koide, T. Komori, Y. Koyama, T. Minetoki, S. Suharnan, A. Tanaka, K. Isono, S. Kuhara, N. Ogasawara, and H. Kikuchi.** 2005. Genome sequencing and analysis of Aspergillus oryzae. Nature. **438:**1157-1161. doi: 10.1038/nature04300.

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. doi: 10.1093/dnares/dsn020.

7. **Nierman, W. C., A. Pain, M. J. Anderson, J. R. Wortman, H. S. Kim, J. Arroyo, M. Berriman, K. Abe, D. B. Archer, C. Bermejo, J. Bennett, P. Bowyer, D. Chen, M. Collins, R. Coulsen, R. Davies, P. S. Dyer, M. Farman, N. Fedorova, N. Fedorova, T. V. Feldblyum, R. Fischer, N. Fosker, A. Fraser, J. L. Garcia, M. J. Garcia, A. Goble, G. H. Goldman, K. Gomi, S. Griffith-Jones, R. Gwilliam, B. Haas, H. Haas, D. Harris, H. Horiuchi, J. Huang, S. Humphray, J. Jimenez, N. Keller, H. Khouri, K. Kitamoto, T. Kobayashi, S. Konzack, R. Kulkarni, T. Kumagai, A. Lafon, J. P. Latge, W. Li, A. Lord, C. Lu, W. H. Majoros, G. S. May, B. L. Miller, Y. Mohamoud, M. Molina, M. Monod, I. Mouyna, S. Mulligan, L. Murphy, S. O'Neil, I. Paulsen, M. A. Penalva, M. Pertea, C. Price, B. L. Pritchard, M. A. Quail, E. Rabbinowitsch, N. Rawlins, M. A. Rajandream, U. Reichard, H. Renauld, G. D. Robson, S. Rodriguez de Cordoba, J. M. Rodriguez-Pena, C. M. Ronning, S. Rutter, S. L. Salzberg, M. Sanchez, J. C. Sanchez-Ferrero, D. Saunders, K. Seeger, R. Squares, S. Squares, M. Takeuchi, F. Tekaia, G. Turner, C. R. Vazquez de Aldana, J. Weidman, O. White, J. Woodward, J. H. Yu, C. Fraser, J. E. Galagan, K. Asai, M. Machida, N. Hall, B. Barrell, and D. W. Denning.** 2005. Genomic sequence of the pathogenic and allergenic filamentous fungus Aspergillus fumigatus. Nature. **438:**1151-1156. doi: 10.1038/nature04332.

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., J. H. de Winde, D. B. Archer, P. S. Dyer, G. Hofmann, P. J. Schaap, G. Turner, R. P. de Vries, R. Albang, K. Albermann, M. R. Andersen, J. D. Bendtsen, J. A. Benen, M. van den Berg, S. Breestraat, M. X. Caddick, R. Contreras, M.**

Cornell, P. M. Coutinho, E. G. Danchin, A. J. Debets, P. Dekker, P. W. van Dijck, A. van Dijk, L. Dijkhuizen, A. J. Driessen, C. d'Enfert, S. Geysens, C. Goosen, G. S. Groot, P. W. de Groot, T. Guillemette, B. Henrissat, M. Herweijer, J. P. van den Hombergh, C. A. van den Hondel, R. T. van der Heijden, R. M. van der Kaaij, F. M. Klis, H. J. Kools, C. P. Kubicek, P. A. van Kuyk, J. Lauber, X. Lu, M. J. van der Maarel, R. Meulenberg, H. Menke, M. A. Mortimer, J. Nielsen, S. G. Oliver, M. Olsthoorn, K. Pal, N. N. van Peij, A. F. Ram, U. Rinas, J. A. Roubos, C. M. Sagt, M. Schmoll, J. Sun, D. Ussery, J. Varga, W. Vervecken, P. J. van de Vondervoort, H. Wedler, H. A. Wosten, A. P. Zeng, A. J. van Ooyen, J. Visser, and H. Stam. 2007. Genome sequencing and analysis of the versatile cell factory Aspergillus niger CBS 513.88. Nat. Biotechnol. **25:**221-231. doi: 10.1038/nbt1282.

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:**W686-9. 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:**W309-12. doi: 10.1093/nar/gkh379.

12. **Yamada, O., R. Takara, R. Hamada, R. Hayashi, M. Tsukahara, and S. Mikami.** 2011. Molecular biological researches of Kuro-Koji molds, their classification and safety. J. Biosci. Bioeng. . doi: 10.1016/j.jbiosc.2011.05.005.