

Genome Sequence of *Schizochytrium* sp. CCTCC M209059, an Effective Producer of Docosahexaenoic Acid-Rich Lipids

Xiao-Jun Ji,^a Kai-Qiang Mo,^a Lu-Jing Ren,^a Gan-Lu Li,^a Jian-Zhong Huang,^b He Huang^a

State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing, Jiangsu, People's Republic of China^a; Engineering Research Center of Industrial Microbiology, Ministry of Education, College of Life Sciences, Fujian Normal University, Fuzhou, Fujian, People's Republic of China^b

X.-J.J. and K.-Q.M. contributed equally to this work.

***Schizochytrium* is an effective species for producing omega-3 docosahexaenoic acid (DHA). Here, we report a genome sequence of *Schizochytrium* sp. CCTCC M209059, which has a genome size of 39.09 Mb. It will provide the genomic basis for further insights into the metabolic and regulatory mechanisms underlying the DHA formation.**

Received 16 June 2015 Accepted 22 June 2015 Published 6 August 2015

Citation Ji X-J, Mo K-Q, Ren L-J, Li G-L, Huang J-Z, Huang H. 2015. Genome sequence of *Schizochytrium* sp. CCTCC M209059, an effective producer of docosahexaenoic acid-rich lipids. *Genome Announc* 3(4):e00819-15. doi:10.1128/genomeA.00819-15.

Copyright © 2015 Ji et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to He Huang, biotech@njtech.edu.cn.

Docosahexaenoic acid (DHA), one of the most typical omega-3 polyunsaturated fatty acids, has received worldwide attention due to its beneficial physiological functions for humans (1, 2). Its traditional sources are coldwater fish oils. However, the product quality derived from fish oil is dependent on season and position and is affected by ocean pollution. Alternatively, DHA can be obtained from marine fungal oils (lipids).

Schizochytrium sp., which was first derived from coastal seawater as a new thraustochytrid by Nakahara et al. (3), has been widely used to produce DHA. It can accumulate up to 50% of its dry weight as lipids, DHA generally constituting 40% or more of these (4). However, the physiological role of DHA to *Schizochytrium* sp. cells has not been elucidated clearly. DHA, in the form of membrane phospholipids, is generally known to play an important role in membrane functions (5). However, only up to 5% of lipids in *Schizochytrium* sp. occur as phospholipids, whereas 90% or more are generally present as triacylglycerols in storage lipids (6–8). Why do *Schizochytrium* sp. cells accumulate more DHA as triacylglycerols instead of as phospholipids? The reason for this is not clear. Furthermore, in *Schizochytrium* spp., the metabolic pathway for DHA biosynthesis (via polyketide synthases) is different from the pathway in other DHA-producing strains (via fatty acid synthases), such as *Thraustochytrium* spp., *Cryptocodinium cohnii*, and so on (9–11).

We therefore sequenced and analyzed the genome of *Schizochytrium* sp. CCTCC M209059, which is an efficient DHA-producing strain stored in the China Center for Type Culture Collection (12, 13), to reveal the metabolic and regulatory mechanisms underlying the DHA formation, both in relation to its physiological functions and its distinctive metabolic pathways.

The genomic DNA of the strain was isolated using the EZNA fungal DNA kit (Omega Bio-tek, Doraville, GA, USA). The purity and concentration of the DNA were measured by a Qubit Fluorometer (Invitrogen, Carlsbad, CA, USA). Genome sequencing was conducted using the Illumina HiSeq2000 DNA sequencing platform at BGI-Shenzhen (Shenzhen, China). The raw sequence

data comprise a total of 61,950,544 reads, assembled into 1,608 contigs through the SOAPdenovo alignment tool (14, 15). The genome assembly is 38,297,968 bp, with an N_{50} equal to 52,007 bp, an N_{90} of 14,718 bp, and a maximum contig size of 236,430 bp. The contigs were further scaffolded with a total of 39,089,698 bp, which were placed in the final 322 scaffolds, with an N_{50} of 595,797 bp, an N_{90} of 144,465 bp, and a maximum scaffold size of 1,674,554 bp. This resulted in a predicted genome size of 39.09 Mb with a G+C content of 56.6%. There are a total of 9,142 predicted protein-coding genes, 178 tRNA genes, and 31 rRNA genes in the genome. Annotation of the genome was performed via Gene Ontology Analysis (16) and the Kyoto Encyclopedia of Genes and Genomes pathway database (17). The genome sequence could provide opportunities for investigating the metabolic and regulatory mechanisms underlying the formation of DHA, which will provide guidance for efficient DHA production with high content and high productivity.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [JTFK00000000](https://www.ncbi.nlm.nih.gov/nuclink/JTFK00000000). The version described in this paper is the first version, [JTFK01000000](https://www.ncbi.nlm.nih.gov/nuclink/JTFK01000000).

ACKNOWLEDGMENTS

This work was financially supported by the National Science Foundation for Distinguished Young Scholars of China (grant 21225626), the National Natural Science Foundation of China (grants 21306085, 21376002, and 21476111), the Jiangsu Provincial Natural Science Foundation (grants BK2012424 and BK20131405), the National High-Tech R&D Program of China (grants 2012AA021704 and 2014AA021701), and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

REFERENCES

1. Gupta A, Barrow CJ, Puri M. 2012. Omega-3 biotechnology: thraustochytrids as a novel source of omega-3 oils. *Biotechnol Adv* 30:1733–1745. <http://dx.doi.org/10.1016/j.biotechadv.2012.02.014>.

2. Horrocks LA, Yeo YK. 1999. Health benefits of docosahexaenoic acid (DHA). *Pharmacol Res* 40:211–225. <http://dx.doi.org/10.1006/phrs.1999.0495>.
3. Nakahara T, Yokochi T, Higashihara T, Tanaka S, Yaguchi T, Honda D. 1996. Production of docosahexaenoic and docosapentaenoic acids by *Schizochytrium* sp. isolated from Yap Islands. *J Am Oil Chem Soc* 11: 1421–1426.
4. Singh A, Ward OP. 1997. Microbial production of docosahexaenoic acid (DHA, C22:6). *Adv Appl Microbiol* 45:271–312. [http://dx.doi.org/10.1016/S0065-2164\(08\)70266-1](http://dx.doi.org/10.1016/S0065-2164(08)70266-1).
5. Stillwell W, Wassall SR. 2003. Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chem Phys Lipids* 126:1–27. [http://dx.doi.org/10.1016/S0009-3084\(03\)00101-4](http://dx.doi.org/10.1016/S0009-3084(03)00101-4).
6. Ashford A, Barclay WR, Weaver CA, Giddings TH, Zeller S. 2000. Electron microscopy may reveal structure of docosahexaenoic acid-rich oil within *Schizochytrium* sp. *Lipids* 35:1377–1386. <http://dx.doi.org/10.1007/s11745-000-0655-2>.
7. Ren LJ, Sun GN, Ji XJ, Hu XC, Huang H. 2014. Compositional shift in lipid fractions during lipid accumulation and turnover in *Schizochytrium* sp. *Bioresour Technol* 157:107–113. <http://dx.doi.org/10.1016/j.biortech.2014.01.078>.
8. Sun LN, Ren LJ, Zhuang XY, Ji XJ, Yan JC, Huang H. 2014. Differential effects of nutrient limitations on biochemical constituents and docosahexaenoic acid production of *Schizochytrium* sp. *Bioresour Technol* 159: 199–206. <http://dx.doi.org/10.1016/j.biortech.2014.02.106>.
9. Ji XJ, Ren LJ, Nie ZK, Huang H, Ouyang PK. 2014. Fungal arachidonic acid-rich oil: research, development and industrialization. *Crit Rev Biotechnol* 34:197–214. <http://dx.doi.org/10.3109/07388551.2013.778229>.
10. Metz JG, Kuner J, Rosenzweig B, Lippmeier JC, Roessler P, Zirkle R. 2009. Biochemical characterization of polyunsaturated fatty acid synthesis in *Schizochytrium*: release of the products as free fatty acids. *Plant Phy Biol Chem* 47:472–478. <http://dx.doi.org/10.1016/j.plaphy.2009.02.002>.
11. Metz JG, Roessler P, Facciotti D, Levering C, Dittrich F, Lassner M, Valentine R, Lardizabal K, Domergue F, Yamada A, Yazawa K, Knauf V, Browne J. 2001. Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes. *Science* 293: 290–295. <http://dx.doi.org/10.1126/science.1059593>.
12. Huang H, Ren LJ, Xiao AH, Jin LJ, Li J. 2009. A kind of *Schizochytrium* sp. and the method for DHA-rich oil production using thereof. Chinese patent ZL 200910033869.5.
13. Ren LJ, Ji XJ, Huang H, Qu L, Feng Y, Tong QQ, Ouyang PK. 2010. Development of a stepwise aeration control strategy for efficient docosahexaenoic acid production by *Schizochytrium* sp. *Appl Microbiol Biotechnol* 87:1649–1656. <http://dx.doi.org/10.1007/s00253-010-2639-7>.
14. Li R, Li Y, Kristiansen K, Wang J. 2008. SOAP: short oligonucleotide alignment program. *Bioinformatics* 24:713–714. <http://dx.doi.org/10.1093/bioinformatics/btn025>.
15. Li R, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, Shan G, Kristiansen K, Li S, Yang H, Wang J, Wang J. 2010. *De novo* assembly of human genomes with massively parallel short read sequencing. *Genome Res* 20:265–272. <http://dx.doi.org/10.1101/gr.097261.109>.
16. Tatusov RL, Galperin MY, Natale DA, Koonin EV. 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res* 28:33–36. <http://dx.doi.org/10.1093/nar/28.1.33>.
17. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. 1999. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 27: 29–34. <http://dx.doi.org/10.1093/nar/27.1.29>.