



Whole-Genome Sequence of the Fruiting Myxobacterium *Cystobacter fuscus* DSM 52655

Anke Treuner-Lange,^a Marc Bruckkotten,^a Oliver Rupp,^b Alexander Goesmann,^b
Lotte Søgaard-Andersen^a

Department of Ecophysiology, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany^a;
Bioinformatics and Systems Biology, Justus-Liebig University Giessen, Giessen, Germany^b

ABSTRACT Among myxobacteria, the genus *Cystobacter* is known not only for fruiting body formation but also for formation of secondary metabolites, such as cystobactamids and cystothiazols. Here, we present the complete genome sequence of the *Cystobacter fuscus* strain DSM 52655, which comprises 12,349,744 bp and 9,836 putative protein-coding sequences.

Members of the *Myxococcales* order provide a rich source of secondary metabolites with antibacterial and cytotoxic activities (1). Many *Myxococcales* species also have the capacity to generate spore-filled fruiting bodies in response to starvation (2–4). Interestingly, these fruiting bodies have very different morphologies (2). For instance, the model organism *Myxococcus xanthus* forms haystack-shaped cell aggregates (2). In contrast, the fruiting bodies of *Cystobacter fuscus* are shiny spherical sporangioles often clustered in groups within a gelatinous slime matrix (2). Little is known about the genetic basis underlying these morphological differences; however, comparative genome investigations of up to 10 different *Myxococcales* genomes have indicated that the developmental program leading to fruiting body formation and sporulation may not be highly conserved (5–7).

So far, 20 complete genomes and 36 draft genomes from members of the *Myxococcales* order are available (5, 8–32). To generate additional resources for accurate genome comparisons, we sequenced and annotated the complete genome of *Cystobacter fuscus* DSM 52655, which was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

After confirming the formation of brown sporangioles clustered within a light brownish slime capsule by *C. fuscus* DSM 52655, we collected genomic DNA (33) and sequenced it using PacBio single-molecule real-time (SMRT) sequencing (34) on the PacBio RSII platform at the Max Planck Genome Centre, Cologne, Germany. Six SMRT-cells were used. Additionally, 12,193,527 100-bp paired-end Illumina reads were obtained using the HiSeq2000 platform. After quality evaluation and filtering of 188,247 PacBio subreads, the assembly process using the HGAP assembly pipeline (35) resulted in one contig with 62-fold coverage. The final contig was inspected manually using the Gepard dotplot generator (36) and YASS (37), manually closed using Illumina reads and the Pilon tool (38), and oriented to DnaA as the first locus tag. Prokka (39) was used to generate the annotation. BLASTp searches against the RefSeq database were used to assign functional annotations and to identify frameshifts. The corresponding genes were removed from the annotation.

The complete genome sequence of *C. fuscus* DSM 52655 contains 12,349,744 bp with a GC content of 68.5%. A total of 9,836 protein-coding sequences (CDSs) were identified together with 67 tRNA genes and 12 rRNA operons. The *C. fuscus* genome is similar in size to the incomplete genomes of *C. fuscus* DSM 2262 (GCA_000335475.2)

Received 24 September 2017 Accepted 26 September 2017 Published 26 October 2017

Citation Treuner-Lange A, Bruckkotten M, Rupp O, Goesmann A, Søgaard-Andersen L. 2017. Whole-genome sequence of the fruiting myxobacterium *Cystobacter fuscus* DSM 52655. *Genome Announc* 5:e01196-17. <https://doi.org/10.1128/genomeA.01196-17>.

Copyright © 2017 Treuner-Lange et al. This is an open-access article distributed under the terms of the *Creative Commons Attribution 4.0 International license*.

Address correspondence to Lotte Søgaard-Andersen, sogaard@mpi-marburg.mpg.de. A.T.-L. and M.B. contributed equally to this work.

and *C. ferrugineus* Cbfe23 (GCA_001887355.1) with sizes of 12.3 Mb and 12.1 Mb, respectively. Aligning the *C. fuscus* genome to other *Myxococcales* genomes using NUCmer (40) revealed overall synteny to the *C. fuscus* DSM 2262 and *C. ferrugineus* Cbfe23 genomes, with 79.5% and 75.6%, respectively, of the sequences aligning. The next best match found was to *Archangium gephyra* DSM 2261 with ~36% of the *C. fuscus* DSM 52655 genome aligning.

The *C. fuscus* DSM 52655 genome sequence provides a resource for the identification of gene clusters encoding enzymes involved in secondary metabolite synthesis and will ultimately help to understand the genetic basis underlying differences in fruiting body morphology.

Accession number(s). This genome sequence was deposited in GenBank under accession number [CP022098](#).

ACKNOWLEDGMENTS

The Max Planck Society supported this work.

Bioinformatics support by the BMBF-funded project “Bielefeld-Gießen Center for Microbial Bioinformatics—BiGi” (grant 031A533) within the German Network for Bioinformatics Infrastructure (de.NBI) is gratefully acknowledged.

REFERENCES

- Landwehr W, Wolf C, Wink J. 2016. Actinobacteria and myxobacteria—two of the most important bacterial resources for novel antibiotics. *Curr Top Microbiol Immunol* 398:273–302. https://doi.org/10.1007/82_2016_503.
- Reichenbach H. 2005. Order VIII. *Myxococcales*. Tchan, Pocheon and Prévot 1948, vol 398AL, p 1059–1144. In Brenner DJ, Krieg NR, Staley JT (ed), Bergey's manual of systematic bacteriology, vol 2. Springer-Verlag, New York, NY.
- Dawid W. 2000. Biology and global distribution of myxobacteria in soils. *FEMS Microbiol Rev* 24:403–427. <https://doi.org/10.1111/j.1574-6976.2000.tb00548.x>.
- Reichenbach H. 1999. The ecology of the myxobacteria. *Environ Microbiol* 1:15–21. <https://doi.org/10.1046/j.1462-2920.1999.00016.x>.
- Huntley S, Hamann N, Wegener-Feldbrügge S, Treuner-Lange A, Kube M, Reinhardt R, Klages S, Müller R, Ronning CM, Nierman WC, Søgaard-Andersen L. 2011. Comparative genomic analysis of fruiting body formation in *Myxococcales*. *Mol Biol Evol* 28:1083–1097. <https://doi.org/10.1093/molbev/msq292>.
- Huntley S, Wuichet K, Søgaard-Andersen L. 2014. Genome evolution and content in the myxobacteria, p 31–50. In Yang Z, Higgs P (ed), Myxobacteria—genomics, cellular and molecular biology. Caister Academic Press, Norfolk, UK.
- Arias Del Angel JA, Escalante AE, Martínez-Castilla LP, Benítez M. 2017. An Evo-Devo perspective on multicellular development of myxobacteria. *J Exp Zool B Mol Dev Evol* 328:165–178. <https://doi.org/10.1002/jezb.22727>.
- Hwang C, Copeland A, Lucas S, Lapidus A, Barry K, Glavina Del Rio T, Dalin E, Tice H, Pitluck S, Sims D, Bretton T, Bruce DC, Detter JC, Han CS, Schmutz J, Larimer FW, Land ML, Hauser LJ, Kyrpides N, Lykidis A, Richardson P, Belieau A, Sanford RA, Löeffler FE, Fields MW. 2015. Complete genome sequence of *Anaeromyxobacter* sp. Fw109-5, an anaerobic, metal-reducing bacterium isolated from a contaminated subsurface environment. *Genome Announc* 3(1):e01449-14. <https://doi.org/10.1128/genomeA.01449-14>.
- Goldman BS, Nierman WC, Kaiser D, Slater SC, Durkin AS, Eisen JA, Eisen J, Ronning CM, Barbazuk WB, Blanchard M, Field C, Halling C, Hinkle G, Iartchuk O, Kim HS, Mackenzie C, Madupu R, Miller N, Shvartsbeyn A, Sullivan SA, Vaudin M, Wiegand R, Kaplan HB. 2006. Evolution of sensory complexity recorded in a myxobacterial genome. *Proc Natl Acad Sci U S A* 103:15200–15205. <https://doi.org/10.1073/pnas.0607335103>.
- Schneiker S, Perlova O, Kaiser O, Gerth K, Alici A, Altmeier MO, Bartels D, Bekel T, Beyer B, Bode E, Bode HB, Bolten CJ, Choudhuri JV, Doss S, Elnakady YA, Frank B, Gaigalat L, Goemann A, Groeger C, Gross F, Jelsbak L, Jelsbak L, Kalinowski J, Kegler C, Knauber T, Konietzny S, Kopp M, Krause L, Krug D, Linke B, Mahmud T, Martinez-Arias R, McHardy AC, Merai M, Meyer F, Mormann S, Muñoz-Dorado J, Perez J, Pradella S, Rachid S, Raddatz G, Rosenau F, Ruckert C, Sasse F, Scharfe M, Schuster SC, Suen G, Treuner-Lange A, Velicer GJ, Vorholter FJ, Weissman KJ, Welch RD, Wenzel SC, Whitworth DE, Wilhelm S, Wittmann C, Blocker H, Puhler A, Müller R. 2007. Complete genome sequence of the myxobacterium *Sorangium cellulosum*. *Nat Biotechnol* 25:1281–1289. <https://doi.org/10.1038/nbt1354>.
- Han K, Li ZF, Peng R, Zhu LP, Zhou T, Wang LG, Li SG, Zhang XB, Hu W, Wu ZH, Qin N, Li YZ. 2013. Extraordinary expansion of a *Sorangium cellulosum* genome from an alkaline milieu. *Sci Rep* 3:2101. <https://doi.org/10.1038/srep02101>.
- Li ZF, Li X, Liu H, Liu X, Han K, Wu ZH, Hu W, Li FF, Li YZ. 2011. Genome sequence of the halotolerant marine bacterium *Myxococcus fulvus* HW-1. *J Bacteriol* 193:5015–5016. <https://doi.org/10.1128/JB.05516-11>.
- Sanford RA, Cole JR, Tiedje JM. 2002. Characterization and description of *Anaeromyxobacter dehalogenans* gen. nov., sp. nov., an aryl-halo-respiring facultative anaerobic myxobacterium. *Appl Environ Microbiol* 68:893–900. <https://doi.org/10.1128/AEM.68.2.893-900.2002>.
- Huntley S, Zhang Y, Treuner-Lange A, Kneip S, Sensen CW, Søgaard-Andersen L. 2012. Complete genome sequence of the fruiting myxobacterium *Coralloccoccus coralloides* DSM 2259. *J Bacteriol* 194:3012–3013. <https://doi.org/10.1128/JB.00397-12>.
- Ivanova N, Daum C, Lang E, Abt B, Kopitz M, Saunders E, Lapidus A, Lucas S, Glavina Del Rio T, Nolan M, Tice H, Copeland A, Cheng JF, Chen F, Bruce D, Goodwin L, Pitluck S, Mavromatis K, Pati A, Mikhailova N, Chen A, Palaniappan K, Land M, Hauser L, Chang YJ, Jeffries CD, Detter JC, Bretton T, Rohde M, Göker M, Bristow J, Markowitz V, Eisen JA, Hugenholtz P, Kyrpides NC, Klenk HP. 2010. Complete genome sequence of *Haliangium ochraceum* type strain (SMP-2). *Stand Genomic Sci* 2:96–106. <https://doi.org/10.4056/sigs.69.1277>.
- Huntley S, Kneip S, Treuner-Lange A, Søgaard-Andersen L. 2013. Complete genome sequence of *Myxococcus stipitatus* strain DSM 14675, a fruiting myxobacterium. *Genome Announc* 1(1):e00100-13. <https://doi.org/10.1128/genomeA.00100-13>.
- Thomas SH, Wagner RD, Arakaki AK, Skolnick J, Kirby JR, Shimkets LJ, Sanford RA, Löffler FE. 2008. The mosaic genome of *Anaeromyxobacter dehalogenans* strain 2CP-C suggests an aerobic common ancestor to the delta-proteobacteria. *PLoS One* 3:e2103. <https://doi.org/10.1371/journal.pone.0002103>.
- Chen XJ, Han K, Feng J, Zhuo L, Li YJ, Li YZ. 2016. The complete genome sequence and analysis of a plasmid-bearing myxobacterial strain *Myxococcus fulvus* 124B02 (M 206081). *Stand Genomic Sci* 11:1. <https://doi.org/10.1166/s40793-015-0121-y>.
- Garcia R, Gemperlein K, Müller R. 2014. *Minicystis rosea* gen. nov., sp. nov., a polyunsaturated fatty acid-rich and steroid-producing soil myxobacterium. *Int J Syst Evol Microbiol* 64:3733–3742. <https://doi.org/10.1099/ijss.0.068270-0>.

20. Yamamoto E, Muramatsu H, Nagai K. 2014. *Vulgatibacter incomptus* gen. nov., sp. nov. and *Labilithrix luteola* gen. nov., sp. nov., two myxobacteria isolated from soil in Yakushima Island, and the description of *Vulgatibacteraceae* fam. nov., *Labilitrichaceae* fam. nov. and *Anaeromyxobacteraceae* fam. nov. *Int J Syst Evol Microbiol* 64:3360–3368. <https://doi.org/10.1099/ijss.0.063198-0>.
21. Sharma G, Subramanian S. 2017. Unravelling the complete genome of *Archangium gephyra* DSM 2261T and evolutionary insights into myxobacterial chitinases. *Genome Biol Evol* 9:1304–1311. <https://doi.org/10.1093/gbe/evw066>.
22. Sharma G, Khatri I, Subramanian S. 2016. Complete genome of the starch-degrading myxobacteria *Sandaracinus amylolyticus* DSM 53668T. *Genome Biol Evol* 8:2520–2529. <https://doi.org/10.1093/gbe/evw151>.
23. Sharma G, Narwani T, Subramanian S. 2016. Complete genome sequence and comparative genomics of a novel myxobacterium *Myxococcus hanspus*. *PLoS One* 11:e0148593. <https://doi.org/10.1371/journal.pone.0148593>.
24. Zaburannyi N, Bunk B, Maier J, Overmann J, Müller R. 2016. Genome analysis of the fruiting body-forming myxobacterium *Chondromyces crocatus* reveals high potential for natural product biosynthesis. *Appl Environ Microbiol* 82:1945–1957. <https://doi.org/10.1128/AEM.03011-15>.
25. Tonomura M, Ehara A, Suzuki H, Amachi S. 2015. Draft genome sequence of *Anaeromyxobacter* sp. strain PSR-1, an arsenate-respiring bacterium isolated from arsenic-contaminated soil. *Genome Announc* 3(3):e00472-15. <https://doi.org/10.1128/genomeA.00472-15>.
26. Müller S, Willett JW, Bahr SM, Darnell CL, Hummels KR, Dong CK, Vlamakis HC, Kirby JR. 2013. Draft genome sequence of *Myxococcus xanthus* wild-type strain DZ2, a model organism for predation and development. *Genome Announc* 1(3):e00217-13. <https://doi.org/10.1128/genomeA.00217-13>.
27. Müller S, Willett JW, Bahr SM, Scott JC, Wilson JM, Darnell CL, Vlamakis HC, Kirby JR. 2013. Draft genome of a type 4 pilus defective *Myxococcus xanthus* strain, DZF1. *Genome Announc* 1(3):e00392-13. <https://doi.org/10.1128/genomeA.00392-13>.
28. Stevens DC, Young J, Carmichael R, Tan J, Taylor RE. 2014. Draft genome sequence of gephyronic acid producer *Cystobacter violaceus* strain Cb vi76. *Genome Announc* 2(6):e01299-14. <https://doi.org/10.1128/genomeA.01299-14>.
29. Tatusova T, Ciufo S, Fedorov B, O'Neill K, Tolstoy I. 2014. RefSeq microbial genomes database: new representation and annotation strategy. *Nucleic Acids Res* 42:D553–D559. <https://doi.org/10.1093/nar/gkt1274>.
30. Kudo K, Yamaguchi N, Makino T, Ohtsuka T, Kimura K, Dong DT, Amachi S. 2013. Release of arsenic from soil by a novel dissimilatory arsenate-reducing bacterium, *Anaeromyxobacter* sp. strain PSR-1. *Appl Environ Microbiol* 79:4635–4642. <https://doi.org/10.1128/AEM.00693-13>.
31. O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, Astashyn A, Badretdin A, Bao Y, Blinkova O, Brover V, Chetvernin V, Choi J, Cox E, Ermolaeva O, Farrell CM, Goldfarb T, Gupta T, Haft D, Hatcher E, Hlavina W, Joardar VS, Kodali VK, Li W, Maglott D, Masterson P, McGarvey KM, Murphy MR, O'Neill K, Pujar S, Rangwala SH, Rausch D, Riddick LD, Schoch C, Shkeda A, Storz SS, Sun H, Thibaud-Nissen F, Tolstoy I, Tully RE, Vatsan AR, Wallin C, Webb D, Wu W, Landrum MJ, Kimchi A, Tatusova T, DiCuccio M, Kitts P, Murphy TD, Pruitt KD. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res* 44:D733–D745. <https://doi.org/10.1093/nar/gkv1189>.
32. Adaikpoh BI, Dowd SE, Stevens DC. 2017. Draft genome sequence of *Archangium* sp. strain Cb G35. *Genome Announc* 5(8):e01678-16. <https://doi.org/10.1128/genomeA.01678-16>.
33. Wilson K. 2001. Preparation of genomic DNA from bacteria. *Curr Protoc Mol Biol Chapter 2:Unit 2.4.* https://doi.org/10.1002/0471142727_mb0204s56.
34. Au KF, Underwood JG, Lee L, Wong WH. 2012. Improving PacBio long read accuracy by short read alignment. *PLoS One* 7:e46679. <https://doi.org/10.1371/journal.pone.0046679>.
35. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Non-hybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
36. Krumsiek J, Arnold R, Rattei T. 2007. Gepard: a rapid and sensitive tool for creating dotplots on genome scale. *Bioinformatics* 23:1026–1028. <https://doi.org/10.1093/bioinformatics/btm039>.
37. Noé L, Kucherov G. 2005. YASS: enhancing the sensitivity of DNA similarity search. *Nucleic Acids Res* 33:W540–W543. <https://doi.org/10.1093/nar/gki478>.
38. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
39. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
40. Kurtz S, Philipp A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. *Genome Biol* 5:R12. <https://doi.org/10.1186/gb-2004-5-2-r12>.