



Complete Genome Sequence of the Fruiting Myxobacterium *Myxococcus macrosporus* Strain DSM 14697, Generated by PacBio Sequencing

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ABSTRACT Members of the *Myxococcales* order initiate a developmental program in response to starvation that culminates in formation of spore-filled fruiting bodies. To investigate the genetic basis for fruiting body formation, we present the complete 8.9-Mb genome sequence of *Myxococcus macrosporus* strain DSM 14697, generated using the PacBio sequencing platform.

Most members of the *Myxococcales* order initiate a developmental program in response to starvation that results in the formation of a multicellular fruiting body inside which cells differentiate to spores (1, 2). Analyses using *Myxococcus xanthus* as a model organism have provided important insights into regulation of fruiting body formation (3, 4). However, comparative genome investigations of different *Myxococcales* genome sequences have indicated that the developmental program that results in fruiting body formation is not highly conserved (5–7).

Only 20 genomes of the *Myxococcales* have been completely sequenced (5, 8–24). In addition, 36 *Myxococcales* draft genomes are available (25–32). To generate additional resources for accurate genomic comparisons and eventually decipher and compare the genetic programs for fruiting body formation, we sequenced and annotated the complete genome of *Myxococcus macrosporus* strain DSM 14697, which was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

After verification of the formation of haystack-shaped fruiting bodies by *M. macrosporus* DSM 14697, 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. Two SMRT cells were used. After quality evaluation and filtering of 184,213 subreads, the assembly process using the HGAP assembly pipeline (35) resulted in one contig with 83-fold coverage, which allowed a manual closure of the contig. The genome was verified for completion and oriented with DnaA as the first locus tag. Genome annotation was done using Prokka (36). BLASTP searches against the RefSeq database were used to assign functional annotation and identify possible frameshifts in genes. The corresponding genes were removed from the annotation.

The complete genome sequence of *M. macrosporus* DSM 14697 contains 8,973,512 bp with a GC content of 70.6%. A total of 7,143 protein-coding sequences (CDSs) were identified together with 79 tRNA genes and 12 rRNA operons. The size of the *M. macrosporus* genome is similar to those of other sequenced genomes of fruiting myxobacteria, which range in size from 9.0 Mb to 16.0 Mb. Aligning the *M. macrosporus* genome with other completely sequenced *Myxococcales* genomes by using NUCmer (37) revealed overall synteny, particularly to other *Myxococcus* species in the following

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order (% alignment): *Myxococcus fulvus* HW-1 (93.5), *M. xanthus* DK 1622 (80.6), *M. hantschii* (73.4), *M. fulvus* 124B02 (43.9), and *M. stipitatus* DSM_14675 (38.3). The best matches outside the genus *Myxococcus* are to *Coralloccoccus coralloides* DSM_2259 (31.4) and *Archangium gephyra* DSM_2261 (20.3).

The *M. macrosporus* genome sequence will contribute to the investigation of the genetic programs leading to fruiting body formation.

Accession number(s). The genome sequence was deposited in GenBank under accession number CP022203.

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REFERENCES

- 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>.
- Konovalova A, Petters T, Søgaard-Andersen L. 2010. Extracellular biology of *Myxococcus xanthus*. *FEMS Microbiol Rev* 34:89–106. <https://doi.org/10.1111/j.1574-6976.2009.00194.x>.
- Kroos L. 2017. Highly signal-responsive gene regulatory network governing *Myxococcus* development. *Trends Genet* 33:3–15. <https://doi.org/10.1016/j.tig.2016.10.006>.
- 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>.
- 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/jez.b.22727>.
- 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, United Kingdom.
- Hwang C, Copeland A, Lucas S, Lapidus A, Barry K, Glavina Del Rio T, Dalin E, Tice H, Pitluck S, Sims D, Brettin T, Bruce DC, Detter JC, Han CS, Schmutz J, Larimer FW, Land ML, Hauser LJ, Kyrpidis N, Lykidis A, Richardson P, Belieav A, Sanford RA, Loeffler 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, Altmeyer MO, Bartels D, Bekel T, Beyer S, Bode E, Bode HB, Bolten CJ, Choudhuri JV, Doss S, Elnakady YA, Frank B, Gaigalat L, Goesmann 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, Martínez-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. 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.0516-11>.
- Sanford RA, Cole JR, Tiedje JM. 2002. Characterization and description of *Anaeromyxobacter dehalogenans* gen. nov., sp. nov., an aryl-halorespiring 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, Brettin T, Rohde M, Göker M, Bristow J, Markowitz V, Eisen JA, Hugenholtz P, Kyrpidis 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(2):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/10.1186/s40793-015-0121-y>.
- García 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/ijs.0.068270-0>.
- 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., *Labilithricaceae* fam. nov. and *Anaeromyxobacteraceae* fam. nov. *Int J Syst Evol Microbiol* 64:3360–3368. <https://doi.org/10.1099/ijs.0.063198-0>.
- 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/evx066>.
- 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>.
- Sharma G, Narwani T, Subramanian S. 2016. Complete genome se-

- quence and comparative genomics of a novel myxobacterium *Myxococcus hansupus*. PLoS One 11:e0148593. <https://doi.org/10.1371/journal.pone.0148593>.
24. Zaburanyi 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. 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>.
 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. 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>.
 29. 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>.
 30. Tatusova T, Ciufu 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>.
 31. O'Leary NA, Wright MW, Brister JR, Ciufu 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. 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):e01678-18. <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. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
 37. Kurtz S, Phillippy 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>.