

Genome Sequences of Eight Morphologically Diverse Alphaproteobacteria[∇]

Pamela J. B. Brown,¹ David T. Kysela,¹ Aaron Buechlein,² Chris Hemmerich,² and Yves V. Brun^{1,2*}

Department of Biology¹ and Center for Genomics and Bioinformatics,² Indiana University, Bloomington, Indiana 47405

Received 3 June 2011/Accepted 15 June 2011

The Alphaproteobacteria comprise morphologically diverse bacteria, including many species of stalked bacteria. Here we announce the genome sequences of eight alphaproteobacteria, including the first genome sequences of species belonging to the genera *Asticcacaulis*, *Hirschia*, *Hyphomicrobium*, and *Rhodomicrobium*.

Prosthecae or stalks are found in a morphologically diverse group of Gram-negative bacteria belonging primarily to the class *Alphaproteobacteria*. The stalked *Alphaproteobacteria* typically have a characteristic dimorphic life cycle in which two dissimilar cell types are produced by asymmetric cell division (2, 4, 8–10, 17, 18). The swarmer progeny cells are motile and do not replicate DNA. The start of the new cell division cycle is coincident with the differentiation of the swarmer cell into a stalked cell. Stalks are true extensions of the cell body and are an integral part of the cell, bounded by both the cell membranes and the cell wall, and form a thin cylindrical extension of the cell surface layer (10). Stalks are essential for the reproduction of a subset of prosthecate bacteria, including *Rhodomicrobium* and *Hyphomicrobium*, which divide by budding,

in which the daughter cell develops at and is later released from the tip of the stalk. The stalks of both budding and nonbudding prosthecate bacteria have been implicated in nutrient uptake and are likely to be particularly advantageous in oligotrophic habitats (5, 14–16, 18).

To facilitate an enhanced understanding of the function of stalks, the mechanism of budding, and regulation of dimorphic life cycles, the genomes of three nonbudding stalked bacteria, three budding stalked bacteria, and two closely related nonstalked bacteria were sequenced. The nonbudding stalked bacteria include *Brevundimonas subvibrioides*, *Asticcacaulis biprosthecum*, and *Asticcacaulis excentricus*. The budding stalked bacteria include *Hirschia baltica*, *Rhodomicrobium vannielii*, and *Hyphomicrobium denitrificans*. Finally, the nonstalked,

TABLE 1. Characteristics of genomes sequenced in this study

Organism	Reference	Genome analysis				GenBank accession no.	
		Sequencing status ^a	Size (Mb)	No. of scaffolds	% GC		No. of CDS ^b
<i>Asticcacaulis biprosthecum</i> C19	9	PD	5.30	6	60	4,712	NZ_ADUH00000000
<i>Asticcacaulis excentricus</i> CB48	10	F	2.59		59	2,330	Chromosome 1, NC_014816
			1.32		60	1,121	Chromosome 2, NC_014817
			0.24	4	59	172	pASTEX01 NC_014818
			0.16		57	140	pASTEX02 NC_014819
<i>Brevundimonas diminuta</i> ATCC 11568	13	PD	3.24	8	65	3,002	NZ_ADUI00000000
<i>Brevundimonas subvibrioides</i> ATCC 15264	1	F	3.45	1	68	3,327	NC_014375
<i>Caulobacter segnis</i> ATCC 21756	1	F	4.66	1	67	4,139	NC_014100
<i>Hirschia baltica</i> ATCC 49814	12	F	3.46	2	45	3119	Chromosome, NC_012982
			0.08		43	68	pHba101 NC_012983
<i>Hyphomicrobium denitrificans</i> ATCC 51888	11	F	3.64	1	60	3,512	NC_014313
<i>Rhodomicrobium vannielii</i> ATCC 17100	18	F	4.01	1	62	3,565	NC_014664

^a F, finished; PD, permanent high-quality draft.

^b Number of annotated protein coding sequences (CDS).

* Corresponding author. Mailing address: Department of Biology, Indiana University, Bloomington, IN 47405. Phone: (812) 855-8860. Fax: (812) 855-6705. E-mail: ybrun@indiana.edu.

[∇] Published ahead of print on 24 June 2011.

nonbudding bacteria *Caulobacter segnis* and *Brevundimonas diminuta* were sequenced. Characteristics of the sequenced genomes are provided in Table 1.

Finished genomes were sequenced as part of the Department of Energy (DOE) Joint Genome Institute (JGI) Community Sequencing Program 2008 using a combination of Sanger, 454, and Illumina methods as described at the JGI website (http://www.jgi.doe.gov/sequencing/protocols/protos_production.html) and were annotated using the JGI-Oak Ridge National Laboratory annotation pipeline (7). Permanent draft genomes were sequenced by the Center for Genomics and Bioinformatics at Indiana University using standard 454 methods to obtain 17× coverage for *A. biprosthicum* and 58× coverage for *B. diminuta*. Permanent draft genomes were annotated using the Integrative Services for Genomic Analysis annotation pipeline (3). All genome annotations were loaded into the JGI Integrated Microbial Resource for analysis (6). Further analysis and comparisons of the genomes sequenced in this work are expected to provide insights into the generation of bacterial morphology, survival in oligotrophic environments, and the evolution of differing modes of bacterial cell growth.

Nucleotide sequence accession numbers. GenBank accession numbers for all of the chromosomes and plasmids sequenced in this study are shown in Table 1.

Y.V.B. was funded by the National Institutes of Health (GM051986 and GM077648) and by the National Science Foundation (MCB0731950). The sequencing of finished genomes was conducted as part of the DOE JGI 2008 Community Sequencing Project (787681) awarded to Y.V.B. The DOE JGI is supported by the Office of Science of the U.S. Department of Energy under contract DE-AC02-05CH11231. P.J.B.B. and D.T.K. were supported by National Institutes of Health National Research Service Awards AI072992 and GM083581. This work was supported in part by the Indiana Metabolomics and Cytomics Initiative of Indiana University, funded in part through a major grant from the Lilly Endowment, Inc.

REFERENCES

1. Abraham, W. R., et al. 1999. Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poin-dexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. *Int. J. Syst. Bacteriol.* **49**(Pt. 3): 1053–1073.
2. Curtis, P. D., and Y. V. Brun. 2010. Getting in the loop: regulation of development in *Caulobacter crescentus*. *Microbiol. Mol. Biol. Rev.* **74**:13–41.
3. Hemmerich, C., A. Buechlein, R. Podicheti, K. V. Revanna, and Q. Dong. 2010. An Ergatis-based prokaryotic genome annotation web server. *Bioinformatics* **26**:1122–1124.
4. Hirsch, P. 1974. Budding bacteria. *Annu. Rev. Microbiol.* **28**:391–444.
5. Larson, R. J., and J. L. Pate. 1976. Glucose transport in isolated prosthecae of *Asticcacaulis biprosthicum*. *J. Bacteriol.* **126**:282–293.
6. Markowitz, V. M., et al. 2010. The integrated microbial genomes system: an expanding comparative analysis resource. *Nucleic Acids Res.* **38**:D382–D390.
7. Mavromatis, K., et al. 2009. The DOE-JGI standard operating procedure for the annotations of microbial genomes. *Stand. Genomic Sci.* **1**:63–67.
8. Moore, R. L. 1981. The biology of *Hyphomicrobium* and other prosthecate, budding bacteria. *Annu. Rev. Microbiol.* **35**:567–594.
9. Pate, J. L., J. S. Porter, and T. L. Jordan. 1973. *Asticcacaulis biprosthicum* sp. nov. Life cycle, morphology and cultural characteristics. *Antonie Van Leeuwenhoek* **39**:569–583.
10. Poindexter, J. S. 1964. Biological properties and classification of the *Caulobacter* group. *Bacteriol. Rev.* **28**:231–295.
11. Rainey, F. A., N. Ward-Rainey, C. G. Gliesche, and E. Stackebrandt. 1998. Phylogenetic analysis and intrageneric structure of the genus *Hyphomicrobium* and the related genus *Filomicrobium*. *Int. J. Syst. Bacteriol.* **48**(Pt. 3):635–639.
12. Schlesner, H., C. Bartels, M. Sittig, M. Dorsch, and E. Stackebrandt. 1990. Taxonomic and phylogenetic studies on a new taxon of budding, hyphal *Proteobacteria*, *Hirschia baltica* gen. nov., sp. nov. *Int. J. Syst. Bacteriol.* **40**:443–451.
13. Segers, P., et al. 1994. Classification of *Pseudomonas diminuta* Leifson and Hugh 1954 and *Pseudomonas vesicularis* Busing, Doll, and Freytag 1953 in *Brevundimonas* gen. nov. as *Brevundimonas diminuta* comb. nov. and *Brevundimonas vesicularis* comb. nov., respectively. *Int. J. Syst. Bacteriol.* **44**: 499–510.
14. Tam, E., and J. L. Pate. 1985. Amino acid transport by prosthecae of *Asticcacaulis biprosthicum*: evidence for a broad-range transport system. *J. Gen. Microbiol.* **131**:2687–2699.
15. Wagner, J. K., and Y. V. Brun. 2007. Out on a limb: how the *Caulobacter* stalk can boost the study of bacterial cell shape. *Mol. Microbiol.* **64**:28–33.
16. Wagner, J. K., S. Setayeshgar, L. A. Sharon, J. P. Reilly, and Y. V. Brun. 2006. A nutrient uptake role for bacterial cell envelope extensions. *Proc. Natl. Acad. Sci. U. S. A.* **103**:11772–11777.
17. Westmacott, D., and S. B. Primrose. 1976. Synchronous growth of *Rhodospseudomonas palustris* from the swarmer phase. *J. Gen. Microbiol.* **94**:117–125.
18. Whittenbury, R., and C. S. Dow. 1977. Morphogenesis and differentiation in *Rhodomicrobium vannielii* and other budding and prosthecate bacteria. *Bacteriol. Rev.* **41**:754–808.