

# Draft Genome of *Phaeobacter gallaeciensis* ANG1, a Dominant Member of the Accessory Nidamental Gland of *Euprymna scolopes*<sup>∇</sup>

Andrew J. Collins and Spencer V. Nyholm\*

Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06269

Received 22 April 2011/Accepted 27 April 2011

***Phaeobacter gallaeciensis* strain ANG1 represents the dominant member of the bacterial consortium within the reproductive accessory nidamental gland (ANG) of the squid *Euprymna scolopes*. We present a 4.59-Mb assembly of its genome, which may provide clues as to how it benefits its host.**

The accessory nidamental glands (ANGs) of female cephalopods are reproductive organs containing dense consortia of bacteria dominated by *Alphaproteobacteria* from the *Roseobacter* clade (5, 11, 13). Although the function of this organ has not been demonstrated, the production of antimicrobial and/or antifouling compounds from ANG bacterial isolates has been proposed (4, 5). Here, we describe the draft genome of *Phaeobacter gallaeciensis* ANG1, a member of the *Rhodobacteraceae*, isolated from the ANG of a sexually mature Hawaiian bobtail squid, *Euprymna scolopes*.

The *Roseobacter* clade is a ubiquitous, diverse group that can comprise up to 20% of bacterial populations in marine environments (7). Besides being implicated in a number of large-scale ecological roles, i.e., carbon cycling (12) and sulfur metabolism (10), roseobacters are also found as members of many eukaryotic-bacterial symbioses. For example, they form obligate associations with marine algae (1), are major colonizers of corals (2), and are commonly found as dominant members in the ANGs of several cephalopods—including loliginid squid (5), cuttlefish (11), and the Hawaiian bobtail squid *Euprymna scolopes* (unpublished data).

We cultured three isolates from the ANG of *E. scolopes* and identified them by sequencing the full-length 16S rRNA gene. The isolate that was sequenced was Gram negative and positive for oxidase and catalase. The 16S rRNA gene was 99.7% identical to 16S gene sequences found in three separate *E. scolopes* ANG clone libraries and 99.2% identical to a coastal isolate, *Phaeobacter gallaeciensis* SCH0407, in GenBank (accession no. AY881240). The genome of this isolate, *P. gallaeciensis* ANG1, was sequenced using Illumina mated paired-end technology. A total of  $1.73 \times 10^8$  36-bp reads were assembled using the CLC Genomic Workbench assembler (CLC Bio, Denmark), resulting in 1,370-fold coverage of a 4.59-Mb genome contained in 135 contigs. Glimmer (8), GeneMark (6), and the RAST server (3) were used to predict open reading frames (ORFs). A total of 4,389

protein-coding genes, 35 tRNAs, and one ribosomal operon were identified.

*Phaeobacter gallaeciensis* ANG1 has complete Embden-Myerhoff-Parnas, Entner-Doudoroff, and pentose phosphate pathways and a complete tricarboxylic acid (TCA) cycle. The genome contains an abundance of predicted ABC transporters, particularly for peptides, amino acids, and polyamines (i.e., putrescine, spermidine). Other transport systems include a twin-arginine transport system, the Sec pathway, and a type IV and a type VI secretion system. In addition, all cobalamin synthesis genes are present, suggesting that *P. gallaeciensis* ANG1 may provide this nutrient for its host. While there is high similarity to two other *Phaeobacter gallaeciensis* genomes available (strains BS107 and 2.10), strain ANG1 lacks the genes needed to synthesize the antibiotic tropodithietic acid (TDA) (9), and classical pathways for antibiotic production were not found.

This genome shows the metabolic and transport potential of a major bacterial constituent of the accessory nidamental gland of *E. scolopes*. Further analyses of this genome may provide significant clues to understanding the role of *P. gallaeciensis* in this symbiotic organ.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited in DDBJ/EMBL/GenBank under accession no. AFCF00000000. The version described in this paper is the first version, AFCF01000000.

This work was funded by the University of Connecticut Research Foundation and NSF grant IOS-0958006 to S.V.N.

We thank Joerg Graf, Lindsey Bomar, Sagar Faldu, Pascal LaPierre, and Monica Shah for assistance with this project.

## REFERENCES

1. Alavi, M., T. Miller, K. Erlandson, R. Schneider, and R. Belas. 2001. Bacterial community associated with *Pfiesteria*-like dinoflagellate cultures. *Environ. Microbiol.* **3**:380–396.
2. Apprill, A., H. Q. Marlow, M. Q. Martindale, and M. S. Rappe. 2009. The onset of microbial associations in the coral *Pocillopora meandrina*. *ISME J.* **3**:685–699.
3. Aziz, R. K., et al. 2008. The RAST server: rapid annotations using subsystem technology. *BMC Genomics* **9**:75.
4. Barbieri, E., K. Barry, A. Child, and N. Wainwright. 1997. Antimicrobial activity in the microbial community of the accessory nidamental gland and egg cases of *Loligo pealei* (Cephalopoda: Loliginidae). *Biol. Bull.* **193**:275–276.
5. Barbieri, E., et al. 2001. Phylogenetic characterization of epibiotic bacteria in the accessory nidamental gland and egg capsules of the squid *Loligo pealei* (Cephalopoda: Loliginidae). *Environ. Microbiol.* **3**:151–167.

\* Corresponding author. Mailing address: Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269. Phone: (860) 486-4886. Fax: (860) 486-4331. E-mail: spencer.nyholm@uconn.edu.

<sup>∇</sup> Published ahead of print on 6 May 2011.

6. **Borodovsky, M., R. Millis, J. Besemer, and A. Lomsadze.** 2003. Prokaryotic gene prediction using GeneMark and GeneMark.hmm. *Curr. Protoc. Bioinformatics* 4.5.1–4.5.16.
7. **Buchan, A., J. M. Gonzalez, and M. A. Moran.** 2005. Overview of the marine *Roseobacter* lineage. *Appl. Environ. Microbiol.* 71:5665–5677.
8. **Delcher, A. L., K. A. Bratke, E. C. Powers, and S. L. Salzberg.** 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23:673–679.
9. **Geng, H., J. B. Bruhn, K. F. Nielsen, L. Gram, and R. Belas.** 2008. Genetic dissection of tropodithietic acid biosynthesis by marine roseobacters. *Appl. Environ. Microbiol.* 74:1535–1545.
10. **Gonzalez, J. M., R. P. Kiene, and M. A. Moran.** 1999. Transformation of sulfur compounds by an abundant lineage of marine bacteria in the alpha subclass of the class *Proteobacteria*. *Appl. Environ. Microbiol.* 65:3810–3819.
11. **Grigioni, S., R. Boucher-Rodoni, A. Demarta, M. Tonolla, and R. Peduzzi.** 2000. Phylogenetic characterization of bacterial symbionts in the accessory nidamental glands of the sepioid *Sepia officinalis* (Cephalopoda: Decapoda). *Mar. Biol.* 136:217–222.
12. **King, G. M.** 2003. Molecular and culture based analyses of aerobic carbon monoxide oxidizer diversity. *Appl. Environ. Microbiol.* 69:7257–7265.
13. **Pichon, D., V. Gaia, M. D. Norman, and R. Boucher-Rodoni.** 2005. Phylogenetic diversity of epibiotic bacteria in the accessory nidamental glands of squids (Cephalopoda: Loliginidae and Idiosepiidae). *Mar. Biol.* 147:1323–1332.