Genome Sequence of *Ruegeria* sp. Strain KLH11, an *N*-Acylhomoserine Lactone-Producing Bacterium Isolated from the Marine Sponge *Mycale laxissima*[∇]

Jindong Zan,¹ W. Florian Fricke,² Clay Fuqua,³ Jacques Ravel,² and Russell T. Hill^{1*}

Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Science, Baltimore, Maryland 21202¹; Institute for Genome Sciences, Department of Microbiology & Immunology, University of Maryland School of Medicine, Baltimore, Maryland 21201²; and Department of Biology, Indiana University, Bloomington, Indiana 47405³

Received 14 June 2011/Accepted 29 June 2011

Ruegeria sp. strain KLH11, isolated from the marine sponge *Mycale laxissima*, produces a complex profile of *N*-acylhomoserine lactone quorum-sensing (QS) molecules. The genome sequence provides insights into the genetic potential of KLH11 to maintain complex QS systems, and this is the first genome report of a cultivated symbiont from a marine sponge.

Sponges can harbor diverse assemblages of microbes, which in some cases can comprise up to 60% of the total biomass of the holobiont (6). This dense microbial community associated with sponges provides conditions in which quorum sensing (QS) may be important (3, 8). QS is a process by which bacteria coordinate group activities such as bioluminescence, antibiotic production, and virulence at high cell density via signal molecules, typified by N-acylhomoserine lactone (AHL) in Proteobacteria (2). We isolated a Ruegeria sp. strain, KLH11 [99% 16S rRNA gene sequence similarity with Ruegeria lacuscaerulensis (GenBank accession no. HQ908678)], which can produce a complex profile of long-chain AHLs (3). The Silicibacter-Ruegeria subgroup belongs to the Roseobacter clade, which is widespread and numerous in surface waters of the oceans and includes organisms that are involved in global sulfur cycling (1). Many roseobacters have been fully sequenced and annotated (5). Ruegeria sp. strain KLH11 is the first readily culturable sponge symbiont to have its genome sequenced. This microorganism has been isolated from several samples of the sponge Mycale laxissima and is termed a symbiont using the term as defined by Taylor et al. (6).

The genome was sequenced by a combination of Roche/454 and Sanger whole-genome shotgun sequencing. Sequences were assembled with a Celera assembler (4), and genes were predicted and annotated using the automated CloVR-Microbe pipeline (7).

The draft genome has a total assembly size of ca. 4.5 Mb, with an average G+C content of 57.3 mol%, and is predicted to have 4,493 coding sequences (CDS), of which 3,243 (ca. 72.0%) were functionally annotated. This assembly is comprised of 13 scaffolds, of which the largest is ca. 3.1 Mb, having 3,193 CDS. One scaffold of 81 kbp shows similarity to the megaplasmid identified in *Ruegeria pomeroyi* DSS-3 (accession number CP000032) and could indicate the presence of extrachromosomal elements in the genome. Approximately 82.0% of all the predicted CDS have ATG sequences at the presumptive start position, and 17.8% may use GTG or TTG. One complete rRNA operon, one partial 16S rRNA gene, two 5S rRNA genes, and 52 tRNAs were identified. About 34 genes were found to be related to the synthesis of bacterial flagella and regulation of bacterial motility. No candidates for chemotaxis functions were observed in the genome. Two sets of quorum-sensing circuits were identified, implying the potential for a complex quorum-sensing mechanism. Roughly 44 genes were predicted to function in the biosynthesis and degradation of polysaccharides.

Comparison of KLH11 genome sequences to closely related free-living bacteria, such as *R. pomeroyi* DSS-3, provides an opportunity to define the unique set of genes important for host-symbiont interactions and the ecological roles and biogeochemical functions. Complex quorum-sensing circuits in KLH11 make it a perfect model for studying the ecological role of QS in the complex symbiosis between sponges and their associated bacteria.

Nucleotide sequence accession number. The data of the genome sequence of KLH11 were deposited in GenBank under the accession number ACCW00000000.

Sequencing was funded by the Gordon and Betty Moore Foundation and the Institute for Genome Sciences at the University of Maryland School of Medicine. This work was supported by the NSF Microbial Observatories Program (MCB-0238515), the Microbial Interactions and Processes Program (MCB-0703467), and the BIO/IOS Program (IOS-0919728). The UNC National Underwater Research Center provided sponge sampling support.

This is contribution no. 4541 from the University of Maryland Center for Environmental Science.

REFERENCES

- Buchan, A., J. M. González, and M. A. Moran. 2005. Overview of the marine Roseobacter lineage. Appl. Environ. Microbiol. 71:5665–5677.
- Fuqua, C., and E. P. Greenberg. 2002. Listening in on bacteria: acyl-homoserine lactone signalling. Nat. Rev. Mol. Cell Biol. 3:685–695.
- Mohamed, N. M., et al. 2008. Diversity and quorum-sensing signal production of Proteobacteria associated with marine sponges. Environ. Microbiol. 10:75–86.
- Myers, E. W., et al. 2000. A whole-genome assembly of *Drosophila*. Science 287:2196–2204.

^{*} Corresponding author. Mailing address: Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Science, Columbus Center Suite 236, 701 East Pratt Street, Baltimore, MD 21202. Phone: (410) 234-8843. Fax: (410) 234-8896. E-mail: hill@umces.edu.

^v Published ahead of print on 8 July 2011.

- 5. Newton, R. J., et al. 2010. Genome characteristics of a generalist marine
- Newbil, K. J., et al. 2010. Genome characteristics of a generatist marine bacterial lineage. ISME J. 4:784–798.
 Taylor, M. W., R. Radax, D. Steger, and M. Wagner. 2007. Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol. Mol. Biol. Rev. 71:295–347.
 White, J., et al. 2011. CloVR-Microbe: assembly, gene finding and functional

annotation of raw sequence data from single microbial genome projectsstandard operating procedure, version 1.0. Nature Proc. doi:10.1038/npre.

Standard operating procedure, version 1.6. (value 116: doi:10.1056/hpre. 2011.5887.1.
 Zan, J., C. Fuqua, and R. T. Hill. 2011. Diversity and functional analysis of luxS genes in vibrios from marine sponges Mycale laxissima and Ircinia stro-bilina. ISME J. doi:10.1038/ismej/2011.31.