

## Genome Sequence of *Moraxella catarrhalis* RH4, an Isolate of Seroresistant Lineage

## Aldert Zomer,<sup>a,b</sup> Stefan P. W. de Vries,<sup>a</sup> Kristian Riesbeck,<sup>c</sup> Andreas L. Meinke,<sup>d</sup> Peter W. M. Hermans,<sup>a</sup> and Hester J. Bootsma<sup>a</sup>

Laboratory of Pediatric Infectious Diseases, Radboud University Medical Centre, Nijmegen, The Netherlands<sup>a</sup>; Centre for Molecular and Biomolecular Informatics, Nijmegen Centre for Molecular Life Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands<sup>b</sup>; Medical Microbiology, Department of Laboratory Medicine, Malmö, Skåne University Hospital, Lund University, Malmö, Sweden<sup>c</sup>; and Intercell AG, Vienna, Austria<sup>d</sup>

Here we report the annotated genome sequence of *Moraxella catarrhalis* strain RH4, a seroresistant-lineage strain isolated from the blood of an infected patient. This genome sequence will allow us to gain further insight into the genetic diversity of clinical *M. catarrhalis* isolates and will facilitate study of *M. catarrhalis* pathogenesis.

The Gram-negative diplococcus *Moraxella catarrhalis* is an emerging human-restricted respiratory tract pathogen. It is the third-most-common cause of childhood otitis media and is frequently associated with exacerbations of chronic obstructive pulmonary disease (COPD) in adults. We reported the first completely assembled and annotated *M. catarrhalis* genome in 2010 (6), of strain BBH18 (erroneously referred to as RH4 at the time), a sputum isolate from a COPD patient during an exacerbation (8). In 2011, an additional 10 genome sequences of clinical *Moraxella* isolates were published (5) and compared to the BBH18 genome and the partial genome sequence of strain ATCC 43617 (10). This indicated a modest diversity in gene content and chromosomal organization between these isolates. Here we present the annotated genome sequence of the clinically relevant RH4 strain, which was originally isolated from the blood of an infected patient (4).

The draft genome sequence of *M. catarrhalis* RH4 was obtained using Illumina 50-bp paired-end technology (a total of 13,826,736 reads, with 700× coverage). Reads were assembled with the Ray assembler software program (3), resulting in a total of 31 contigs (>100 bp in size). Contigs were ordered using the program Projector 2 (9) with the BBH18 sequence as a scaffold, and the correct order was verified by gap-spanning PCRs. When possible, gaps were filled or corrected by Sanger sequencing of PCR products, followed by use of GapFiller (2). After manual contig assembly, 9 contigs covering a total of 1,836,691 bp were obtained, which is within the size range of the reported genomes (1.78 to 1.96 Mbp).

The RH4 genome sequence was annotated using the RAST (rapid annotations using subsystems technology) server (1) and manually corrected for errors in open reading frame (ORF) calling. The total genome has a G+C content of 41.6% and is composed of 1,904 genes, including 1,845 protein-encoding genes, 4 rRNA operons, and at least 43 tRNAs. RH4 has a novel sequence variant for the *abcZ* allele and thus a novel multilocus sequence type (MLST) but clearly belongs to the seroresistant lineage (11). The RH4 genome contains the bro-1 β-lactamase gene and all of the major known *M. catarrhalis* virulence factors, among which UspA1, UspA2H, MID/Hag, and in contrast to, for instance, BBH18, a complete mha locus. Compared to all Moraxella genomes published to date, the RH4 genome contains 10 unique genes, 8 of which are located consecutively on a 10.1-kb fragment. In addition to 4 putative restriction-modification protein-encoding genes, this cluster contains a putative CiaB-encoding gene, where the ortholog in Campylobacter jejuni is involved in internalization into mammalian cells (7).

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited in DDBJ/EMBL/GenBank under the accession no. AMSO0000000. The version described in this article is the first version, AMSO01000000.1.

## ACKNOWLEDGMENTS

This work was supported by a Vienna Spot of Excellence (VSOE) grant (ID337956), the Anna and Edwin Berger foundation (to K.R.), and the Swedish Medical Research Council (grant number 521-2010-4221; to K.R.).

## REFERENCES

- 1. Aziz RK, et al. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75. doi:10.1186/1471-2164-9-75.
- 2. Boetzer M, Pirovano W. 2012. Toward almost closed genomes with GapFiller. Genome Biol. 13:R56. doi:10.1186/gb-2012-13-6-r56.
- 3. Boisvert S, Laviolette F, Corbeil J. 2010. Ray: simultaneous assembly of reads from a mix of high-throughput sequencing technologies. J. Comput. Biol. 17:1519–1533.
- 4. Christensen JJ, Ursing J, Bruun B. 1994. Genotypic and phenotypic relatedness of 80 strains of *Branhamella catarrhalis* of worldwide origin. FEMS Microbiol. Lett. 119:155–159.
- Davie JJ, et al. 2011. Comparative analysis and supragenome modeling of twelve *Moraxella catarrhalis* clinical isolates. BMC Genomics 12:70. doi: 10.1186/1471-2164-12-70.
- de Vries SP, et al. 2010. Genome analysis of *Moraxella catarrhalis* strain RH4, a human respiratory tract pathogen. J. Bacteriol. 192:3574–3583.
- 7. Konkel ME, Kim BJ, Rivera-Amill V, Garvis SG. 1999. Bacterial secreted proteins are required for the internalization of *Campylobacter jejuni* into cultured mammalian cells. Mol. Microbiol. **32**:691–701.
- 8. Mollenkvist A, et al. 2003. The *Moraxella catarrhalis* immunoglobulin Dbinding protein MID has conserved sequences and is regulated by a mechanism corresponding to phase variation. J. Bacteriol. **185**:2285–2295.
- 9. van Hijum SA, Zomer AL, Kuipers OP, Kok J. 2005. Projector 2: contigR mapping for efficient gap-closure of prokaryotic genome sequence assemblies. Nucleic Acids Res. 33:W560–W566.
- Wang W, et al. 2007. Metabolic analysis of *Moraxella catarrhalis* and the effect of selected in vitro growth conditions on global gene expression. Infect. Immun. 75:4959–4971.
- 11. Wirth T, et al. 2007. The rise and spread of a new pathogen: seroresistant *Moraxella catarrhalis*. Genome Res. 17:1647–1656.

Received 29 September 2012 Accepted 3 October 2012

Address correspondence to Hester J. Bootsma, h.bootsma@cukz.umcn.nl. A.Z. and S.P.W.D.V. contributed equally to this work.

Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JB.01833-12