Genome Sequence of the Emerging Pathogen *Aeromonas caviae*[∇]

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Aeromonas caviae is a Gram-negative, motile and rod-shaped facultative anaerobe that is increasingly being recognized as a cause of diarrhea in children. Here we present the first genome sequence of an *A. caviae* strain that was isolated as the sole pathogen from a child with profuse diarrhea.

Aeromonads are Gram-negative organisms belonging to the Gammaproteobacteria. Nineteen species of Aeromonas can be distinguished by biochemical and molecular techniques (5); most do not have a strong association with disease. However, Aeromonas salmonicida is a well-established fish pathogen, and Aeromonas caviae, Aeromonas hydrophila, and Aeromonas veronii account for 85% of isolates derived from human infections (5). While Aeromonas sp. can be isolated from ca. 2% of healthy individuals, association with stools from patients with diarrhea has been reported to be as high as 10.8% (4, 5). Here we report the genome sequence of A. caviae Ae398. This strain was isolated from a 17-month-old male at the Hospital Universitário Pedro Ernesto, Rio de Janeiro, Brazil. The patient presented with gastroenteritis and diarrhea, without mucus or blood, approximately three times a day for 3 weeks. A. caviae Ae398 was isolated as the sole pathogen from a stool specimen collected 3 weeks after onset of symptoms and was identified by conventional biochemical tests (3). The isolate presented extracelullar enzymatic activities, resistance to several antibiotics, and adherence to epithelial cell monolayers (2, 3, 7, 9, 10).

Whole-genome shotgun sequencing of *A. caviae* Ae398 was performed by a combination of 454 GS-FLX (162,066 shotgun reads of average length 358 bp) and Illumina sequencing (25,272,910 51-bp paired-end reads), leading to a final assembly of 149 contigs >200 bp. The assembly represented 4,339,218 bp, with a mean contig size of 29,793 bp, an N50 value of 76,364 bp (where N50 is the contig length such that at least 50% of the bases of the assembly are contained within contigs of this size or greater), and an average G+C content of 61.4%. Nucmer pairwise genome alignments (6) show the *A. caviae* Ae398 genome displays the highest overall synteny with

* Corresponding author. Mailing address: School of Immunity and Infection, University of Birmingham, Birmingham B15 2TT, United Kingdom. Phone: 44 1214724524. Fax: 44 1214143599. E-mail: i.r .henderson@bham.ac.uk. *A. hydrophila* ATCC 7966 (70.4% aligned) and *A. salmonicida* A449 (61.7% aligned) and average nucleotide identities of 88.8% and 86.8% across aligned regions, respectively.

The deduced size of the *A. caviae* Ae398 genome (4.43 Mb) is similar to those of *A. hydrophila* ATCC 7966 (4.47 Mb) and *A. salmonicida* A449 (4.70 Mb). *A. caviae* Ae398 harbors at least one conjugative plasmid of >30 kb, although it remains in several contigs in the assembly. Five different insertion (IS) element types are present in *A. caviae* Ae398, whereas *A. salmonicida* possesses 88 copies of 10 different IS elements and *A. hydrophila* completely lacks IS elements (8, 11). An \sim 33-kb putative prophage bounded by 55-bp repeats is found at the tRNA-Leu attachment site in *A. caviae* Ae398; the 3' region shares high identity with *phi*O18P (1), whereas the 5' region is most similar to structural genes from non-*Aeromonas* phage genomes.

A. caviae contains many putative virulence genes, including those encoding a type 2 secretion system (11), an RTX toxin, and polar flagella. The genome sequence of A. caviae Ae398 is an important milestone in understanding the diversity and pathogenesis of A. caviae, opening new avenues for investigating the pathogenic processes of this organism and allowing robust diagnostics to be developed.

Nucleotide sequence accession numbers. The assembly was deposited at the WGS division of DDBJ/EMBL/GenBank under accession no. CACP01000001 to CACP01000149.

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REFERENCES

- Beilstein, F., and B. Dreiseikelmann. 2008. Temperate bacteriophage PhiO18P from an *Aeromonas media* isolate: characterization and complete genome sequence. Virology 373:25–29.
- Castilho, M. C., et al. 2009. High frequency of hemolytic and cytotoxic activity in *Aeromonas* spp. isolated from clinical, food and environmental in Rio de Janeiro, Brazil. Antonie Van Leeuwenhoek 96:53–61.
- Freitas, A. C., S. M. Souza, L. C. Macedo, E. C. Pinto, and S. S. Pereira. 1998. Aeromonas species associated with gastroenteritis in children: prev-

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alence, characteristics and virulence properties. Rev. Microbiol. 29:152-157.

- Gracey, M., V. Burke, and J. Robinson. 1982. Aeromonas-associated gastroenteritis. Lancet ii:1304–1306.
- Janda, J. M., and S. L. Abbott. 2010. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. Clin. Microbiol. Rev. 23:35–73.
- 6. Kurtz, S., et al. 2004. Versatile and open software for comparing large genomes. Genome Biol. 5:R12.
- 7. Palu, A. P., et al. 2006. Antimicrobial resistance in food and clinical Aeromonas isolates. Food Microbiol. 23:504–509.
- 8. Reith, M. E., et al. 2008. The genome of Aeromonas salmonicida subsp.

salmonicida A449: insights into the evolution of a fish pathogen. BMC Genomics 9:427.

- Rocha-De-Souza, C. M., et al. 2001. Identification of a 43-kDa outer-membrane protein as an adhesin in *Aeromonas caviae*. J. Med. Microbiol. 50:313– 319.
- Rocha-De-Souza, C. M., et al. 2003. Influence of polarisation and differentiation on interaction of 43-kDa outer-membrane protein of *Aeromonas caviae* with human enterocyte-like Caco-2 cell line. Int. J. Mol. Med. 11:661– 667.
- Seshadri, R., et al. 2006. Genome sequence of *Aeromonas hydrophila* ATCC 7966T: jack of all trades. J. Bacteriol. 188:8272–8282.