JB Journals.ASM.org

GENOME ANNOUNCEMENT

Draft Genome Sequence of the Virulent Strain 01-B526 of the Fish Pathogen *Aeromonas salmonicida*

Steve J. Charette,[a,b,c] Francis Brochu,[a] Brian Boyle,[a] Geneviève Filion,[a,b] Katherine H. Tanaka,[a,b] and Nicolas Derome[a,d]

Institut de Biologie Intégrative et des Systèmes, Pavillon Charles-Eugène-Marchand, Université Laval, Quebec City, Quebec, Canada[a]; Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec (Hôpital Laval), Quebec City, Quebec, Canada[b]; Département de Biochimie, de Microbiologie et de Bio-Informatique, Faculté des Sciences et de Génie, Université Laval, Quebec City, Quebec, Canada[c]; and Département de Biologie, Faculté des Sciences et de Génie, Université Laval, Quebec City, Quebec, Canada[d]

***Aeromonas salmonicida* is an important fish pathogen, mainly of salmonids. This bacterium causes a disease named furunculosis, which is particularly detrimental for the aquaculture industry. Here, we present the draft genome sequence of *A. salmonicida* 01-B526, a strain isolated from a brook trout that is more virulent than *A. salmonicida* reference strain A449, for which a genome sequence is available.**

The Gram-negative bacterium *Aeromonas salmonicida* subsp. *salmonicida* is the causative agent of furunculosis, especially in salmonids (11, 17). The complete genome sequence of *A. salmonicida* reference strain A449 has already been published (16). Unfortunately, this strain was not virulent when facing the alternative host model *Dictyostelium discoideum* amoeba, probably due to the absence of proteolytic activity (6). In this context, it appeared important to obtain the genomic sequence of a true virulent strain of *A. salmonicida*, such as 01-B526, a strain isolated from an infected brook trout in the province of Quebec (Canada) displaying high virulence against both fish and amoeba (6, 7).

The total genomic DNA of *A. salmonicida* 01-B526 was extracted using the DNeasy blood and tissue kit (Qiagen, Streetsville, ON, Canada). Whole-genome 3-kb paired-end DNA sequencing of *A. salmonicida* 01-B526 was performed using the Roche/454 pyrosequencing method on the Genome Sequencer FLX system with titanium chemistry at the Plateforme d'Analyse Génomique of the Institut de Biologie Intégrative et des Systèmes (IBIS; Université Laval). In total, 214,046,091 bases were analyzed using the *de novo* assembler module (gsAssembler) of Newbler version 2.5.3 (454 Life Sciences). A total of 1,108 contigs were produced (173 contigs were larger than 500 bases, and 935 contigs ranged from 100 to 499 bases). Paired-end data (average pair distance of 2.38 kb) joined 135 large contigs into 31 scaffolds. A reference assembly of the same data set of *A. salmonicida* A449 (GenBank accession numbers CP000646.1, CP000645.1, and CP000644.1) using the gsMapper module of Newbler version 2.5.3 produced 108 contigs. This high number of contigs despite an average coverage of more than 40× is due to the presence of a high number of repeated elements and especially insertion sequences (IS) in the *A. salmonicida* genome (16). Manual gap closing is under way to complement *de novo* and reference assemblies.

The ongoing assembly of the data set shows that the *A. salmonicida* 01-B526 chromosome has 4.75 Mb compared to 4.70 Mb for *A. salmonicida* A449 (16). 01-B526 holds a large plasmid, pAsa5, of 155 kb (16). This strain also possesses 3 small plasmids, pAsa1, pAsa2, and pAsa3, of 5,424 bases, 5,247 bases, and 5,616 bases, respectively (5). The genome structure analysis of 01-B526 was further investigated with PCR and EcoRI restriction profiles (5). Restriction analyses suggested the additional presence of the pAsal1 plasmid (5) in *A. salmonicida* 01-B526 (data not shown). This plasmid has been missed in sequence analysis for two reasons: the very high homology of a big part of this plasmid with pAsa3, and the presence of one IS also found elsewhere in the genome. These kinds of elements cannot be easily managed by next-generation assemblers and most often require manual intervention (1). The total genome assembly has a mean G+C content of 58.5%. The annotation of the sequences was made by the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (2–4, 8–10, 12–15).

In the future, a deep comparative analysis of the genome sequences of A449 and 01-B526 might allow the identification of new virulence factors used by *A. salmonicida* to infect fish. The fact that the 01-B526 chromosome is 50 kb bigger than the one of A449 is of particular interest.

Nucleotide sequence accession number. The nucleotide sequence for the draft genome sequence was deposited in DDBJ/EMBL/GenBank under accession number AGVO00000000.

ACKNOWLEDGEMENTS

We are grateful to C. Uhland and P. Belhumeur (Université de Montréal, Montréal, Canada) for the 01-B526 strain.

This project was funded by Discovery grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to both S.J.C. and N.D. and by a grant from the Réseau Aquaculture Québec (RAQ). F.B. and K.H.T. received scholarships from the Undergraduate Student Research Awards Program of the NSERC. S.J.C. is a research scholar of the Fonds de la Recherche en Santé du Québec (FRSQ).

REFERENCES

1. **Alkan C, Sajjadian S, Eichler EE.** 2011. Limitations of next-generation genome sequence assembly. Nat. Methods **8**:61–65.
2. **Angiuoli SV, et al.** 2008. Toward an online repository of standard operating procedures (SOPs) for (meta) genomic annotation. OMICS **12**: 137–141.
3. **Besemer J, Lomsadze A, Borodovsky M.** 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes.

Received 14 November 2011 **Accepted** 18 November 2011

Address correspondence to Steve J. Charette, steve.charette@bcm.ulaval.ca.

doi:10.1128/JB.06276-11

Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res. **29**:2607–2618.
4. **Borodovsky M, McIninch J.** 1993. GeneMark: parallel gene recognition for both DNA strands. Comput. Chem. **17**:122–133.
5. **Boyd J, et al.** 2003. Three small, cryptic plasmids from *Aeromonas salmonicida* subsp. *salmonicida* A449. Plasmid **50**:131–144.
6. **Daher RK, et al.** 2011. Alteration of virulence factors and rearrangement of pAsa5 plasmid caused by the growth of *Aeromonas salmonicida* in stressful conditions. Vet. Microbiol. **152**:353–360.
7. **Dautremepuits C, Fortier M, Croisetiere S, Belhumeur P, Fournier M.** 2006. Modulation of juvenile brook trout (*Salvelinus fontinalis*) cellular immune system after *Aeromonas salmonicida* challenge. Vet. Immunol. Immunopathol. **110**:27–36.
8. **Delcher AL, Bratke KA, Powers EC, Salzberg SL.** 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics **23**:673–679.
9. **Eddy SR.** 2002. A memory-efficient dynamic programming algorithm for optimal alignment of a sequence to an RNA secondary structure. BMC Bioinformatics **3**:18.
10. **Griffiths-Jones S, et al.** 2005. Rfam: annotating non-coding RNAs in complete genomes. Nucleic Acids Res. **33**:D121–D124.
11. **Hiney M, Olivier G.** 1999. Furunculosis (*Aeromonas salmonicida*), p 341–425. *In* Woo P, Bruno D (ed), Fish diseases and disorders III: viral, bacterial and fungal infections. CAB Publishing, Oxford, United Kingdom.
12. **Klimke W, et al.** 2009. The National Center for Biotechnology Information's protein clusters database. Nucleic Acids Res. **37**:D216–D223.
13. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**: 955–964.
14. **Lukashin AV, Borodovsky M.** 1998. GeneMark.hmm: new solutions for gene finding. Nucleic Acids Res. **26**:1107–1115.
15. **Pruitt KD, Tatusova T, Klimke W, Maglott DR.** 2009. NCBI reference sequences: current status, policy and new initiatives. Nucleic Acids Res. **37**:D32–D36.
16. **Reith ME, et al.** 2008. The genome of *Aeromonas salmonicida* subsp. *salmonicida* A449: insights into the evolution of a fish pathogen. BMC Genomics **9**:427.
17. **Wiklund T, Dalsgaard I.** 1998. Occurrence and significance of atypical *Aeromonas salmonicida* in non-salmonid and salmonid fish species: a review. Dis. Aquat. Organ. **32**:49–69.