



# Draft Genome Sequence of *Pseudomonas fluorescens* ML11A, an Endogenous Strain from Brook Charr with Antagonistic Properties against *Aeromonas salmonicida* subsp. *salmonicida*

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**ABSTRACT** *Pseudomonas fluorescens* ML11A, isolated from brook charr, showed a strong *in vitro* inhibitory effect against *Aeromonas salmonicida* subsp. *salmonicida*, a bacterial fish pathogen. Its genome harbors gene clusters for siderophore and bacteriocin biosynthesis and shares 99% whole-genome identity with *P. fluorescens* A506, a biological control strain used in agriculture.

**A** *eromonas salmonicida* subsp. *salmonicida* is an opportunistic pathogen of farmed salmonid fish with acute episodes resulting in fatal septicemia (1). An increasing number of strains of this bacterium are resistant to multiple antibiotics (2–5). Alternative control and prevention strategies are needed to limit the propagation of antimicrobial resistance (6). Since the host microbiota plays a major role in mitigating colonization and invasion by pathogens (7, 8), administering beneficial bacteria from the microbiota to susceptible hosts appears to be a promising solution against furunculosis, as proven in other host–pathogen combinations (9–12).

A bacterial strain, ML11A, was recovered from brook charr (*Salvelinus fontinalis*) skin mucus (Pisciculture de la Jacques-Cartier Inc., Cap-Santé, QC, Canada). This strain was initially identified as *Pseudomonas* sp. based on 16S rRNA homology with *P. azotoformans* (13). *Pseudomonas* sp. ML11A showed a strong *in vitro* diffusible inhibitory effect against 10 *A. salmonicida* subsp. *salmonicida* strains from North America and Europe. To further investigate the biological safety of *Pseudomonas* sp. ML11A for future use in fish farms and its mechanism of action against *A. salmonicida* subsp. *salmonicida*, its genome was sequenced.

Whole-genome shotgun paired-end libraries (2 × 300 bp) were prepared with a KAPA Hyper Prep Kit (Kapa Biosystems, Wilmington, MA, USA) and sequenced on an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA). Sequence reads were quality-filtered, trimmed, and *de novo* assembled with the A5 pipeline (14). A total of 75 contigs were generated with an average coverage of 22.6-fold. The average contig size was 88,741 bp, and the  $N_{50}$  contig size was 176,212 bp. The size of the assembled genome is 6,655,593 bp with a G+C content of 59.5%, within range of known values for *Pseudomonas* genomes (15).

*Pseudomonas* sp. ML11A was subsequently identified as *P. fluorescens* on the basis of 99.2% shared average nucleotide identity (16) with *P. fluorescens* A506 (17), a strain approved in the United States and Canada as a biocontrol agent against fire blight of apple and pear (*Erwinia amylovora*) (18). The draft genome of *P. fluorescens*

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ML11A was annotated using the RAST annotation server (<http://rast.nmpdr.org>) and the NCBI Prokaryotic Genome Annotation Pipeline ([https://www.ncbi.nlm.nih.gov/genome/annotation\\_prok](https://www.ncbi.nlm.nih.gov/genome/annotation_prok)). It contains 6,054 protein-coding genes, 15 rRNA genes, and 64 tRNA genes. Of all the protein-coding genes, 51% were assigned to 547 SEED subsystems, among which putatively active gene clusters for antibacterial compounds such as colicin V (19) and siderophore pyoverdine (20) were found.

**Accession number(s).** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [MRXZ00000000](https://www.ncbi.nlm.nih.gov/nuclseq/MRXZ00000000). The version described in this paper is the first version, MRXZ01000000.

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## REFERENCES

- Cipriano RC, Bullock GL. 2001. Furunculosis and other diseases caused by *Aeromonas salmonicida*. Fish disease eaflet 66. U.S. Geological Survey.
- Trudel MV, Vincent AT, Attéré SA, Labbé M, Derome N, Culley AI, Charette SJ. 2016. Diversity of antibiotic-resistance genes in Canadian isolates of *Aeromonas salmonicida* subsp. *salmonicida*: dominance of pSN254b and discovery of pAsa8. *Sci Rep* 6:35617. <https://doi.org/10.1038/srep35617>.
- Vincent AT, Trudel MV, Paquet VE, Boyle B, Tanaka KH, Dallaire-Dufresne S, Daher RK, Frenette M, Derome N, Charette SJ. 2014. Detection of variants of the pRAS3, pAB5S9, and pSN254 plasmids in *Aeromonas salmonicida* subsp. *salmonicida*: multidrug resistance, interspecies exchanges, and plasmid reshaping. *Antimicrob Agents Chemother* 58: 7367–7374. <https://doi.org/10.1128/AAC.03730-14>.
- Vincent AT, Emond-Rheault JG, Barbeau X, Attéré SA, Frenette M, Lagüe P, Charette SJ. 2016. Antibiotic resistance due to an unusual ColE1-type replicon plasmid in *Aeromonas salmonicida*. *Microbiology* 162:942–953. <https://doi.org/10.1099/mic.0.000286>.
- Tanaka KH, Vincent AT, Trudel MV, Paquet VE, Frenette M, Charette SJ. 2016. The mosaic architecture of *Aeromonas salmonicida* subsp. *salmonicida* pAsa4 plasmid and its consequences on antibiotic resistance. *PeerJ* 4:e2595. <https://doi.org/10.7717/peerj.2595>.
- WHO. 2014. Antimicrobial resistance: global report on surveillance. WHO, Geneva, Switzerland.
- Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. 2012. Probiotic mechanisms of action. *Ann Nutr Metab* 61: 160–174. <https://doi.org/10.1159/000342079>.
- Stecher B, Maier L, Hardt WD. 2013. 'Blooming' in the gut: how dysbiosis might contribute to pathogen evolution. *Nat Rev Microbiol* 11:277–284. <https://doi.org/10.1038/nrmicro2989>.
- Boutin S, Bernatchez L, Audet C, Derôme N. 2012. Antagonistic effect of indigenous skin bacteria of brook charr (*Salvelinus fontinalis*) against *Flavobacterium columnare* and *F. psychrophilum*. *Vet Microbiol* 155: 355–361. <https://doi.org/10.1016/j.vetmic.2011.09.002>.
- Boutin S, Audet C, Derome N. 2013. Probiotic treatment by indigenous bacteria decreases mortality without disturbing the natural microbiota of *Salvelinus fontinalis*. *Can J Microbiol* 59:662–670. <https://doi.org/10.1139/cjm-2013-0443>.
- Goulden EF, Hall MR, Pereg LL, Høj L. 2012. Identification of an antagonistic probiotic combination protecting ornate spiny lobster (*Panulirus ornatus*) larvae against *Vibrio owensii* infection. *PLoS One* 7:e39667. <https://doi.org/10.1371/journal.pone.0039667>.
- Schubiger CB, Orfe LH, Sudheesh PS, Cain KD, Shah DH, Call DR. 2015. Entericidin is required for a probiotic treatment (*Enterobacter* sp. strain C6-6) to protect trout from cold-water disease challenge. *Appl Environ Microbiol* 81:658–665. <https://doi.org/10.1128/AEM.02965-14>.
- Anzai Y, Kim H, Park JY, Wakabayashi H, Oyaizu H. 2000. Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol* 50:1563–1589. <https://doi.org/10.1099/00207713-50-4-1563>.
- Tritt A, Eisen JA, Facciotti MT, Darling AE. 2012. An integrated pipeline for *de novo* assembly of microbial genomes. *PLoS One* 7:e42304. <https://doi.org/10.1371/journal.pone.0042304>.
- Silby MW, Winstanley C, Godfrey SAC, Levy SB, Jackson RW. 2011. *Pseudomonas* genomes: diverse and adaptable. *FEMS Microbiol Rev* 35:652–680. <https://doi.org/10.1111/j.1574-6976.2011.00269.x>.
- Konstantinidis KT, Tiedje JM. 2005. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci U S A* 102: 2567–2572. <https://doi.org/10.1073/pnas.0409727102>.
- Stockwell VO, Johnson KB, Sugar D, Loper JE. 2010. Control of fire blight by *Pseudomonas fluorescens* A506 and *Pantoea vagans* C9-1 applied as single strains and mixed inocula. *Phytopathology* 100:1330–1339. <https://doi.org/10.1094/PHYTO-03-10-0097>.
- Health Canada - Pest Management Regulatory Agency. 2011. ARCHIVED - Proposed Registration Decision PRD2011-18, *Pseudomonas fluorescens* Strain A506. [http://hc-sc.gc.ca/cps-spc/pest/part/consultations/\\_prd2011-18/prd2011-18-eng.php#a11](http://hc-sc.gc.ca/cps-spc/pest/part/consultations/_prd2011-18/prd2011-18-eng.php#a11).
- Cascales E, Buchanan SK, Duché D, Kleanthous C, Lloubès R, Postle K, Riley M, Slatin S, Cavard D. 2007. Colicin biology. *Microbiol Mol Biol Rev* 71:158–229. <https://doi.org/10.1128/MMBR.00036-06>.
- Schalk IJ, Guillon L. 2013. Pyoverdine biosynthesis and secretion in *Pseudomonas aeruginosa*: implications for metal homeostasis. *Environ Microbiol* 15:1661–1673. <https://doi.org/10.1111/1462-2920.12013>.