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Genome sequence of the virulent *Aeromonas salmonicida* atypical strain T30 isolated from sablefish with furunculosis

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ABSTRACT *Aeromonas salmonicida*, a Gram-negative bacterium, causes the disease furunculosis in multiple fish species. We present the complete genome sequence of the atypical *A. salmonicida* strain T30, which was isolated from furunculosis in sablefish in Manchester, WA, USA. Analyzing this genome will help to identify the bacterium's role in marine aquaculture.

KEYWORDS *Aeromonas salmonicida*, sablefish, genome sequence

Aeromonas salmonicida is one of the most important primary pathogens in aquaculture. The "typical" *A. salmonicida* subspecies *salmonicida* was first discovered as a significant pathogen in fish (1). "Atypical" strains are mainly distinguished from "typical" strains by the absence of characteristic brown pigment on agar cultures (2). Atypical *A. salmonicida* impacts marine and freshwater fish due to virulence factors (3–6).

Sablefish (*Anoplopoma fimbria*) is the highest-valued finfish per pound in Alaska and West Coast commercial fisheries because of their rich oil content (7). Sablefish aquaculture is expanding, and *A. salmonicida* infections often occur due to the bacterium's ubiquitous occurrence in aquatic environments (8). The atypical *A. salmonicida* strain T30, first isolated from net-pen-reared sablefish with an active *A. salmonicida* infection at NOAA's Manchester Marine Research Station (Port Orchard, WA), has been verified as virulent through disease challenge studies (8). It was provided by Joseph Dietrich (NOAA, NWFSC), inoculated, and stored in our laboratory. A single colony of *A. salmonicida* T30 was inoculated into tryptic soy broth and incubated at 20°C for 48 h for DNA extraction. Bacterial genomic DNA was extracted using the Genomic DNA Kit (K182002; Invitrogen, MA, USA). DNA quantity and quality were assessed by NanoDrop 2000 (Thermo Scientific, NJ).

DNA was sent (Novogene Corporation, Inc., Sacramento, CA, USA) for whole genome sequencing. The DNA library was prepared using the NEBNext Ultra DNA library prep kit (E7370L; New England BioLabs) and sequenced using the Illumina MiSeq sequencer by Novogene (150bp paired-end reads, 7,547,418 raw reads, 1.1 G). Trimming, error correction, contig creation, and quality control of sequence reads were conducted using PATRIC (9). *De novo* assembly was performed through the Unicycler v0.4.8 pipeline using PATRIC. The assembly produced 231 contigs with a genome of 4,491,114 bp, a GC content of 58.81%, and an N50 of 48,077. The genome was annotated using NCBI PGAP v.6.5 with the best-placed reference protein set, GeneMarkS-2+. A total of 4,197 protein-coding sequences, 88 tRNA genes, 2 rRNA genes, and 4 ncRNA genes were predicted. The annotation included 857 hypothetical proteins and 3,563 proteins with functional assignments. The proteins with functional assignments included 1,078 proteins with Enzyme Commission numbers, 890 with Gene Ontology assignments, and 777 proteins that were mapped to KEGG pathways. Many of the annotated genes have homology to known transporters, virulence factors, drug targets, and antibiotic resistance genes (Table 1).

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TABLE 1 Summary of special interest genes

Specialty genes	Source	No. of genes
Antibiotic resistance	CARD	7
Antibiotic resistance	NDARO	4
Antibiotic resistance	PATRIC	42
Drug target	DrugBank	36
Drug target	TTD	4
Transporter	TCDB	32
Virulence factor	PATRIC_VF	15
Virulence factor	VFDB	6
Virulence factor	Victors	33

The closest reference and representative genomes were identified by Mash/MinHas (10). PATRIC global protein families were selected from these genomes to determine the phylogenetic placement (Fig. 1). The protein sequences from these families were aligned with MUSCLE (11), and the nucleotides for each of those sequences were mapped to the protein alignment. The joint set of amino acid and nucleotide alignments was concatenated into a data matrix. RaxML (12) was used to analyze this matrix, with fast bootstrapping used to generate the support values in the tree (13).

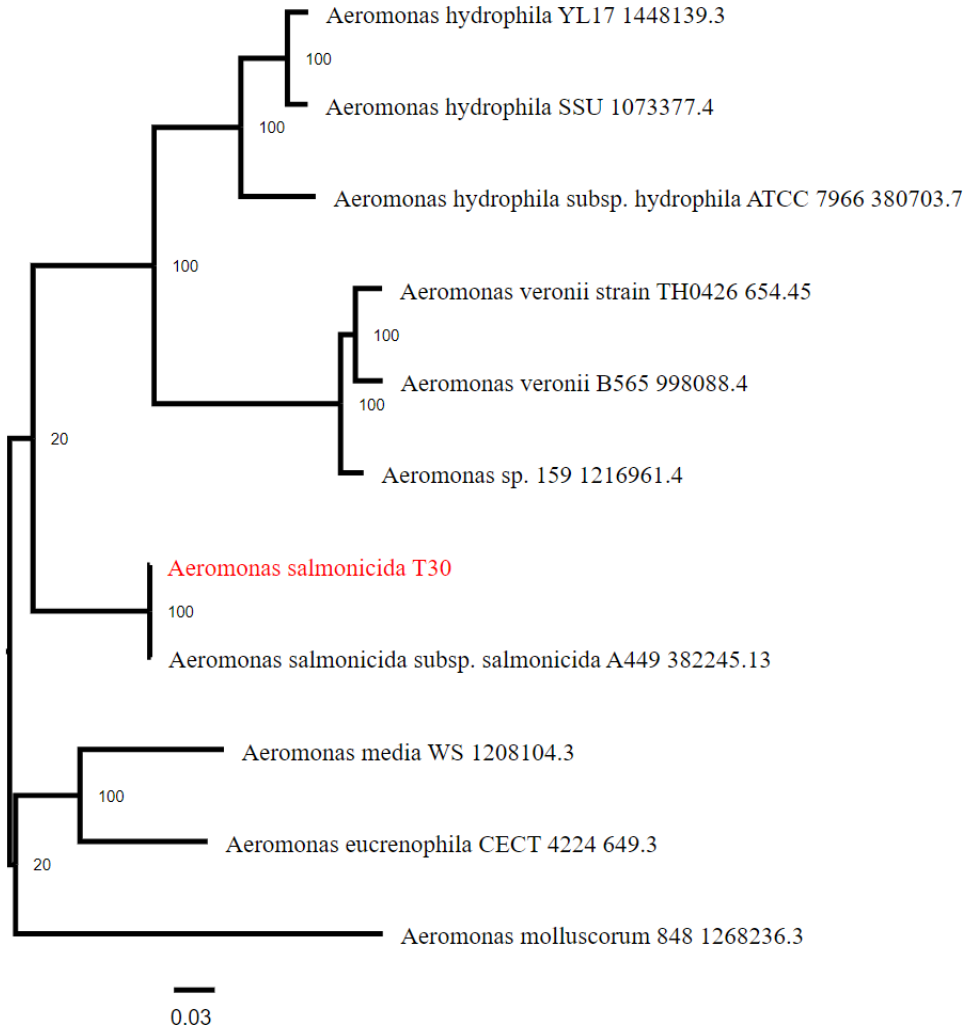

FIG 1 Phylogenetic tree. PATRIC global protein families (PGFams) were selected from the closest reference and representative genomes of *A. salmonicida* to determine the phylogenetic placement.

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Jie Ma, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft, Writing – review and editing | Veronica L. Myrsell, Investigation, Writing – original draft | Joseph Dietrich, Resources, Writing – review and editing | Kenneth D. Cain, Conceptualization, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

This whole-genome sequencing project has been deposited at GenBank under accession no. JARYHA000000000. The raw data for this project can be found under SRA accession no. PRJNA961212.

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