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Complete Genome Sequence of *Francisella endociliophora* Strain FSC1006, Isolated from a Laboratory Culture of the Marine Ciliate *Euplotes raikovi*

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A strain of *Francisella endociliophora* was isolated from a laboratory culture of the marine ciliate *Euplotes raikovi*. Here, we report the complete genome sequence of the bacterial strain FSC1006 (*Francisella* Strain Collection, Swedish Defence Research Agency, Umeå, Sweden).

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In 2011, Schrallhammer and colleagues (1) published a 16S rRNA gene sequence from a bacterium associated with the marine ciliate *Euplotes raikovi* that was phylogenetically positioned within the genus *Francisella* and described as "Candidatus *F. noatunensis* subsp. *endociliophora* subsp. nov." (1). The genus *Francisella* includes several species with little genetic variation but with diverse environmental niches spanning from marine fish pathogens, potential endosymbionts of protozoa, and human pathogens, *viz*. *Francisella tularensis* subsp. *tularensis*, the causative agent of tularemia included among the tier 1 agents on the United States Select Agents list (2–4). The laboratory culture of *E. raikovi* was used to isolate strain FSC1006 (*Francisella* Strain Collection, Swedish Defence Research Agency, Umeå, Sweden). Briefly, the sample was bead beaten and acid treated (5) before being serially diluted in phosphate-buffered saline (PBS) and spread on Thayer-Martin agar culture plates (6). The culture plates were incubated at room temperature (20°C) for 1 to 2 weeks and monitored for bacterial growth. The strain FSC1006 grows in nutrient broth (NB) and McLeod medium at room temperature (RT) but not at 37°C.

DNA was extracted as previously described by Larsson et al. (7) and sent for sequencing to the Uppsala Genome Center (Uppsala, Sweden). A Pacific Biosciences RSII system (10-kb library, 2-h movie length) generated a total of 144,357 PacBio reads, with an average read length of 3.9 kb, using two single-molecular real-time (SMRT) cells. The SMRT Analysis system version 2.2.0.p3 was used to assemble a draft genome consisting of a single contig. Finally, the contig ends were aligned to determine the joining point of the circular genome. The complete genome consists of 2,015,987 nucleotides and has a mean G+C content of 32.4%. Annotation was performed using the NCBI annotation service.

F. endociliophora FSC1006 contains 1,831 predicted protein-coding sequences and 49 predicted noncoding RNAs. The average nucleotide identity (ANI) was calculated by pairwise genome comparisons for the publicly available genomes within *Francisella* clades I and II (8), using the MUMmer and BLAST algorithms with JSpecies version 1.2.1 (9). The similarities between *F. endociliophora* and the clade I and clade II genomes were 78.4 to 77.5% and 78.3 to 77.6%, respectively. Commonly, a threshold of >95 to 96% identity is used to classify genomes as belonging to the same species (10). Multiple genome alignments were computed using progressiveMauve, with default parameters (11), and a phylogenetic tree was generated using the neighbor-joining method. The phylogeny shows that *F. endociliophora* does not belong to any of the two previously known *Francisella* clades and instead forms a new separate branching clade in the *Francisella* genus.

This complete genome sequence is of great importance for the understanding of the environmental diversity of *Francisella* species. A broadening of our understanding is needed in order to further explore the ecology and epidemiology of *Francisella* spp. (12). We argue, based on the phylogeny and low similarity, that this isolate should be classified as a new species, *F. endociliophora*, instead of being a subspecies of *Francisella noatunensis*.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. CP009574. The version described in this paper is the first version, CP009574.1.

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REFERENCES

1. **Schrallhammer M, Schweikert M, Vallesi A, Verni F, Petroni G.** 2011. Detection of a novel subspecies of *Francisella noatunensis* as endosymbiont of the ciliate *Euplotes raikovi*. Microb. Ecol. **61:**455–464. http://dx.doi.org/10.1007/s00248-010-9772-9.
2. **Ahlinder J, Ohrman C, Svensson K, Lindgren P, Johansson A, Forsman M, Larsson P, Sjödin A.** 2012. Increased knowledge of *Francisella* genus diversity highlights the benefits of optimised DNA-based assays. BMC Microbiol. **12:**220. http://dx.doi.org/10.1186/1471-2180-12-220.
3. **Colquhoun DJ, Duodu S.** 2011. *Francisella* infections in farmed and wild aquatic organisms. Vet. Res. **42:**47. http://dx.doi.org/10.1186/1297-9716-42-47.
4. **Keim P, Johansson A, Wagner DM.** 2007. Molecular epidemiology, evolution, and ecology of *Francisella*. Ann. N. Y. Acad. Sci. **1105:**30–66. http://dx.doi.org/10.1196/annals.1409.011.
5. **Humrighouse BW, Adcock NJ, Rice EW.** 2011. Use of acid treatment and a selective medium to enhance the recovery of *Francisella tularensis* from water. Appl. Environ. Microbiol. **77:**6729–6732. http://dx.doi.org/10.1128/AEM.05226-11.
6. **Sandström G, Tärnvik A, Wolf-Watz H, Löfgren S.** 1984. Antigen from *Francisella tularensis*: nonidentity between determinants participating in cell-mediated and humoral reactions. Infect. Immun. **45:**101–106.
7. **Larsson P, Elfsmark D, Svensson K, Wikström P, Forsman M, Brettin T, Keim P, Johansson A.** 2009. Molecular evolutionary consequences of niche restriction in *Francisella tularensis*, a facultative intracellular pathogen. PLoS Pathog. **5:**e1000472. http://dx.doi.org/10.1371/journal.ppat.1000472.
8. **Sjödin A, Svensson K, Ohrman C, Ahlinder J, Lindgren P, Duodu S, Johansson A, Colquhoun DJ, Larsson P, Forsman M.** 2012. Genome characterisation of the genus *Francisella* reveals insight into similar evolutionary paths in pathogens of mammals and fish. BMC Genomics **13:**268. http://dx.doi.org/10.1186/1471-2164-13-268.
9. **Richter M, Rosselló-Móra R.** 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc. Natl. Acad. Sci. U. S. A. **106:**19126–19131. http://dx.doi.org/10.1073/pnas.0906412106.
10. **Kim M, Oh H-S, Park S-C, Chun J.** 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int. J. Syst. Evol. Microbiol. **64:**346–351. http://dx.doi.org/10.1099/ijs.0.059774-0.
11. **Darling AE, Mau B, Perna NT.** 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One **5:**e11147. http://dx.doi.org/10.1371/journal.pone.0011147.
12. **Ahlinder J, Ohrman C, Svensson K, Lindgren P, Johansson A, Forsman M, Larsson P, Sjödin A.** 2012. Increased knowledge of *Francisella* genus diversity highlights the benefits of optimised DNA-based assays. BMC Microbiol. **12:**220. http://dx.doi.org/10.1186/1471-2180-12-220.