

GENOME ANNOUNCEMENT

Complete Genome Sequence of *Aggregatibacter (Haemophilus) aphrophilus* NJ8700[∇]

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We report the finished and annotated genome sequence of *Aggregatibacter aphrophilus* strain NJ8700, a strain isolated from the oral flora of a healthy individual, and discuss characteristics that may affect its dual roles in human health and disease. This strain has a rough appearance, and its genome contains genes encoding a type VI secretion system and several factors that may participate in host colonization.

Aggregatibacter aphrophilus (formerly *Haemophilus aphrophilus*) (11) is well known as an etiologic agent in infectious endocarditis caused by gram-negative bacteria (7). Most often, however, it is found as a nonpathogenic, commensal resident of dental plaque and the oropharyngeal flora. The complete genome sequence of *A. aphrophilus* NJ8700 was achieved using a hybrid approach of a shotgun sequencing strategy combined with 454 pyrosequencing (two runs). The 454 sequences were assembled with the Newbler assembler (454 Life Sciences), and the Sanger reads were added to the resulting contigs using MUMmer (8) and custom scripts. The contigs were linked together into scaffolds using Bambus (15), and gaps between contigs were closed by direct sequencing using a technique described by N. Nagarajan et al. (submitted for publication), achieving a 25-fold coverage. Automated annotation was done at the Institute for Genomic Research/J. Craig Venter Institute through the Annotation Engine Service.

The *A. aphrophilus* NJ8700 genome is 2,313,035 bp in length, with a GC content of 42.23% and 2,320 predicted coding sequences. Approximately 88.4% of nucleotides are predicted to encode proteins. The genome contains 57 tRNAs, including one gene for tRNA^{Sec} (AAP_1961), and five rRNA nontandem cistrons. Like other *Pasteurellaceae* (3, 4), the genome has four RNA subunit genes (*rpoA*, *rpoB*, *rpoC*, and *rpoZ*; AAP_2188, AAP_1813, AAP_1812, and AAP_1427), and five sigma factor genes (AAP_1594, AAP_1967, AAP_2019, AAP_2021, and AAP_2324).

The *A. aphrophilus* NJ8700 genome contains genes encoding

a type VI secretion system (T6SS) (AAP_1851 to -1862, AAP_2123), which is the first instance of its presence in a member of the *Pasteurellaceae* (1, 2, 9, 10, 16, 17, 20). There are several open reading frames (ORFs) similar to *vgrG* (AAP_0259, AAP_0279 to -0281, AAP_0288, AAP_292, AAP_1540, AAP_1541, AAP_2121) that encode other possible substrates. The *flp-tad* cluster (AAP_0177 to -0190) is similar to the *tad* locus involved in the rough colony phenotype in *Aggregatibacter actinomycetemcomitans* (12, 13, 14, 21). Also present is a locus required for the assembly of type IVa pili, including *pilF*, *pilA*, *pilB*, *pilC*, and *pilD* (AAP_0008, AAP_1464 to -1467). The *A. aphrophilus* genome contains genes encoding several adhesins that may participate in host colonization (EmaA, AAP_0065; Aae, AAP_0152; YadA and Hia, AAP_0523 and AAP_0527).

Genes for the production of PGA (poly-*N*-acetylglucosamine), i.e., *hmsD*, *pgaC*, *pgaB*, and *pgaA* (AAP_1678 to -1681); *N*-acetylneuraminase lyase (*nanA*, AAP_A0548); and the *dspB* enzyme that degrades PGA (AAP_0383 and AAP_0384), all implicated in biofilm formation, are present (6).

A. aphrophilus NJ8700 has several loci implicated in iron utilization, including one for a predicted hemoglobin binding protein, *hgpA* (AAP_1269), and a hemoglobin/transferrin binding receptor (AAP_2099). Genes for the hemophore receptor HasR (AAP_1311) and a heme utilization protein (AAP_2308) are present. A gene for the TbpA (transferrin binding protein; AAP_1194, AAP_1226) may signal an ability to use transferrin. *A. aphrophilus* carries genes encoding potential siderophore receptors (AAP_0347, AAP_0905), a TonB-dependent hemoglobin/transferrin/lactoferrin family receptor (AAP_1145), and a receptor for ferrienterochelin/colicins (AAP_1677). Two ORFs may encode chelatin transporters (AAP_1146 to -1149; AAP_783 to -785), along with the ferric-dicitrate transport system (*fecBCDE*, AAP_1294 to -1297). The genome harbors the genes coding for the Fur

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regulator (AAP_0360) and periplasmic-binding transport systems: the *afe* locus (AAP_0393, AAP_0395 to -0397), the *hit* locus (AAP_1640, AAP_1644 to -1654), and *afu* loci (AAP_0695 to -0697, AAP_1193 to -1196) (5, 18, 19, 22).

There are three regions of the bacterial chromosome where phage/prophage gene clusters were identified, including the *accA*-GMP gene intergenic region (acetyl coenzyme A carboxylase, AAP_0460; GMP synthase, AAP_0517), which harbors a prophage (M. Di Bonaventura et al., submitted for publication).

Nucleotide sequence accession number. The complete genome sequence of *Aggregatibacter (Haemophilus) aphrophilus* strain NJ8700 has been assigned GenBank accession number CP001607.

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