

Complete Genome Sequence of the Endosymbiont of *Acanthamoeba* Strain UWC8, an Amoeba Endosymbiont Belonging to the “*Candidatus* Midichloriaceae” Family in *Rickettsiales*

Zhang Wang, Martin Wu

Department of Biology, University of Virginia, Charlottesville, Virginia, USA

The endosymbiont of *Acanthamoeba* strain UWC8 is an obligate amoeba endosymbiont belonging to the family of “*Candidatus* Midichloriaceae” in *Rickettsiales*. We report here the complete genome sequence of this bacterium, which should catalyze future studies of amoeba-symbiont interactions.

Received 17 July 2014 Accepted 21 July 2014 Published 7 August 2014

Citation Wang Z, Wu M. 2014. Complete genome sequence of the endosymbiont of *Acanthamoeba* strain UWC8, an amoeba endosymbiont belonging to the “*Candidatus* Midichloriaceae” family in *Rickettsiales*. *Genome Announc.* 2(4):e00791-14. doi:10.1128/genomeA.00791-14.

Copyright © 2014 Wang and Wu. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Martin Wu, mw4yv@virginia.edu.

“*Candidatus* Midichloriaceae” is a recently defined family in *Rickettsiales* that is distinct from previously described *Rickettsiaceae*, *Anaplasmataceae*, and *Holosporaceae* families (1). It consists of intracellular bacteria residing within a wide range of host species, including ticks, fleas, stink bugs, amoebae, cnidarian, sponges, fish, and humans (2–11). “*Ca. Midichloriaceae*” is closely related to mitochondria (3). Despite being of significant ecological and evolutionary interest, only “*Candidatus* Midichloria mitochondrii” in this family has been sequenced (3). Here we report the complete genome sequence of the endosymbiont of *Acanthamoeba* UWC8 that belongs to the “*Ca. Midichloriaceae*” family.

The *Acanthamoeba* UWC8 strain harboring the endosymbiont was obtained from ATCC and cultured according to ATCC’s protocol. The amoeba cells from 10 T-75 tissue flasks were harvested and resuspended in the endosymbiont extraction buffer (0.3 M-sucrose, 10 mM Tris-HCl, 5 mM MgCl₂, pH 7.5). The cells were subsequently homogenized using a Dounce tissue grinder (Wheaton), and endosymbiont cells were isolated by differential centrifugation. The genome was sequenced by a 454 GS-FLX System using a 3-kb paired-end library, generating 104,222 reads totaling 31 Mbp with an average coverage depth of 20×. The genome was assembled by GS *De Novo* Assembler (Newbler) version 2.5.3. PCR and Sanger sequencing were used to close the gaps between contigs when necessary.

The complete genome of the endosymbiont of *Acanthamoeba* UWC8 consists of a single molecule of 1,615,277 bp with a G+C content of 34.7%. The genome was annotated using the IGS Annotation Engine (12): 89.5% of the genome was predicted to encode 1,608 open reading frames (ORFs), 36 tRNA genes, and one rRNA operon, and, of these, 1,049 (65.2%) ORFs can be assigned a putative function. The genome is predicted to encode major metabolic pathways, including a complete TCA cycle, electron transport chain, and pentose phosphate pathway. While most enzymes of glycolysis are present in the genome, the key enzyme phosphofructokinase (*PfkA*) converting beta-D-fructose-6P to beta-D-fructose-1,6-2P is missing. Instead, the fructose-1,6-bisphosphatase (*fbp*) catalyzing the opposite reaction is encoded in the genome, suggesting that gluconeogenesis instead of glycol-

ysis is functional. Consistent with its obligate intracellular lifestyle, the endosymbiont is deficient in the biosynthesis of many of its own metabolic substances, including amino acids, nucleotides, and several coenzymes. Accordingly, it encodes a large number of ABC-type transporters and solute carrier families importing most of these nutrients from the host. The genome encodes complete pathways for synthesizing both peptidoglycan and lipopolysaccharide (LPS), suggesting that a complete cell envelope structure can be assembled. In addition, the genome encodes 4 genes in capsule biosynthesis and secretion that are absent in other *Rickettsiales*, which is consistent with the previous finding that capsules or slime layers are produced by this bacterium (13). This genome represents a valuable resource for future studies of this organism and amoeba-symbiont interactions in general.

Nucleotide sequence accession number. The complete genome sequence of the endosymbiont of *Acanthamoeba* UWC8 has been deposited at DDBJ/EMBL/GenBank under the accession number CP004403.

ACKNOWLEDGMENTS

We are grateful to Dr. Michelle Giglio for her help on annotating the genome sequence.

This research was funded by an award from the Thomas F. and Kate Miller Jeffress Memorial Trust to M.W.

REFERENCES

1. Montagna M, Sasser D, Epis S, Bazzocchi C, Vannini C, Lo N, Sacchi L, Fukatsu T, Petroni G, Bandi C. 2013. “*Candidatus* Midichloriaceae” fam. nov. (*Rickettsiales*), an ecologically widespread clade of intracellular Alphaproteobacteria. *Appl. Environ. Microbiol.* 79:3241–3248. <http://dx.doi.org/10.1128/AEM.03971-12>.
2. Beninati T, Lo N, Sacchi L, Genchi C, Noda H, Bandi C. 2004. A novel alpha-proteobacterium resides in the mitochondria of ovarian cells of the tick *Ixodes ricinus*. *Appl. Environ. Microbiol.* 70:2596–2602. <http://dx.doi.org/10.1128/AEM.70.5.2596-2602.2004>.
3. Sasser D, Lo N, Epis S, D’Auria G, Montagna M, Comandatore F, Horner D, Peretó J, Luciano AM, Franciosi F, Ferri E, Crotti E, Bazzocchi C, Daffonchio D, Sacchi L, Moya A, Latorre A, Bandi C. 2011. Phylogenomic evidence for the presence of a flagellum and cbb(3)

- oxidase in the free-living mitochondrial ancestor. *Mol. Biol. Evol.* 28: 3285–3296. <http://dx.doi.org/10.1093/molbev/msr159>.
4. Fraune S, Bosch TC. 2007. Long-term maintenance of species-specific bacterial microbiota in the basal metazoan *Hydra*. *Proc. Natl. Acad. Sci. U. S. A.* 104:13146–13151. <http://dx.doi.org/10.1073/pnas.0703375104>.
 5. Lloyd SJ, LaPatra SE, Snekvik KR, St-Hilaire S, Cain KD, Call DR. 2008. Strawberry disease lesions in rainbow trout from southern Idaho are associated with DNA from a *Rickettsia*-like organism. *Dis. Aquat. Organ* 82:111–118. <http://dx.doi.org/10.3354/dao01969>.
 6. Erickson DL, Anderson NE, Cromar LM, Jolley A. 2009. Bacterial communities associated with flea vectors of plague. *J. Med. Entomol.* 46: 1532–1536. <http://dx.doi.org/10.1603/033.046.0642>.
 7. Vannini C, Ferrantini F, Schleifer KH, Ludwig W, Verni F, Petroni G. 2010. “*Candidatus anadelfobacter veles*” and “*Candidatus cyrtobacter comes*,” two new *Rickettsiales* species hosted by the protist ciliate *Euplotes harpa* (Ciliophora, Spirotrichea). *Appl. Environ. Microbiol.* 76: 4047–4054. <http://dx.doi.org/10.1128/AEM.03105-09>.
 8. Mediannikov OI, Ivanov LI, Nishikawa M, Saito R, Sidel'nikov IuN, Zdanovskaia NI, Mokretsova EV, Tarasevich IV, Suzuki H. 2004. Microorganism “Montezuma” of the order *Rickettsiales*: the potential causative agent of tick-borne disease in the Far East of Russia. *Zh. Mikrobiol. Epidemiol. Immunobiol.*:7–13.
 9. Mariconti M, Epis S, Gaibani P, Dalla Valle C, Sasseria D, Tomao P, Fabbì M, Castelli F, Marone P, Sambri V, Bazzocchi C, Bandi C. 2012. Humans parasitized by the hard tick *Ixodes ricinus* are seropositive to *Midichloria mitochondrii*: is *Midichloria* a novel pathogen, or just a marker of tick bite? *Pathog. Glob. Health* 106:391–396. <http://dx.doi.org/10.1179/2047773212Y.0000000050>.
 10. Sunagawa S, Woodley CM, Medina M. 2010. Threatened corals provide underexplored microbial habitats. *PLoS One* 5:e9554. <http://dx.doi.org/10.1371/journal.pone.0009554>.
 11. Boscaro V, Petroni G, Ristori A, Verni F, Vannini C. 2013. “*Candidatus Defluviella procrastinata*” and “*Candidatus Cyrtobacter zanobii*”, two novel ciliate endosymbionts belonging to the “*Midichloria* clade”. *Microb. Ecol.* 65:302–310. <http://dx.doi.org/10.1007/s00248-012-0170-3>.
 12. Galens K, Orvis J, Daugherty S, Creasy HH, Angiuoli S, White O, Wortman J, Mahurkar A, Giglio MG. 2011. The IGS standard operating procedure for automated Prokaryotic annotation. *Stand. Genomics Sci.* 4:244–251. <http://dx.doi.org/10.4056/signs.1223234>.
 13. Fritsche TR, Horn M, Seyedirashti S, Gautom RK, Schleifer KH, Wagner M. 1999. In situ detection of novel bacterial endosymbionts of *Acanthamoeba* spp. phylogenetically related to members of the order *Rickettsiales*. *Appl. Environ. Microbiol.* 65:206–212.