



Draft Genome Sequence and Annotation of the Obligate Bacterial Endosymbiont *Caedibacter taeniospiralis*, Causative Agent of the Killer Phenotype in *Paramecium tetraurelia*

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ABSTRACT *Caedibacter taeniospiralis* is an obligate endosymbiont living in the cytoplasm of *Paramecium tetraurelia*. *C. taeniospiralis* causes the so-called killer trait, eliminating intraspecific competitors of its host when released into the medium by the concerted action of the unusual protein structure R-body (refractile body) in addition to an as-yet-unknown toxin.

Caedibacter taeniospiralis (Thiotrichales, Gammaproteobacteria) lives in the cytoplasm of its host, Paramecium tetraurelia (Ciliophora). This unicellular, relatively large (150- μ m) eukaryote separates vegetative and sexual functions, possesses germline and somatic nuclei, and is a long-established model in genetics and epigenetics research (1). A special feature of the Paramecium-Caedibacter symbiosis is the "killer trait." A proportion of the endosymbiont population produces an unusual protein structure, the R-body (refractile body) (2). This structure, most likely acting as a delivery device for an unidentified toxin, is responsible for killing symbiont-free, sensitive paramecia after the ingestion of bacteria released from infected strains (3). Caedibacter-harboring cells are protected from the lethal effect of their symbionts. Cultivation of *C. taeniospiralis* outside its host has not been accomplished so far.

Total DNA was isolated from exponentially growing paramecia. Their infection status was verified by fluorescence *in situ* hybridization, which enabled detection of intracellular *C. taeniospiralis* 51^T, as described elsewhere (4). To minimize contamination from food bacteria, *P. tetraurelia* cultures (strain 51K = CCAP 1660/3F) were fed with β -lactam-hypersensitive *Escherichia coli* Δ tolC (5) and treated with 10 μ g ml⁻¹ ampicillin before DNA isolation (6). Library preparation for whole-genome sequencing used the tagmentation procedure (7). We generated a library of ~500 to 800 bp for subsequent sequencing on an Illumina MiSeq instrument (2 × 300 nucleotides [nt]). After assembly using the ABySS-pe program (8), 24 contigs were assigned to the genome of *C. taeniospiralis*. These can be differentiated from the host genome by their nearly identical coverage and by their GC content of 41.5%, diverging to the host genome's GC content of 28% (9). Draft genome sequences had a total sequence length of 1.3 Mb (N_{50} , 55.531 bp), including the previously known plasmid, pKAP, of 41.7 kb (10). The genome contains three rRNA clusters in 16S-23S-5S configurations.

Gene annotation of the draft sequence was carried out using Prodigal (11), tRNAscan-SE (12), and RNAmmer (13) software tools. Next to 36 tRNA genes, 1,080

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Address correspondence to Martina Schrallhammer, martina.schrallhammer@biologie.uni-freiburg.de, or Martin Simon, martin.simon@uni-saarland.de. protein coding sequences could be identified, of which, 787 could be functionally annotated by a similarity search against the eggNOG database with the eggNOG mapper software (14).

The genome sequence of *C. taeniospiralis* strain 51K will improve our understanding of this organism and its symbiotic interaction with *P. tetraurelia*, including and in addition to the killer trait. The availability of this sequence information enables phylogenomic analyses of the genus *Caedibacter* and will provide a valuable resource for the identification and analysis of toxin candidates, which might have unusual biological properties that explain the need for the R-body as a delivery device.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number PGGB00000000. The version described in this paper is version PGGB01000000.

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REFERENCES

- Simon M, Plattner H. 2014. Unicellular eukaryotes as models in cell and molecular biology: critical appraisal to their past and future value. Int Rev Cell Mol Biol 309:141–198. https://doi.org/10.1016/B978-0-12-800255-1 .00003-X.
- Schrallhammer M, Schweikert M. 2009. The killer effect of *Paramecium* and its causative agents, p 1–21. *In* Fujishima M (ed), Endosymbionts in Paramecium, microbiology monographs. Springer, New York, NY.
- Schrallhammer M, Galati S, Altenbuchner J, Schweikert M, Görtz HD, Petroni G. 2012. Tracing the role of R-bodies in the killer trait: absence of toxicity of R-body producing recombinant *E. coli* on paramecia. Eur J Protistol 48:290–296. https://doi.org/10.1016/j.ejop.2012.01.008.
- Dusi E, Krenek S, Schrallhammer M, Sachse R, Rauch G, Kaltz O, Berendonk TU. 2014. Vertically transmitted symbiont reduces host fitness along temperature gradient. J Evol Biol 27:796–800. https://doi.org/10 .1111/jeb.12336.
- Lagkouvardos I, Shen J, Horn M. 2014. Improved axenization method reveals complexity of symbiotic associations between bacteria and acanthamoebae. Environ Microbiol Rep 6:383–388. https://doi.org/10.1111/ 1758-2229.12162.
- DOE Joint Genome Institute. 2012. Bacterial DNA isolation CTAB protocol. http://lofdmq2n8tc36m6i46scovo2e.wpengine.netdna-cdn.com/wp -content/uploads/2014/02/JGI-Bacterial-DNA-isolation-CTAB-Protocol -2012.pdf.
- Picelli S, Björklund AK, Reinius B, Sagasser S, Winberg G, Sandberg R. 2014. Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. Genome Res 24:2033–2040. https://doi.org/ 10.1101/gr.177881.114.
- 8. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I. 2009.

ABySS: a parallel assembler for short read sequence data. Genome Res 19:1117–1123. https://doi.org/10.1101/gr.089532.108.

- Aury JM, Jaillon O, Duret L, Noel B, Jubin C, Porcel BM, Ségurens B, Daubin V, Anthouard V, Aiach N, Arnaiz O, Billaut A, Beisson J, Blanc I, Bouhouche K, Câmara F, Duharcourt S, Guigo R, Gogendeau D, Katinka M, Keller AM, Kissmehl R, Klotz C, Koll F, Le Mouël A, Lepère G, Malinsky S, Nowacki M, Nowak JK, Plattner H, Poulain J, Ruiz F, Serrano V, Zagulski M, Dessen P, Bétermier M, Weissenbach J, Scarpelli C, Schächter V, Sperling L, Meyer E, Cohen J, Wincker P. 2006. Global trends of wholegenome duplications revealed by the ciliate *Paramecium tetraurelia*. Nature 444:171–178. https://doi.org/10.1038/nature05230.
- Jeblick J, Kusch J. 2005. Sequence, transcription activity, and evolutionary origin of the R-body coding plasmid pKAP298 from the intracellular parasitic bacterium *Caedibacter taeniospiralis*. J Mol Evol 60:164–173. https://doi.org/10.1007/s00239-004-0002-2.
- Hyatt D, Chen GL, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. https://doi.org/10.1186/1471 -2105-11-119.
- Lowe TM, Chan PP. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44: W54–W57. https://doi.org/10.1093/nar/gkw413.
- Lagesen K, Hallin PF, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108. https://doi.org/10.1093/nar/gkm160.
- Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, von Mering C, Bork P. 2017. Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. Mol Biol Evol 34:2115–2122. https://doi .org/10.1093/molbev/msx148.