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Genome Sequence of *Haloplasma contractile*, an Unusual Contractile Bacterium from a Deep-Sea Anoxic Brine Lake[∇]

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We present the draft genome of *Haloplasma contractile*, isolated from a deep-sea brine and representing a new order between Firmicutes and Mollicutes. Its complex morphology with contractile protrusions might be strongly influenced by the presence of seven MreB/Mbl homologs, which appears to be the highest copy number ever reported.

Haloplasma contractile was isolated from Shaban Deep (2), one of the most extreme environments on Earth (3). It is the sole representative of *Haloplasmatales* and has a unique morphology and cellular dynamics. Its cellular contractility suggests an involvement of cytoskeleton elements in a flagellum-independent motility previously reported solely for *Spiroplasma*.

Haloplasma contractile was grown in DSMZ medium 1231 under optimal growth conditions (2). Genomic DNA was extracted from the biomass obtained by using a blood & cell culture DNA minikit (Qiagen), following the manufacturer's instructions. Genome sequencing was performed using a combination of Roche 454 GS (FLX Titanium) and Illumina (single and paired-end) sequencing platforms. A total of 81,201,517 bp (mean read length, 289 bp) was obtained from Roche 454, providing approximately 21-fold coverage. Single and paired-end Illumina data provided 193,522,440 bp (mean read length, 30 bp) and 196,095,200 bp (mean read length, 35 bp), corresponding to 468-fold coverage. Roche 454 data were assembled using Newbler Assembler, version 2.5 (Roche), while Illumina data were assembled with SOAPdenovo (<http://soap.genomics.org.cn/soapdenovo.html>). Assemblies were merged using AMOS Minimus2 (<http://sourceforge.net/apps/mediawiki/amos/index.php?title=Minimus2>).

The sequences assembled into 40 scaffolds (N50 contig size of approximately 348.8 kb, where N50 is the contig length such that at least 50% of the bases of the assembly are contained within contigs of this size or greater). Genes were identified using Prodigal software (6) followed by mpiBLAST (<http://www.mpiblast.org/>) and EBI-Interproscan (<http://www.ebi.ac.uk/InterProScan/>) annotation matching data in public databases. This approach provided annotation for 84% of all 3,984 predicted genes. The draft genome has a G+C content of 33%.

The most striking features of *Haloplasma contractile* are its

unique morphology and cellular dynamics. The underlying genetic aspects of microbial cell morphology are still not well understood but seem to be related to cell wall, cytoskeleton, and membrane-bound linking proteins (4). The *dcw* gene cluster plays a particularly important role, with different morphologies reflecting the presence/absence of specific genes, as well as gene arrangement and proximity (8, 11). In *H. contractile*, this cluster mostly retained the relative gene order and content predicted for the ancestral cluster (9), like *Bacillus subtilis* (11) but different from the mollicutes, which display multiple deletions (1). However, multiple insertions split the *dcw* cluster in *H. contractile* and disrupted the *murD-ftsW-murG* gene sequences. Such disruptions seem to have originated all nonrod morphologies currently known (10, 11).

The *dcw* cluster of *H. contractile* includes all genes required for peptidoglycan synthesis, although cellular contractility and the results of previous tests seemed to preclude the presence of peptidoglycan. Synthesis *in vivo* might not occur or may be restricted or limited to the noncontractile central body.

The presence of genes involved in the synthesis of cytoskeletal elements (e.g., actin and tubulin homologs) was also confirmed. MreB/Mbl seems especially relevant, due to its typical helical placement and major role in cell contractility in *Spiroplasma* (7). The seven MreB/Mbl homologs detected in *H. contractile* are the highest copy number ever reported and might strongly influence morphology and contractility. Previous studies had shown their number to vary between one and three except for *Spiroplasma*, where five different homologs were detected (5, 7).

Nucleotide sequence accession number. Nucleotide sequences are available in GenBank, project identification number 66977, with accession number AFNU000000000.

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