

EXTENDED GENOME REPORT

Open Access



Complete genome sequence analysis of *Archaeoglobus fulgidus* strain 7324 (DSM 8774), a hyperthermophilic archaeal sulfate reducer from a North Sea oil field

Nils-Kåre Birkeland^{1*} , Peter Schönheit², Lianna Poghosyan¹, Anne Fiebig^{3,4} and Hans-Peter Klenk^{3,5}

Abstract

Archaeoglobus fulgidus is the type species of genus *Archaeoglobus* Stetter 1998, a hyperthermophilic sulfate reducing group within the *Archaeoglobi* class of the euryarchaeota phylum. Members of this genus grow heterotrophically or chemolithoautotrophically with sulfate or thiosulfate as electron acceptors. Except for *A. fulgidus* strain 7324 and the candidate species "*Archaeoglobus lithotrophicus*", which both originate from deep oil-fields, the other members of this genus have been recovered from marine hydrothermal systems. Here we describe the features of the *A. fulgidus* strain 7324 genome as compared to the *A. fulgidus* VC16 type strain. The 2.3 Mbp genome sequence of strain 7324 shares about 93.5% sequence identity with that of strain VC16^T but is about 138 Kbp longer, which is mostly due to two large 'insertions' carrying one extra *cdc6* (cell-cycle control protein 6) gene, extra CRISPR elements and mobile genetic elements, a high-GC ncRNA gene (*hgcC*) and a large number of hypothetical gene functions. A comparison with four other *Archaeoglobus* spp. genomes identified 1001 core *Archaeoglobus* genes and more than 2900 pan-genome orthologous genes.

Keywords: Anaerobic, Hyperthermophile, Sulfate reduction, SRB, Petroleum, Oil-field

Introduction

Archaeoglobus fulgidus strain 7324 was recovered from hot oil-field water originating from a deep oil-well in the North Sea [1]. It shares many features with the *A. fulgidus* type strain VC16, e.g. dissimilatory sulfate reduction, utilization of lactate and pyruvate as carbon sources, irregular coccoid to disc-shaped cells, and blue-green fluorescence under the UV microscope due to the presence of Coenzyme F₄₂₀. Strain VC16^T was isolated from a shallow marine hydrothermal system at Volcano island, Italy [2]. The complete genome sequence of strain VC16^T was reported in 1997 as the third archaeal genome to be fully sequenced [3] and *A. fulgidus* has since served as a prototype for studies of archaeal and hyperthermophilic sulfate reduction [4]. Here we report a summary of the features of *A. fulgidus* strain 7324, together with the

description of the complete genomic sequencing and annotation and comparison with the genome of the *A. fulgidus* type strain and other *Archaeoglobus* spp.

Organism information

Classification and features

Genus *Archaeoglobus* comprises five validly published species; *A. fulgidus* [2], *Archaeoglobus profundus* [5], *Archaeoglobus veneficus* [6], *Archaeoglobus infectus* [7], *Archaeoglobus sulfaticallidus* [8], and one candidate species termed "*Archaeoglobus lithotrophicus*" [9]. All are hyperthermophilic sulfate-reducers capable of heterotrophic or chemolithoautotrophic growth on H₂ and CO₂. The *Archaeoglobus* 'clade' also encompasses a few non-sulfate reducing anaerobic hyperthermophiles; *Geoglobus acetivorans* [10] and "*Geoglobus ahangari*" [11, 12], which are both Fe(III) reducers, and *Ferroglobus placidus*, which is capable of using ferrous iron, H₂ and sulfide as electron donors with nitrate as electron acceptor [13]. Fig. 1 shows the phylogenetic affiliation of all current members of the

* Correspondence: nils.birkeland@uib.no

¹Department of Biology, University of Bergen, P.O. Box 7803, NO-5020 Bergen, Norway

Full list of author information is available at the end of the article

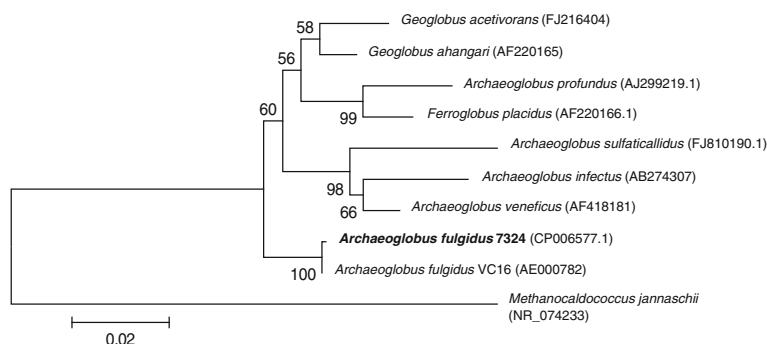


Fig. 1 16S rRNA-based phylogenetic tree showing the position of the *Archaeoglobus fulgidus* strains within the *Archaeoglobaceae* family, using *Methanocaldococcus jannaschii* as outgroup. Only species with validly published names are included. The sequences were aligned using Clustal X [46] and the tree was inferred using the Neighbor-joining algorithm in MEGA 6.06 [47]. The bar indicated number of substitutions per site. Bootstrap values $\geq 60\%$ are indicated at nodes and are based on 100 replicates. Sequence accession numbers are indicated in brackets. *A. fulgidus* strain 7324 is in bold

Archaeoglobaceae family, including strain 7324. All *Archaeoglobus* species form small irregularly shaped cells. A scanning electron micrograph of *A. fulgidus* strain 7324 is shown in Fig. 2, revealing a similar cell shape as originally determined by transmission electron microscopy [1]. Strain 7324 has not been phylogenetically characterized by 16S rRNA gene sequencing before, but a wet lab genomic DNA: DNA hybridization with *A. fulgidus* strain Z, which, like the type strain, was recovered from the Vulcano

island [14], revealed a genome hybridization value of 100% [1]. This close relationship was now confirmed via digital DNA-DNA hybridization [15] between strains VC16^T and 7324 with a GLM-based DDH estimate of 93.9%. All three *A. fulgidus* strains share common physiological characteristics, like growth from 60 °C to above 84 °C, use of sulfate and thio-sulfate as electron acceptors, optimal growth with lactate or pyruvate as carbon sources, and production of trace amounts of methane. Although the

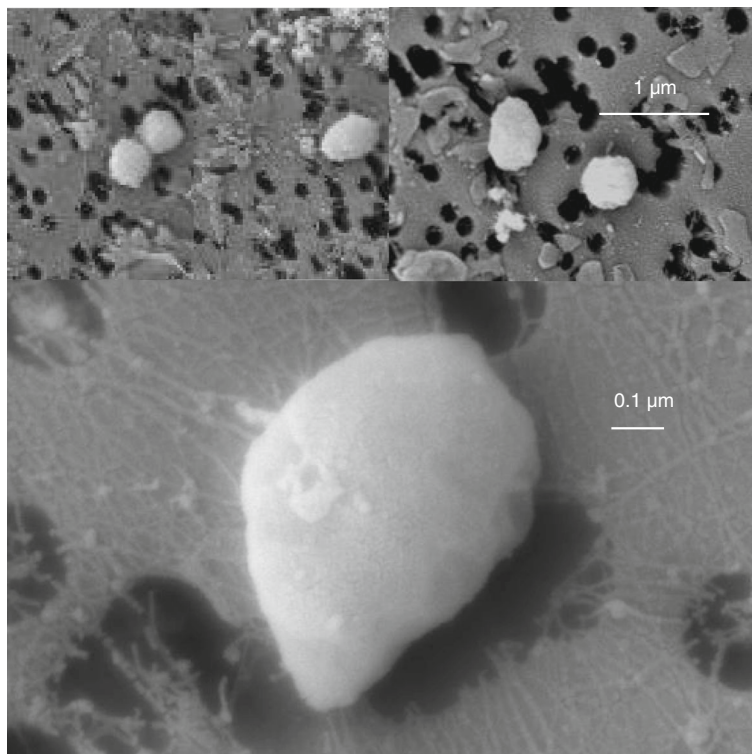


Fig. 2 Scanning electron micrographs of cells of *A. fulgidus* strain 7324. Bars equal 1 µm (upper panel) or 0.1 µm (lower panel)

optimal growth temperature of strain 7324 was initially determined to 76 °C, we have routinely been cultivating it at 80 °C. In contrast to the other isolates, strain 7324 rapidly lyses after the stationary phase [1]. The main features of the organism are listed in Table 1.

Genome sequencing information

Genome project history

A. fulgidus strain 7324 was chosen for whole genome sequencing because it was isolated from a deep and hot oil reservoir while the closely related type strain, VC-16, was isolated from a shallow marine hot vent. A genome comparison might reveal particular adaptations of strain 7324 to the deep biosphere. The genome project information is given in the Genomes OnLine Database (Gp0102124). The genome sequence is deposited in GenBank (CP006577.1). A summary of the project information is shown in Table 2.

Growth conditions and genomic DNA preparation

A. fulgidus strain 7324 was from our own collection at the University of Bergen. It was cultivated in anaerobic medium containing lactate and sulfate as described previously [1]. The incubation temperature was 80 °C. Genomic DNA was isolated using a modification of the cetyl trimethylammonium bromide method as described [16].

Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [17]. The initial assembly of 454 raw data suggested a contamination of the sequenced sample. Using blast search, all contigs (>500 nt in length) could be assigned either to *A. fulgidus* or *Thermococcus litoralis*, an archaeon that shares the same habitat [18]. To overcome this issue, two additional blast searches including all contigs longer than 500 nt were performed

Table 1 Classification and general features of *Archaeoglobus fulgidus* strain 7324 (DSM 8774)

MIGS ID	Property	Term	Evidence code ^a
	Current classification	Domain <i>Archaea</i>	TAS [40]
		Phylum <i>Euryarchaeota</i>	TAS [41]
		Class <i>Archaeoglobi</i>	TAS [42]
		Order <i>Archaeoglobales</i>	TAS [43]
		Family <i>Archaeoglobaceae</i>	TAS [44]
		Genus <i>Archaeoglobus</i>	TAS [2]
		Species <i>Archaeoglobus fulgidus</i>	TAS [2]
		Strain 7324	TAS [1]
	Gram stain	Negative	IDA
	Cell shape	Irregular coccoid to disc shaped	TAS [1]
	Motility	Non-motile	TAS [1]
	Sporulation	Nonsporulating	NAS
	Temperature range	60 to 85 °C	TAS [1]
	Optimum temperature	76 °C ^b	TAS [1]
	Carbon source	Lactate, pyruvate, valerate	TAS [1]
	Energy source	Lactate, pyruvate, valerate + H ₂	TAS [1]
	Terminal electron receptor	Sulfate and thiosulfate	TAS [1]
MIGS-6	Habitat	Deep oil-fields	TAS [1]
MIGS-6.3	Salinity	3–500 mM NaCl (optimum at 300 mM)	TAS [1]
MIGS-22	Oxygen	Strictly anaerobic	TAS [1]
MIGS-15	Biotic relationship	Free-living	TAS [1]
MIGS-14	Pathogenicity	None	NAS
MIGS-4	Geographic location	Norway/North Sea	TAS [1]
MIGS-5	Sample collection time	1993 or earlier	TAS [1]
MIGS-4.3	Depth	Subsurface	TAS [1]

^aEvidence codes – *IDA* Inferred from Direct Assay, *TAS* Traceable Author Statement (i.e., a direct report exists in the literature), *NAS*, Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [45]

^bThe strain grows well and has been routinely cultivated the last two decades in our labs at 80 °C

Table 2 Project information

MIGS ID	Property	Term
MIGS 31	Finishing quality	Finished
MIGS-28	Libraries used	454 Titanium paired-end, Solexa paired end
MIGS 29	Sequencing platforms	454-GS-FLX, Solexa, Illumina
MIGS 31.2	Fold coverage	103x
MIGS 30	Assemblers	Newbler, Velvet v. 1.0.18; Consed v.20.0
MIGS 32	Gene calling method	GeneMark
	Locus Tag	AFULGI
	Genbank ID	CP006577.1
	GenBank Date of Release	01.10.2014
	GOLD ID	Gp0102124
	BIOPROJECT	PRJNA208006
MIGS 13	Source Material Identifier	DSM 8774
	Project relevance	Environmental, evolution of anaerobic respiration

against the previously sequenced genome of *A. fulgidus* VC-16^T (NCBI/GenBank:AE000782) and all available genomic sequences of *Thermococcus* species in Genbank (Dec. 2010). Only sequences in length sharing more than 90% sequence identity with *A. fulgidus* VC-16^T and having no hits in the *Thermococcus* blast database were kept. A total of 84 Newbler contigs could be assigned to *A. fulgidus*. Illumina raw reads were assembled to 223 contigs. Both draft assemblies were merged in a hybrid approach using the phred/phrap/consed pipeline [19]. After manual curation, a total of 27 ordered gaps were closed by bridging PCRs at LGC Genomics (Berlin). The final consensus sequence represents a single circular chromosomal element (103x coverage).

Genome annotation

Coding genes were predicted by GeneMark [20] as part of the genome annotation pipeline in the Integrated Microbial Genomes Expert Review system [21]. The tRNAs were identified by tRNAscan-SE-1.23 [22], while ribosomal RNA genes within the genome were predicted using the tool RNAmmer [23]. Other non-coding RNA genes were predicted using Infernal [24]. CRISPR elements were identified by the program CRT [25]. Manual functional annotation was performed within the IMG platform [21] and the Artemis Genome Browser [26].

Genome properties

The genome of *A. fulgidus* strain 7324 comprises one circular chromosome with a total size of 2,316,287 bp, which is 137,887 bp larger than *A. fulgidus* VC16^T DSM

3404 [3]. The mole percent G + C is 48.08, which is slightly higher than the 47% value estimated previously with thermal denaturation [1] and slightly lower than for the type strain DSM 3404 (48.6%); in any case within the 1% threshold with the species' type strain VC16^T sensu Meier-Kolthoff et al. [27]. No plasmids were detected. The strain 7324 genome is the largest of the genome-sequenced *Archaeoglobus* species, the smallest one being the *A. profundus* genome with a total size of 1.56 Mbp [28]. Out of the total 2615 genes annotated in the 7324 genome, 2558 were identified as protein coding genes and 56 as RNA genes (Table 3). Only 67.29% of the genes could be assigned to COG functional categories as listed in Table 4. Five CRISPR repeat regions were identified, as compared with only three in strain VC16^T (AE000782). There is only one rRNA operon (Fig. 3). As for VC16^T, there is no apparent GC skew in the genome, which could indicate the presence of multiple DNA replication origins and explain previous difficulties in precise mapping of replication origin(s) in this species using a marker rescue analysis approach [29, 30].

Insights from the genome sequence

Genes encoding central metabolic pathways like dissimilatory sulfate reduction, lactate oxidation, a complete TCA cycle and the Acetyl-CoA pathway were identified in strain 7324, confirming previous physiological characterization and similarity with strain VC16^T [1]. A genome alignment between strains VC16^T and 7324 revealed a large degree of genomic similarity and sequence synteny, interrupted mostly by two large additional regions of about 64 and 109 Kbp (InsI and InsII, respectively) in strain 7324 as compared to VC16^T (Fig. 4). Both these regions are flanked by a

Table 3 Genome statistics

Attribute	Value	% of Total
Genome size (bp)	2,316,287	n/a
DNA coding (bp)	2,077,792	89.70
DNA G + C (bp)	1,113,590	48.08
DNA scaffolds	1	n/a
Total genes	2615	100
Protein coding genes	2558	97.86
RNA genes	56	2.14
Pseudo genes	1	0.04
Genes in internal clusters	342	13.08
Genes with function prediction	1880	71.89
Genes assigned to COGs	1759	67.29
Genes with Pfam domains	1982	75.82
Genes with signal peptides	80	3.06
Genes with transmembrane helices	490	18.75
CRISPR repeats	5	n/a

Table 4 Number of genes associated with general COG functional categories

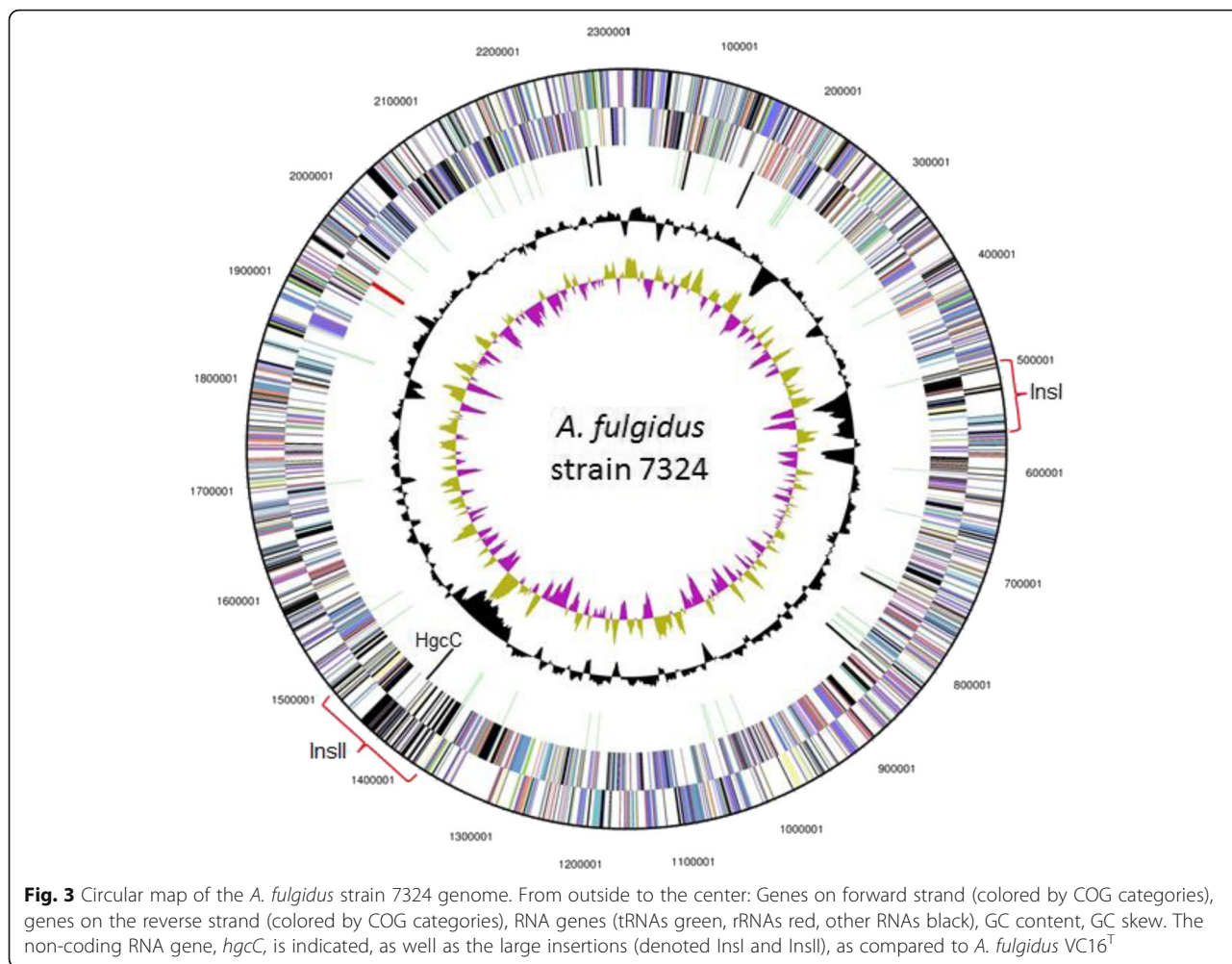
Code	Value	%age	Description
J	195	10.3	Translation, ribosomal structure and biogenesis
A	1	0.05	RNA processing and modification
K	90	4.75	Transcription
L	75	3.96	Replication, recombination and repair
B	7	0.37	Chromatin structure and dynamics
D	15	0.79	Cell cycle control, Cell division, chromosome partitioning
V	68	3.59	Defense mechanisms
T	54	2.85	Signal transduction mechanisms
M	42	2.22	Cell wall/membrane biogenesis
N	19	1	Cell motility
U	19	1	Intracellular trafficking and secretion
O	68	3.59	Posttranslational modification, protein turnover, chaperones
C	178	9.4	Energy production and conversion
G	48	2.54	Carbohydrate transport and metabolism
E	150	7.92	Amino acid transport and metabolism
F	64	3.38	Nucleotide transport and metabolism
H	138	7.29	Coenzyme transport and metabolism
I	111	5.86	Lipid transport and metabolism
P	82	4.33	Inorganic ion transport and metabolism
Q	32	1.69	Secondary metabolites biosynthesis, transport and catabolism
R	249	13.15	General function prediction only
S	170	8.98	Function unknown
-	855	32.71	Not in COGs

disrupted tRNA gene, which implies that InsI and II represent genomic insertions. They also possess a considerably lower G + C content (42%) as compared to the average of the genome (Fig. 3), indicating a result of recombination with an AT-rich organism. This is further supported by identification of a non-coding high-GC RNA gene in InsII belonging to the *hgcC* family of ncRNA (RFAM v12 accession code RF00062) typically found in AT-rich hyperthermophiles (Figs. 3 and 5b). This ncRNA family was originally identified in the genomes of *Pyrococcus furiosus* and *Methanocaldococcus jannaschii* [31] but its function is still unresolved.

InsII also contains a gene encoding an extra homologue of the Orc1/Cdc6 family of replication initiation control proteins in addition to the two other *cdc6* homologous in the 7324 and VC16^T genomes. The closest homologue identified by a blast search is from *A. veneficus* (62% amino acid sequence identity). The majority of the other genes are hypothetical or have a general function prediction only. InsI carries two CRISPR repeat regions and 14 genes encoding CRISPR-associated proteins including a Cas6 homologue (Fig. 5a). The rest of this insert mostly contains hypothetical genes.

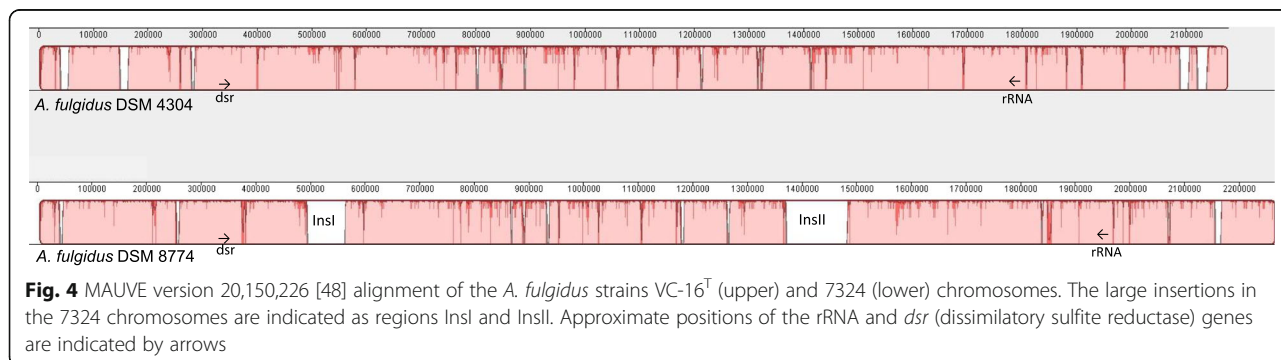
A Venn diagram shows that *A. fulgidus* strains VC16^T and 7324 share a large number of genes (2292) (Fig. 6a), reflecting the high degree of genome similarity. The 263 genes unique to strain 7324 include about 200 hypothetical genes/uncharacterized functions most of them belonging to the large insertions and the CRISPR-associated genes of InsII. The Venn diagram including all the five genome-sequenced strains revealed an *Archaeoglobus* core genome of 1001 genes (Fig. 6b), most of which encode energy-yielding, biosynthetic and regulatory functions. About 200 of the core genes belong to the hypothetical/uncharacterized category in the EggNog database [32]. This is considerably lower than the 32% fraction of unassigned genes for the entire strain 7324, but underpins that a large part of central gene functions in this genus still remain to be disclosed. About 2900 genes belong to the *Archaeoglobus* pan-genome, being unique to one of the genomes or shared by 2 to 4 of the species.

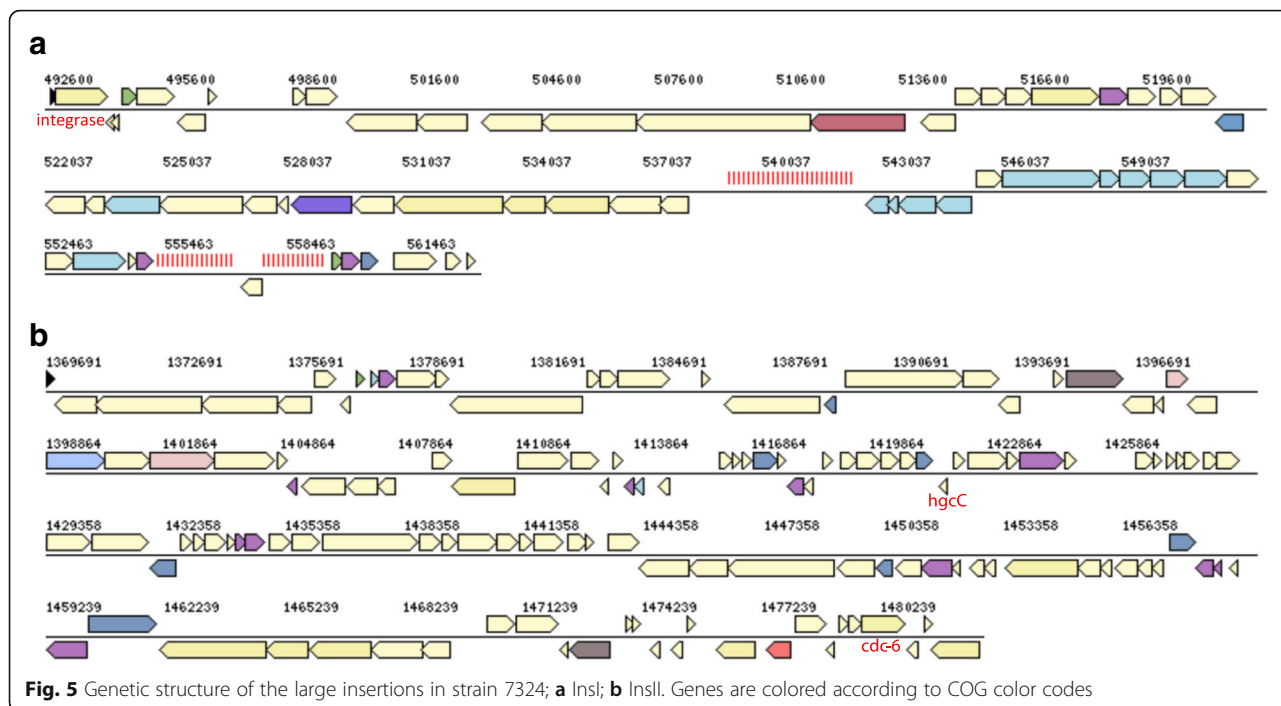
Archaeoglobus fulgidus strain 7324 has been reported to degrade starch [33] and several enzymes involved in starch degradation have been purified from cells grown on starch. These enzymes include cyclodextrin gluconotransferase, cyclodextrinase, maltodextrin phosphorylase, and phosphoglucomutase, ADP-dependent glucokinase, ADP-dependent



phosphofructokinase and pyruvate kinase [34–37]. The enzymes were biochemically characterized and the N-termini (with exception of cyclodextrinase) have been determined. They showed highest sequence identity to proteins from *Thermococcales*, e.g. from *Thermococcus litoralis*. However, in the genome of *A. fulgidus* presented here, genes encoding sugar degrading enzymes have not been identified. Rather, various analyses indicated that *A. fulgidus* strain 7324,

deposited as DSM 8774, was contaminated with a *Thermococcus* strain. The genome of this *Thermococcus* strain has also been sequenced and all genes encoding the sugar degrading enzymes, originally attributed to *A. fulgidus* strain 7324, were found in the *Thermococcus* strain genome. Also, glutamate dehydrogenase from a DSM 8774 culture grown in the presence of small amounts of yeast extract (0.3 gL⁻¹) was previously purified and characterized [38, 39], but



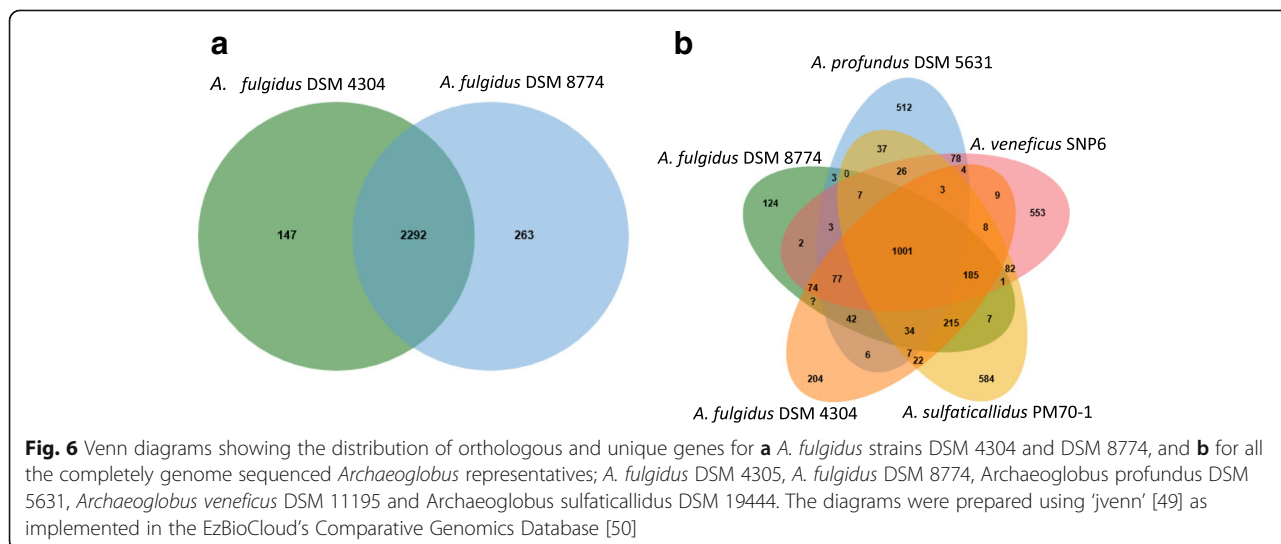


appears also to be encoded by the *Thermococcus* strain genome. The purity of the original *A. fulgidus* 7324 isolate was not assessed by 16S rRNA gene sequencing prior to deposition at DSMZ [1] and whether the *Thermococcus* contamination was present in the original culture or has been introduced at a different stage is not known. The genome analysis of this *Thermococcus* strain, which appears to represent a novel *Thermococcus* species, will be published separately.

Conclusions

The complete genome of *A. fulgidus* strain 7324, recovered from hot water produced from an oil well in

the North Sea was sequenced and annotated. In addition to the *A. fulgidus* type strain, VC16, isolated from a shallow hot vent in the Mediterranean, this is the second *A. fulgidus* genome to be characterized. The two strains share 93.5% genome sequence similarity, and differ mostly by two large insertions of 64 and 109 Kbp in strain 7324 that seem to have originated from an AT-rich archaeon. The insertions carry two additional CRISPR elements, an extra *cdc6* gene, a variety of mobile genetic elements and a large number of hypothetical and unassigned genes. Based on comparison with four other *Archaeoglobus* spp. genomes, the *Archaeoglobus* core genome was estimated



to 1001 genes. No particular traits indicating adaptation to the petroleum reservoir subsurface environment could be identified.

Authors' contributions

NKB, PS and HPK conceived and designed this project, and were involved in interpretation of the data. NKB, HPK and AF performed the sequencing, assembly and the bioinformatics analysis. LP performed the electron microscopy. NKB prepared the first draft of the manuscript. All authors were involved in critical revision of the manuscript and approved the final version.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Department of Biology, University of Bergen, P.O. Box 7803, NO-5020 Bergen, Norway. ²Institut für Allgemeine Mikrobiologie, Christian-Albrechts-Universität Kiel, 24118 Kiel, Germany. ³Leibniz-Institut DSMZ – German Collection of Microorganisms and Cell Cultures, Inhoffenstrasse 7b, 38124 Braunschweig, Germany. ⁴Present Address: IPK Gatersleben, Corrensstr. 3, 06466 Gatersleben, Germany. ⁵Present Address: School of Biology, Newcastle University, Newcastle upon Tyne NE1 7RU, UK.

Received: 24 July 2017 Accepted: 5 December 2017

Published online: 16 December 2017

References

1. Beeder J, Nilsen RK, Rosnes JT, Torsvik T, Lien T. *Archaeoglobus fulgidus* isolated from hot North Sea oil field waters. *Appl Env Microbiol.* 1994;60:1227–31.
2. Stetter KO. *Archaeoglobus fulgidus* gen. nov., sp. nov, a new taxon of extremely thermophilic Archaeobacteria. *Syst Appl Microbiol.* 1988;10:172–3.
3. Klenk HP, Clayton RA, Tomb JF, White O, Nelson KE, Ketchum KA, Dodson RJ, Gwinn M, Hickey EK, Peterson JD, et al. The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. *Nature.* 1997;390:364–70.
4. Parey K, Fritz G, Ermler U, Kroneck PMH. Conserving energy with sulfate around 100 degrees C - structure and mechanism of key metal enzymes in hyperthermophilic *Archaeoglobus fulgidus*. *Metallomics.* 2013;5:302–17.
5. Burggraf S, Jannasch HW, Nicolaus B, Stetter KO. *Archaeoglobus profundus* sp. nov., represents a new species within the sulfate-reducing Archaeobacteria. *Syst Appl Microbiol.* 1990;13:24–8.
6. Huber H, Jannasch H, Rachel R, Fuchs T, Stetter KO. *Archaeoglobus veneficus* sp. nov., a novel facultative chemolithoautotrophic hyperthermophilic sulfite reducer, isolated from abyssal black smokers. *Syst Appl Microbiol.* 1997;20:374–80.
7. Mori K, Maruyama A, Urabe T, Suzuki KI, Hanada S. *Archaeoglobus infectus* sp. nov., a novel thermophilic, chemolithoheterotrophic archaeon isolated from a deep-sea rock collected at Suiyo seamount, Izu-Bonin arc, western Pacific Ocean. *Int J Syst Evol Micr.* 2008;58:810–6.
8. Steinsbu BO, Thorseth IH, Nakagawa S, Inagaki F, Lever MA, Engelen B, Øvreas L, Pedersen RB. *Archaeoglobus sulfatcallidus* sp. nov., a thermophilic and facultatively lithoautotrophic sulfate-reducer isolated from black rust exposed to hot ridge flank crustal fluids. *Int J Syst Evol Micr.* 2010;60:2745–52.
9. Stetter KO, Huber R, Blochl E, Kurr M, Eden RD, Fielder M, Cash H, Vance I. Hyperthermophilic Archaea are thriving in deep North-Sea and Alaskan oil-reservoirs. *Nature.* 1993;365:743–5.
10. Slobodkina GB, Kolganova TV, Querellou J, Bonch-Osmolovskaya EA, Slobodkin AI. *Geoglobus acetivorans* sp. nov., an iron(III)-reducing archaeon from a deep-sea hydrothermal vent. *Int J Syst Evol Micr.* 2009;59:2880–3.
11. Kashefi K, Tor JM, Holmes DE, Van Praagh CVG, Reysenbach AL, Lovley DR. *Geoglobus ahangari* gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the sole electron acceptor. *Int J Syst Evol Micr.* 2002;52:719–28.
12. Tindall BJ, Euzéby JP. Status of strains that contravene rules 27 (3) and 30 of the international code of nomenclature of bacteria. Opinion 81. *Int J Syst Evol Microbiol.* 2008;58:1755–63.
13. Hafenbradl D, Keller M, Dirmeier R, Rachel R, Rossnagel P, Burggraf S, Huber H, Stetter KO. *Ferroglobus placidus* gen. nov., sp. nov., a novel hyperthermophilic archaeum that oxidizes Fe²⁺ at neutral pH under anoxic conditions. *Arch Microbiol.* 1996;166:308–14.
14. Zellner G, Stackebrandt E, Kneifel H, Messner P, Sleytr UB, Demacario EC, Zabel HP, Stetter KO, Winter J. Isolation and characterization of a thermophilic, sulfate reducing Archaeobacterium, *Archaeoglobus fulgidus* strain-Z. *Syst Appl Microbiol.* 1989;11:151–60.
15. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinf.* 2013; doi:10.1186/1471-2105-14-60.
16. Lien T, Madsen M, Rainey FA, Birkeland NK. *Petrotoga mobilis* sp. nov., from a North Sea oil-production well. *Int J Syst Bacteriol.* 1998;48:1007–13.
17. The DOE Joint Genome Institute. <http://www.jgi.doe.gov>. Accessed 15 Feb 2017.
18. Kostyukova AS, Gongadze GM, Polosina YY, Bonch-Osmolovskaya EA, Miroshnichenko ML, Chernyh NA, Obraztsova MV, Svetlichny VA, Messner P, Sleytr UB, et al. Investigation of structure and antigenic capacities of *Thermococcales* cell envelopes and reclassification of "*Caldococcus litoralis*" Z-1301 as *Thermococcus litoralis* Z-1301. *Extremophiles.* 1999;3:239–45.
19. Phrap, Phred and Consed for Windows, MacOS, Linux, and Unix. www.phrap.com. Accessed 15 Feb 2017.
20. Besemer J, Borodovsky M. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. *Nucleic Acids Res.* 2005;33:451–4.
21. Markowitz VM, Mavromatis K, Ivanova NN, Chen IM, Chu K, Kyrpides NC. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics.* 2009;25:2271–8.
22. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinf.* 2010; doi:10.1186/1471-2105-11-119.
23. Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 2007;35:3100–8.
24. Nawrocki EP, Kolbe DL, Eddy SR. Infernal 1.0: inference of RNA alignments. *Bioinformatics.* 2009;25:1335–7.
25. Bland C, Ramsey TL, Sabree F, Lowe M, Brown K, Kyrpides NC, Hugenholtz P. CRISPR recognition tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats. *BMC Bioinf.* 2007; doi:10.1186/1471-2105-8-209.
26. Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream MA, Barrell B. Artemis: sequence visualization and annotation. *Bioinformatics.* 2000;16:944–5.
27. Meier-Kolthoff JP, Klenk HP, Göker M. Taxonomic use of DNA G+C content and DNA-DNA hybridization in the genomic age. *Int J Syst Evol Microbiol.* 2014;64:352–6.
28. von Jan M, Lapidus A, Del Rio TG, Copeland A, Tice H, Cheng JF, Lucas S, Chen F, Nolan M, Goodwin L, et al. Complete genome sequence of *Archaeoglobus profundus* type strain (AV18(T)). *Stand Genomic Sci.* 2010;2:327–46.
29. Maisnier-Patin S, Malandrin L, Birkeland NK, Bernander R. Chromosome replication patterns in the hyperthermophilic euryarchaea *Archaeoglobus fulgidus* and *Methanocaldococcus (Methanococcus) jannaschii* (vol 45, pg 1443, 2001). *Mol Microbiol.* 2002;46:599.
30. Maisnier-Patin S, Malandrin L, Birkeland NK, Bernander R. Chromosome replication patterns in the hyperthermophilic euryarchaea *Archaeoglobus fulgidus* and *Methanocaldococcus (Methanococcus) jannaschii*. *Mol Microbiol.* 2002;45:1443–50.
31. Klein RJ, Misulovin Z, Eddy SR. Noncoding RNA genes identified in AT-rich hyperthermophiles. *P Natl Acad Sci USA.* 2002;99:7542–7.
32. Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Rattai T, Mende DR, Sunagawa S, Kuhn M, et al. eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Res.* 2016;44:286–93.
33. Labes A, Schönheit P. Sugar utilization in the hyperthermophilic, sulfate-reducing archaeon *Archaeoglobus fulgidus* strain 7324: starch degradation to acetate and CO₂ via a modified Embden-Meyerhof pathway and acetyl-CoA synthetase (ADP-forming). *Arch Microbiol.* 2001;176:329–38.
34. Hansen T, Schönheit P. ADP-dependent 6-phosphofructokinase, an extremely thermophilic, non-allosteric enzyme from the hyperthermophilic, sulfate-reducing archaeon *Archaeoglobus fulgidus* strain 7324. *Extremophiles.* 2004;8:29–35.
35. Johnsen U, Hansen T, Schönheit P. Comparative analysis of pyruvate kinases from the hyperthermophilic archaea *Archaeoglobus fulgidus*, *Aeropyrum*

- pernix*, and *Pyrobaculum aerophilum* and the hyperthermophilic bacterium *Thermotoga maritima* - unusual regulatory properties in hyperthermophilic archaea. *J Biol Chem*. 2003;278:25417–27.
36. Labes A, Schönheit P. ADP-dependent glucokinase from the hyperthermophilic sulfate-reducing archaeon *Archaeoglobus fulgidus* strain 7324. *Arch Microbiol*. 2003;180:69–75.
 37. Labes A, Schönheit P. Unusual starch degradation pathway via cyclodextrins in the hyperthermophilic sulfate-reducing archaeon *Archaeoglobus fulgidus* strain 7324. *J Bacteriol*. 2007;189:8901–13.
 38. Aalen N, Steen IH, Birkeland NK, Lien T. Purification and properties of an extremely thermostable NADP(+)-specific glutamate dehydrogenase from *Archaeoglobus fulgidus*. *Arch Microbiol*. 1997;168:536–9.
 39. Steen IH, Hvoslef H, Lien T, Birkeland NK. Isocitrate dehydrogenase, malate dehydrogenase, and glutamate dehydrogenase from *Archaeoglobus fulgidus*. *Method Enzymol*. 2001;331:13–26.
 40. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, bacteria, and Eucarya. *Proc Natl Acad Sci U S A*. 1990;87:4576–9.
 41. Garrity GM, Holt JG. Phylum All. *Euryarchaeota* phy. nov. In: Garrity GM, Boone DR, Castenholz RW, editors. *Bergey's manual of systematic bacteriology*, second edition, volume 1. New York: Springer; 2001. p. 211–355.
 42. Garrity GM, Holt JG. Class VI. *Archaeoglobi* class. nov. In: Garrity GM, Boone DR, Castenholz RW, editors. *Bergey's manual of systematic bacteriology*, second edition, volume 1. The *Archaea* and the deeply branching and phototrophic *Bacteria*. New York: Springer; 2001. p. 349.
 43. Huber H, Stetter KO. Order I. *Archaeoglobales* ord. nov. In: Garrity GM, Boone DR, Castenholz RW, editors. *Bergey's manual of systematic bacteriology*, second edition, volume 1. The *Archaea* and the deeply branching and phototrophic *Bacteria*. New York: Springer; 2001. p. 349.
 44. Huber H, Stetter KO. Family I. *Archaeoglobaceae* fam. nov. In: Garrity GM, Boone DR, Castenholz RW, editors. *Bergey's manual of systematic bacteriology*, second edition, volume 1. The *Archaea* and the deeply branching and phototrophic *Bacteria*. New York: Springer; 2001. p. 349.
 45. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene ontology: tool for the unification of biology. *Nat Genet*. 2000;25:25–9.
 46. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. Clustal W and clustal X version 2.0. *Bioinformatics*. 2007;23:2947–8.
 47. Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*. 2013;30:2725–9.
 48. Darling ACE, Mau B, Blattner FR, Perna NT. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res*. 2004;14:1394–403.
 49. Bardou P, Mariette J, Escudie F, Djemiel C, Klopp C. jvenn: an interactive Venn diagram viewer. *BMV. Bioinformatics*. 2014;15 doi:10.1186/1471-2105-15-293.
 50. EzBioCloud's Comparative Genomics Database. <https://www.ezbiocloud.net/>. Accessed 15 Mar 2017.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

