



Microbe Profile: *Aquifex aeolicus*: an extreme heat-loving bacterium that feeds on gases and inorganic chemicals

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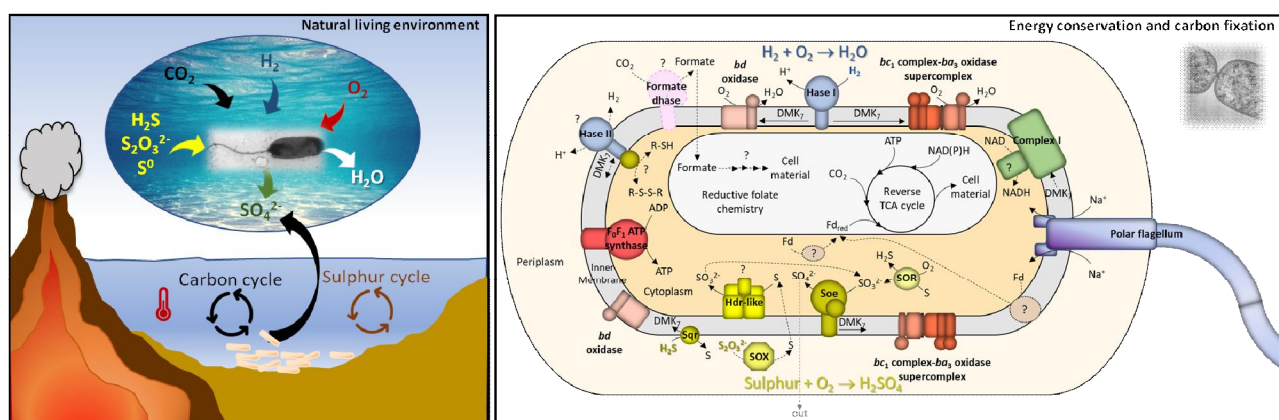
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Aquifex aeolicus: an extreme heat-loving bacterium that feeds on gases and inorganic chemicals

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GRAPHICAL ABSTRACT

Habitat and fundamental metabolic processes of *Aquifex aeolicus*

Natural living environment: *Aquificae* are ubiquitous and profuse in both marine and terrestrial hydrothermal systems, including underwater volcanoes and hot springs. They are predominant in many microbial communities in thermal habitats containing filamentous biomass and in sediments of springs, but are also found in planktonic populations. As chemolithoautotrophs, they play a principal role in the biogeochemical cycles of carbon, sulphur, and nitrogen. *Aquifex aeolicus* is a marine bacterium isolated near the shallow submarine volcanic vents of the island Vulcano (Italy). It feeds on molecular hydrogen, carbon dioxide, molecular oxygen, and mineral nutrients, releasing only water. Instead of H₂, inorganic sulphur compounds can also be oxidized, up to sulphate.

Energy conservation and carbon assimilation: The respiratory chains in *A. aeolicus* include hydrogenases (Hase I and Hase II), O₂ reductases (*ba*₃ and *bd* oxidases), a number of sulphur oxidoreductase enzymes, and the lipid-soluble quinone 2-demethylmenaquinone-7 (DMK₇). The physiological role of complex I, which might function in reverse to produce NADH, remains to be clarified [1]. An electrochemical ion gradient is used for ATP synthesis, motility or other cellular functions. In *A. aeolicus* the polar flagellar motor is driven by Na⁺ ions. Biomass components are produced from inorganic carbon (the CO₂ fixing pathways are boxed in grey) via an ancestral form of the reductive tricarboxylic acid cycle (rTCA) and a still-uncharacterized reductive folate pathway (incomplete Wood-Ljungdahl pathway) [2]. The rTCA cycle is driven by a pool of low-potential, reduced ferredoxins (Fd_{red}), the reduction of which is still enigmatic in *A. aeolicus*.

Keywords : *Aquifex aeolicus*, hyperthermophile, marine bacterium, evolution

ABSTRACT

The bacterium *Aquifex aeolicus* is the model organism for the deeply rooted phylum *Aquificae*. This water-maker is an H₂-oxidizing microaerophile that flourishes in extremely hot marine habitats, and it also thrives on the sulphur compounds commonly found in volcanic environments. *A. aeolicus* has hyper-stable proteins and a fully sequenced genome, with some of its essential metabolic pathways deciphered (including energy conservation). Many of its proteins have also been characterized (especially structurally), including many of the enzymes involved in replication, transcription, RNA processing, and cell envelope biosynthesis. Enzymes that are of promise for biotechnological applications have been widely investigated in this species. *A. aeolicus* has also added to our understanding of the origins of life and evolution.

TAXONOMY

Domain *Bacteria*, phylum *Aquificae*, order *Aquificales*, family *Aquificaceae*, genus *Aquifex*, species *Aquifex aeolicus* VF5. The genus consists of two species described in 1992: the type species *Aquifex pyrophilus* (strain Kol5a; DSM 6858) and *Aquifex aeolicus* which has not yet been validly published [3].

PROPERTIES

A. aeolicus is a Gram-negative, rod-shaped, motile, flagellated, non-spore-forming bacterium of approximately 0.5 x 2-6 µm in cell size. It is a hyperthermophile, and because it grows best at 85 °C (and up to 95 °C), it is one of the most extreme of the hyperthermophilic bacteria. Its optimal growth medium contains 3% (w/v) NaCl at neutral pH. As an obligate chemolithoautotroph, it does not use light or organic molecules to thrive, and instead feeds exclusively on gases and inorganic nutrients. *A. aeolicus* is a Knallgas bacterium, a microaerophilic respiratory microorganism obtaining its energy from hydrogen, but it can also oxidize reduced inorganic sulphur compounds (elemental sulphur and thiosulphate) [1,4,5]. In serum bottles, a maximum biomass of 0.9 OD₆₂₀ units is reached after 10 h of growth (on H₂/O₂).

GENOME

A. aeolicus was the first hyperthermophilic bacterium to have its genome sequenced [4,6]. The genome (circular chromosome) is quite small for a free-living bacterium: with only 1,551,335 base pairs, it is about one-third the size of the *Escherichia coli* genome. At 43%, the GC content of the genome is relatively low, although this content is very high for 16S rRNA (65%) as for other hyperthermophiles. *A. aeolicus* also contains a plasmid of 39,456 base pairs that harbours 63 genes, most of them for proteins of unknown function. On the chromosome, only 1,731 genes have been identified (47 being pseudogenes), with 1,632 coding for proteins and 52 for RNA. Genes for tRNA are reduced to a minimum. Protein-coding regions and intergenic sequences represent 93% and 6.2% of the genome sequence, respectively. Most of the genes seem to be organized within operons, but with an unexpected composition, and numerous genes overlap. Many of the genes that encode pathway constituents (or even subunits of the same complex or enzyme) are scattered around the genome and are thus encoded separately rather than being co-transcribed [4,5].

Keywords : *Aquifex aeolicus*, hyperthermophile, marine bacterium, evolution

PHYLOGENY

Many studies have tried to assess the phylogenetic position of the phylum *Aquificae* and thus of *Aquifex*, but it has not yet been clearly and unequivocally determined. Based on the sequences of 16S rRNA genes as well as those of certain proteins (such as those required for protein synthesis), *Aquificae* are considered to represent one of the earliest divergent lineages within the domain *Bacteria* in the phylogenetic tree of life. In this phylogenetic placement, *Aquifex* is close to the basal phylum *Thermotogae*, which is composed mostly of thermophilic and hyperthermophilic bacteria. Other phylogenetic and phylogenomic analyses have suggested a stronger affiliation with *Proteobacteria* (especially *Epsilonproteobacteria*), thus implying a later divergence of these bacteria [7]. However, because a large proportion of *Aquificae* genes were acquired by lateral gene transfers, this is probably just the result of extensive gene transfers between these two clades, which share the same environmental niches [2].

KEY FEATURES & DISCOVERIES

A. aeolicus has attracted considerable attention because, as a hyperthermophile, its proteins are extremely stable and generally easier to purify than those of its mesophilic counterparts. Many *A. aeolicus* proteins have been produced heterologously or purified from *A. aeolicus* in order to determine their three-dimensional structures, with about 300 structures of *A. aeolicus* proteins deposited in the Protein Data Bank archive. Genetic tools are not available for this bacterium, but biochemical and genomic studies of *A. aeolicus* have led to the discovery or characterization of new metabolic pathways and proteins. Examples of this include the prediction that carbon assimilation is done via a hybrid pathway (see graphical abstract and below, [2]), the discovery of an unknown RNase P enzyme involved in tRNA maturation [5], and the characterization of Soe, a new type of membrane-bound sulphite dehydrogenase [8]. Thermal adaptation and molecular stability are achieved by a combination of factors, including the synthesis of ionic-compatible solutes which protect proteins from heat denaturation, a higher degree of protein oligomerization, and the presence of disulphide bonds. As is typical for hyperthermophiles, *Aquifex* lipids contain ether bonds, which are assumed to be more stable than the ester linkages usually encountered in the lipids of other bacteria. Enzymes from *A. aeolicus* exhibit extraordinary stability, offering potential benefits for future applications. For example, its super-resistant hydrogenase I was used as a model for understanding the immobilization and orientation of enzymes on electrodes for the development of electricity-generating H₂-fed biofuel cells [1], while the cage-forming lumazine synthase was engineered to encapsulate molecules and act as a nanocarrier for delivering materials.

Energy metabolism was extensively investigated in *A. aeolicus* [1], and the bacterium is now a model organism for understanding the oxidation of inorganic sulphur compounds. Several enzymes involved in this dissimilatory sulphur oxidation pathway have been fully or partially characterized: sulphide:quinone reductase (SQR); sulphur oxygenase/reductase (SOR); heterodisulphide reductase-like complex (Hdr); sulphite dehydrogenase (Soe) [8]; and various sulphur transferases. *A. aeolicus* can also use the energy from H₂, which it oxidizes via the membrane-bound [NiFe]-hydrogenase I. This O₂-tolerant enzyme harbours a proximal [4Fe6S] cluster with an unusual structure. As a microaerophile, *A. aeolicus* reduces O₂ via a *bc*₁ complex-*ba*₃-type cytochrome *c* oxidase supercomplex and/or a *bd*-type quinol oxidase. Phylometabolic analysis has predicted that *A. aeolicus* uses a previously unrecognized and unique carbon fixation strategy in which most of the carbon is fixed through the reductive TCA cycle, while a subset of cell material is produced from an incomplete form of the reductive acetyl-CoA (Wood-Ljungdahl) pathway [2].

Keywords : *Aquifex aeolicus*, hyperthermophile, marine bacterium, evolution

OPEN QUESTIONS

• Respiratory chains in *A. aeolicus* have not been fully elucidated. What is the physiological role of various respiratory enzymes? What are the mechanisms used by *A. aeolicus* to regulate these energy pathways? Does it have the ability to use other energy sources or electron acceptors?

• How does *A. aeolicus* assimilate CO₂? Does it use the putative hybrid carbon fixation strategy, combining the rTCA cycle and the incomplete form of the reductive folate pathway? Is this mechanism unique? What drives the thermodynamically unfavourable reaction of reducing the low-potential ferredoxins involved in the rTCA cycle? Does this reduction require an enzyme that performs electron bifurcation?

• What is the evolutionary history of *A. aeolicus*? Why does it seem to require O₂ to grow even though it is a deeply branching bacterium?

• How does *A. aeolicus* cope with environmental changes? How does it interact with other bacterial and archaeal species in its natural habitats? What is its role within thermal microbial consortia?

Recommended reading

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