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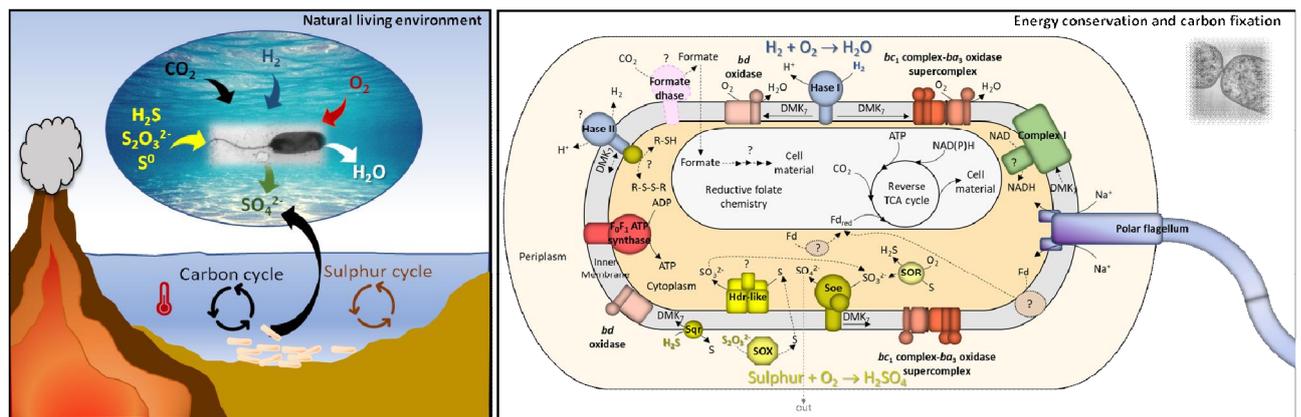
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1 ***Aquifex aeolicus*: an extreme heat-loving bacterium that feeds on gases and inorganic**
2 **chemicals**

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10

11 **GRAPHICAL ABSTRACT**

12 Habitat and fundamental metabolic processes of *Aquifex aeolicus*

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

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

33

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

34 ABSTRACT

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

45

46 TAXONOMY

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

51

52 PROPERTIES

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

63

64 GENOME

65 *A. aeolicus* was the first hyperthermophilic bacterium to have its genome sequenced [4,6].
66 The genome (circular chromosome) is quite small for a free-living bacterium: with only
67 1,551,335 base pairs, it is about one-third the size of the *Escherichia coli* genome. At 43%,
68 the GC content of the genome is relatively low, although this content is very high for 16S
69 rRNA (65%) as for other hyperthermophiles. *A. aeolicus* also contains a plasmid of 39,456
70 base pairs that harbours 63 genes, most of them for proteins of unknown function. On the
71 chromosome, only 1,731 genes have been identified (47 being pseudogenes), with 1,632
72 coding for proteins and 52 for RNA. Genes for tRNA are reduced to a minimum. Protein-
73 coding regions and intergenic sequences represent 93% and 6.2% of the genome sequence,
74 respectively. Most of the genes seem to be organized within operons, but with an unexpected
75 composition, and numerous genes overlap. Many of the genes that encode pathway
76 constituents (or even subunits of the same complex or enzyme) are scattered around the
77 genome and are thus encoded separately rather than being co-transcribed [4,5].

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

78 PHYLOGENY

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

91 KEY FEATURES & DISCOVERIES

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

113 Energy metabolism was extensively investigated in *A. aeolicus* [1], and the bacterium is
114 now a model organism for understanding the oxidation of inorganic sulphur compounds.
115 Several enzymes involved in this dissimilatory sulphur oxidation pathway have been fully or
116 partially characterized: sulphide:quinone reductase (SQR); sulphur oxygenase/reductase
117 (SOR); heterodisulphide reductase-like complex (Hdr); sulphite dehydrogenase (Soe) [8]; and
118 various sulphur transferases. *A. aeolicus* can also use the energy from H₂, which it oxidizes
119 via the membrane-bound [NiFe]-hydrogenase I. This O₂-tolerant enzyme harbours a proximal
120 [4Fe₆S₃] cluster with an unusual structure. As a microaerophile, *A. aeolicus* reduces O₂ via
121 a *bc*₁ complex-*ba*₃-type cytochrome *c* oxidase supercomplex and/or a *bd*-type quinol oxidase.
122 Phylometabolic analysis has predicted that *A. aeolicus* uses a previously unrecognized and
123 unique carbon fixation strategy in which most of the carbon is fixed through the reductive
124 TCA cycle, while a subset of cell material is produced from an incomplete form of the
125 reductive acetyl-CoA (Wood-Ljungdahl) pathway [2].

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

126 **OPEN QUESTIONS**

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

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

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

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

141

142 **Recommended reading**

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

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167 10.1016/j.bbabbio.2020.148279

168

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