Extending the Upper Temperature Limit for Life

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The upper temperature limit for life is a key parameter for delimiting when and where life might have evolved on a hot, early Earth; the depth to which life exists in the Earth's subsurface; and the potential for life in hot, extraterrestrial environments. A combination of geological and microbiological evidence suggests that electron transport to Fe(III) may have been the first form of microbial respiration as life evolved (*1*–*3*). Geological evidence suggests that microorganisms that use Fe(III) as an electron acceptor are key components of the deep, hot biosphere (*1*, *4*). Furthermore, the accumulation of Fe(III) in hot sediments around marine hydrothermal vents might have led to Fe(III) reduction being an important process in modern hydrothermal environments (*1*, *5*).

To learn more about the physiological properties of Fe(III)-reducing microorganisms growing at high temperatures, we attempted to culture microorganisms on Fe(III) from a water sample from an active, "black smoker," hydrothermal (300°C) vent called Finn, located in the Mothra hydrothermal vent field (at 47°55.46'N and 129°06.51'W) along the Endeavor segment of the Juan de Fuca Ridge, in the Northeast Pacific Ocean. An organism, designated strain 121, was isolated at 100°C in an anaerobic medium (*6*) under N_2-CO_2 (80:20) in sealed culture tubes that contained formate (10 mM) as the electron donor and Fe(III) oxide (100 mmol/l) as the electron acceptor. Cell growth and Fe(III) reduction were monitored with previously described methods (*7*) for determining cell numbers with epifluorescene microscopy and Fe(II) production with ferrozine. Fe(III) was reduced to Fe(II) with formation of the magnetic mineral magnetite (Fig. 1A), as confirmed with x-ray diffraction analysis. There was no Fe(III) reduction in the absence of the organism.

Strain 121 is coccoid, about 1.0 μm in diameter, with lophotrichous-like flagellation (Fig. 1B). The cell envelope consists of a cytoplasmic membrane, a periplasmic space, and a single outer surface layer, typical of Archaea (Fig. 1C). Analysis of the 16S ribosomal DNA (rDNA) sequence (1100 base pairs considered; GenBank AY21635) and topR gene fragment (105 amino acids considered; GenBank AY21633) of strain 121 indicated that it was a member of the Archaea, most closely related to *Pyrodictium occultum* (96.0% similar) and *Pyrobaculum aerophilum* (95.3% similar).

Strain 121 grew at temperatures between 85° and 121°C (Fig. 1D). To ensure accuracy, growth temperatures were documented with an electronic thermocouple in the incubator as well as three calibrated mercury thermometers, one of which was inserted through the butyl rubber stopper of a sealed culture tube containing the growth medium. For studies at 121°C, the media was maintained at 121°C for 2 hours before inoculation and was inoculated while still at 121°C.

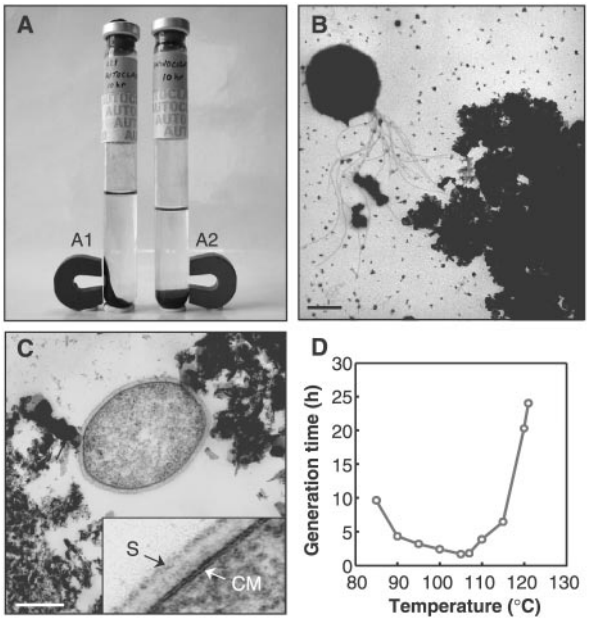

Fig. 1. (**A**) Although sterilization indicator tape showed that the inoculated media (A1) should be sterile after 10 hours in an autoclave at 121°C, Fe(III) continued to be reduced with the formation of magnetite over 3 subsequent days of incubation at 103°C. There was no reduction in an uninoculated control (A2). Negatively stained electron micrograph (**B**) and thin section (**C**) of strain 121 illustrating single layer cell envelope (S) and cytoplasmic membrane (CM). Bar, 1 μm. (**D**) Time for cells of strain 121 to double at different temperatures.

Growth at 121°C is remarkable because sterilization at 121°C, typically in pressurized autoclaves to maintain water in a liquid state, is a standard procedure shown to kill all previously described microorganisms and heat-resistant spores. Only 1% of the cells of *Pyrolobus fumarii*, which is reported to grow at temperatures up to 113°C, were intact after autoclaving for 1 hour, and there was no evidence that the remaining cells were viable (*8*). Autoclaving did not kill strain 121, and it doubled in cell numbers after 24 hours at 121°C. Although growth could not be readily detected at higher temperatures, cultures incubated for up to 2 hours at 130°C still grew when transferred to fresh medium at 103°C. At temperatures below 85°C, the cells remained viable, but did not divide.

The factors that permit strain 121 to grow at such high temperatures are unknown. It is generally assumed that the upper temperature limit for life is related to the instability of key molecules essential for life, but which molecules are most important in defining the upper temperature limit have not been defined (*9*). However, strain 121 offers the possibility to do this work.

The use of Fe(III) as an electron acceptor was key to the isolation of strain 121, as it does not use other known electron acceptors. Culture approaches designed to select for other forms of energy conservation might well yield organisms that can grow at even higher temperatures.

References and Notes

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