

## The upper temperature for life - where do we draw the line?

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**A new archaeal isolate has been reported that is capable of growing at up to 121 °C. This hyperthermophile, dubbed strain 121, grows chemoautotrophically using formate as an electron donor and Fe<sup>III</sup> as an electron acceptor and is closely related to members of the archaeal genera *Pyrodictium* and *Pyrobaculum*. Although the reported maximum growth temperature of strain 121 is 8 °C higher than the previous record holder (*Pyrolobus fumarii*;  $T_{max} = 113$  °C), the two organisms have virtually the same optimal growth temperatures.**

A publication by Kashefi and Lovley [1] has once more brought the issue of the upper temperature limit for life to the attention of the scientific community. The isolation of a hyperthermophilic archaeal strain that grows optimally at 105-107 °C, and maximally at 121 °C is reported. At first inspection, this organism would appear to have moved the bar up by some 8 °C, the previous record having been held by *Pyrolobus fumarii* [2]. However, some caution must be advised in interpreting these results. Strain 121 is reported to have a doubling time of ~ 7 hours at 115 °C and 24 hours at 121 °C. By contrast, *P. fumarii* showed no growth at 115 °C [2], but had a doubling time of ~ 8 hours at 112 °C with a significant number of cells in an exponentially growing culture surviving autoclaving. Therefore, strain 121 shows detectable growth at 8 °C above that reported for *P. fumarii*. Readers should note that measuring accurate growth rates at extremes of temperature is difficult at the best of times, and cannot be made easier by the generation of the amorphous, opaque magnetite that is the product of Fe<sup>II</sup> reduction. However, it is remarkable that strain 121 survives at 130 °C for short periods. To put this in perspective, if 10<sup>7</sup> cells were exposed to 130 °C and completely killed in 2 hours, we would calculate a survival half-life of ~ 6 minutes. The remarkable fact is that a vegetative cell can survive this long at such temperatures.

This work therefore represents another contribution to the exciting search for the upper temperature limit for life: a search that has shown a generally linear rise (see [Figure 1](#), [after References](#)) over the past 30 years.

Although the authors note that growth at 121 °C is remarkable because this is the temperature of sterilization, there is nothing remarkable about this temperature *per se* other than the fact that this is the boiling temperature of water at 1 atmosphere (15 psi) overpressure. Had the original designers of autoclaves chosen a different overpressure (such as 20 psi), a different 'sterilization' temperature would have been embedded in our minds.

It is an exciting thought that the cardinal growth temperatures determined in laboratory studies might be underestimates of true *in vivo* growth temperatures. For example, the well-known extremely thermophilic aerobic *Thermus Aquaticus* [3] is readily isolated from terrestrial 90-100 °C hydrothermal systems, but will not grow reliably in the laboratory above 80 °C (H.W. Morgan, pers. commun.). Nevertheless, terrestrial and shallow marine environments are probably too cool to harbor the most hyperthermophilic organisms, because their

slow growth rates at such relatively low temperatures probably result in their being out-competed by rapidly growing organisms, such as *Pyrococcus* [4].

The deep-sea hydrothermal vent biotopes, from which numerous hyperthermophilic archaea have been isolated, provide ample scope for high temperature growth. The friable metal sulfide walls of 'smoker' chimneys offer huge, if rather steep temperature gradients. The temperature of the interior water column might be as high as 400 °C, whereas the external seawater temperature is typically less than 5 °C, generating a temperature gradient of several hundred degrees over a few tens of centimeters in the chimney walls [5]. Horizontal transect samples from smoker chimneys have shown that biological signals, in the form of amplifiable 16S rRNA genes, are present across much of the transect [5,6]. Unfortunately, the fragility of the chimneys and the difficulties of maneuvering instruments using the remote arms of submersibles mean that it has not been possible to correlate the biological signatures with the thermal gradient. To do so would provide an elegant means of providing a culture-independent measure of the upper temperature limit for life.

### **The molecular basis of thermophily**

The enthusiastic analysis of molecular stability [7-10], which has closely followed the growth of the field of 'thermophily', appears to have waned in the past five years, leaving many fundamental questions unanswered. It would therefore be beneficial for thermophile research in general if the Kashefi and Lovley publication [1] triggered another debate on the molecular mechanisms that dictate the ability (or inability) of an organism to grow at high temperatures. Protein structural analysis [7,10] has indicated that conformational stability might not be the limiting factor; proteins possessing high levels of thermostability ( $T_m$  values in excess of 130 °C [11]) have been characterized. Strain 121 will probably yield more examples of thermostable proteins, the study of which should further contribute to our understanding of the molecular basis of protein stability. For example, a high-resolution crystal structure of the glutamate dehydrogenase will probably be a very useful addition to the protein series that exposed the vital contribution of salt-bridge networks to protein conformational stability [12,13]. Although we believe that we have at least some understanding of protein structural stability, our understanding of the implications of chemical instability at high temperatures is less developed. Studies from the early 1980s indicated that natural amino acids are reasonably chemically stable up to around 150 °C, but are degraded in minutes or even seconds at 250 °C [10,14]. Therefore, the conformational stability of proteins and the inherent chemical stability of their constituent amino acids suggests that hyperthermophiles should not face a particularly high maintenance energy burden owing to an increased protein for protein structural repair or resynthesis [10]. The same might not apply to other cell constituents, such as coenzymes [e.g. NAD/P(H)] and low molecular weight metabolites (e.g. AMP, ADP, ATP, acetyl CoA, acetyl phosphate and carbamoyl phosphate).

It has been suggested that small molecules, not macromolecules, dictate the upper temperature limit of life. This view is based on observations (albeit *in vitro*) that key metabolic intermediates, such as the nicotinamide cofactors, are highly unstable at temperatures as low as 95 °C [15]. Strain 121 should provide an excellent test-bed for analysis of putative mechanisms of cofactor stabilization (such as molecular channeling [16]) or of any propensity to rely on more stable alternative chemical moieties (such as Fe-S proteins instead of nicotinamide cofactors [17]).

## Origins of life

Kashefi and Lovley [1] do not report where the 16S rRNA sequence is positioned in the universal tree of life [18]. However, its high relatedness to *Pyrodictium* and *Pyro-baculum* appears to position it as a relatively deep branch within the Crenarchaeotal lineage. This branching point, together with the apparently 'primitive' form [19] of chemoautotrophic energy transfer (formate-F<sup>111</sup> redox pair) in strain 121, is consistent with the currently favored view [20,21] of a high temperature - rather than low temperature - origin of life (or at least a thermophilic Last Common Ancestor).

## To sum up

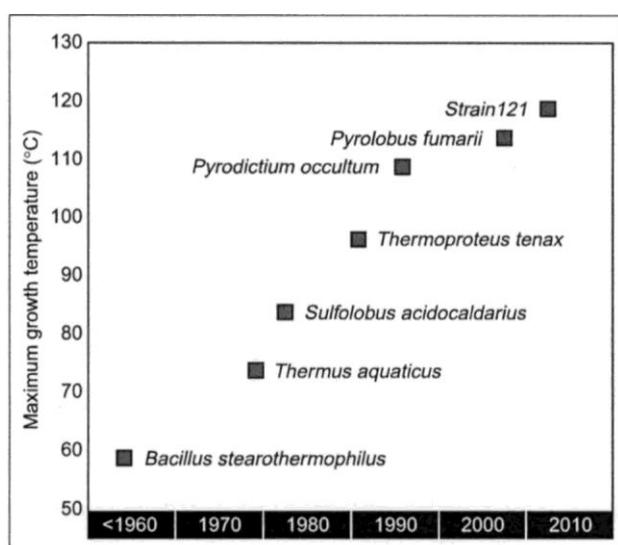
The newly isolated hyperthermophilic archaeal strain 121 grows slowly at 121 °C and even survives short periods at 130 °C. This is another organism that grows best at temperatures well in excess of 100 °C! We should not be astonished so much by the numerical increments but by the biochemical implications of this fact, and we should be excited by the scope provided by this and similar organisms to further our understanding of the evolution and adaptation of molecular structures and systems.

And what about the upper limit of life? It appears improbable that the end-point of this search is represented by strain 121. The consensus view is that the true upper limit, where the energetic burden imposed by molecular repair and resynthesis becomes unsustainable, will probably lie in the region of 140-150 °C [22].

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**Figure 1.** The rise of the upper temperature limit of life. Data points indicate the dates that new organisms were reported that have significantly extended the upper temperature limit of life. Included are *Bacillus stearothermophilus* [23], *Thermus aquaticus* [3], *Sulfolobus acidocaldarius* [24], *Thermoproteus tenax* [25], *Pyrodictium occultum* [26], *Pyrolobus fumarii* [2] and Strain 121 [1].