PERSPECTIVES: MICROBIAL ECOLOGY

How to Avoid Oxygen

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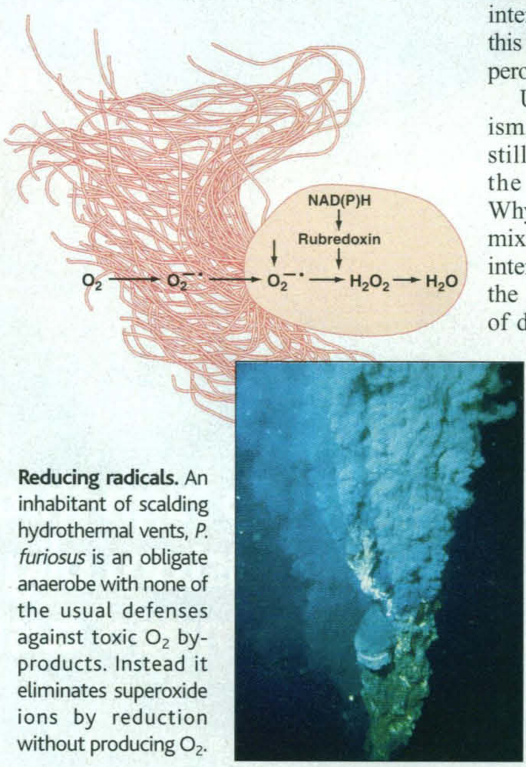
"The universe is not just queerer than we suppose, but even queerer than we can suppose."
–J.B.S. Haldane (*Possible Worlds*, 1927)

Beneath the edge of beyond, where scalding waters well up from deep-sea volcanic vents ("black smokers") and meet the icy-cold abyssal sea, is perhaps the strangest place on our planet. Here, near the upper temperature border of life on Earth, exotic, indeed bizarre, bacteria display the most way-out metabolic characteristics. One of these microbes, *Pyrococcus furiosus*, can grow only in the absence of molecular oxygen (O_2), and grows most rapidly at 100°C. Now, Jenney *et al.* show (*1*) on page 306 of this issue that this strictly anaerobic hyperthermophile (growing at greater than 80°C) has a most unusual property: It is able to detoxify oxygen's potentially damaging reactive species [such as superoxide (O_2^-), the one-electron reduction product] without recourse to the superoxide dismutase (SOD) reaction, the usual route for disposal of the superoxide radical. Moreover, this fascinating organism uses a hitherto undescribed route, the superoxide reductase (SOR) pathway for the reduction of superoxide, first to H_2O_2 and then to H_2O (see the figure). The elegant beauty, and indeed paramount significance, of this unique metabolic pathway is that no O_2 is formed. To a strictly anaerobic organism this is a most innocuous—and welcome—outcome.

Pyrococcus furiosus was first described in 1986 by Fiala and Stetter (*2*). These researchers took samples of hot anaerobic sediments with original temperatures between 90° and 100°C from a beach at Porto di Lavante, Vulcano Island, Italy. They cultured the organism in complex organic medium under a gas phase of H_2:CO_2 (80:20) at high pressure (3 bar), and at 100°C. The rapidly growing coccoid bacterium, with about 50 flagella at one pole, would not grow at 65° or 105°C. Only CO_2 and H_2, not oxygen, were detected as metabolic products, and H_2 inhibition of growth (observed at high H_2 concentrations) could be prevented by the addition of elemental S, whereupon H_2S was generated as a detoxification product. This ability to detoxify H_2 and grow under very hot conditions is essential to life within the H_2-containing volcanic biotopes, although *P. furiosus* is not quite the record-holder for growth at elevated temperatures [*Pyrolobus fumarii* likes it even hotter, up to 113°C (*3, 4*)].

Pyrococcus furiosus is one remarkable member of a growing list of hyperthermophiles. In addition to inhabiting continental and submarine hydrothermal systems worldwide, these hypothermophilic archaeal methanogens and sulfur metabolizers are found in other hell-holes—smoldering mining refuse piles, self-heating coalpits, and boiling mud baths (*5*). Even some non-archaeal bacteria (for example, *Aquifex*) can survive at 90°C.



Reducing radicals. An inhabitant of scalding hydrothermal vents, *P. furiosus* is an obligate anaerobe with none of the usual defenses against toxic O_2 by-products. Instead it eliminates superoxide ions by reduction without producing O_2.

In the present work, a standard SOD assay (*6*) initially suggested that the activity of SOD was high in *P. furiosus*, but purification of the enzyme and amino acid and gene sequence analyses indicated that it was actually homologous with two previously characterized bacterial redox proteins, neelaredoxin and desulphoferrodoxin. Both of these proteins have been reported to have SOD activity, but their in vivo functions are not fully defined. Compared with an authentic SOD from cow, the catalytic properties of this enzyme proved to be quite distinct: The new activity was an oxidoreductase, with no O_2 formed. Alterations in the reaction conditions that virtually eliminated its action had no effect on conventional SOD. The authors conclude that the activity is actually a SOR. Homologs of this *P. furiosus* SOR are found in almost all the genome sequences available from anaerobes, but not in those from aerobes or facultative anaerobes.

The electron transport chain in *P. furiosus* uses reduced rubredoxin as donor (see the figure), with NAD(P)H as the probable ultimate source of reducing power. Rubredoxins, common in anaerobes, have not previously been assigned clear functions, although protection against reactive oxygen species was always a possibility. Hydrogen peroxide, the intermediate product, is further reduced in this catalase-negative organism to water by a peroxidase, perhaps a rubrerythrin (*7*).

Unlike many systems within this organism, the SOR electron transport chain is still catalytically competent 80°C below the optimum temperature for growth. Why? In locales where ecosystems meet, mix, and merge, especially at the turbulent interfaces within the volcanic cauldrons of the deep sea, episodic exposure to the O_2 of down-welling colder waters occurs, and must be detoxified. The unusual temperature dependence of the SOR reaction ensures that *P. furiosus* can do just that (*8*).

A recent laboratory demonstration (*9*) of oxygen- and sulfide-centered free radicals during mixing of sulfidic vent plumes with cooler oxygenated seawater may indicate that these same products occur in real "black smokers." Chemiluminescence is indicative of the excited-state reactions of singlet oxygen (*10*), and the eerie glow amidst the abyssal gloom may well be due to this activated form of oxygen. Further work will help clarify the life-styles of the unusual organisms that inhabit this strange world and may provide clues to the limits for life in the cosmos where conditions may well be even more extreme.

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