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Opinion

Colonization of Black Smokers by Hyperthermophilic Microorganisms

Reinhard Wirth[1,*]

Newly erupted black smokers (hydrothermal vent chimneys) are sterile during their formation, but they house hyperthermophiles in substantial amounts in later stages. No hard data exist on the mechanisms by which hyperthermophiles colonize newly erupted black smokers. Here I propose a scenario – based on various experimental data – for how hyperthermophiles colonize black smokers. Hyperthermophiles which are present in cold sea water in minute amounts are transferred by chance to the outside of black smokers and react within seconds to the high temperature by very fast movements. After reaching an optimal temperature region they scan the surface via a zigzag seek-movement and adhere via their flagella at a suitable place, building up biofilms.

Discovery and Characterization of Black Smokers

Black smokers (see Glossary) are found in submarine **hydrothermal vent** systems [1–5], that were first detected in 1977 [6]. Black smokers are formed by effluents which come into contact with rocks heated by magma chambers directly located below them (Figure 1, Key Figure). The metals and sulfides dissolved in the hydrothermal fluids precipitate in the low-temperature sea water to build up the black smoker chimney (Box 1). The emanating fluid forms jets, so-called **hydrothermal vent plumes**, which can rise to extended areas from their source. More than 500 vent fields are known [2], and they are globally distributed [4].

Black smokers house an ecosystem not expected to exist: extraordinary communities of organisms were reported to exist in these thermal vent areas [6]. This **hydrothermal vent community** was characterized extensively; since sunlight does not penetrate into the depths of most of the hydrothermal vent areas (>1000 m), only chemotrophy can fuel life in these abyssal habitats. Indeed, chemolithotrophic (e.g., methanocaldocci) and chemoorganotrophic (e.g., thermococci and pyrococci), hyperthermophiles have been isolated from black smokers. Right from the first studies of life associated with hydrothermal vents it was noted that very high concentrations (from 10^8 to 10^9 bacteria per ml) of sulfur-oxidizing and heterotrophic bacteria may be found in the form of mats close to black smokers [6]; concentrations of hyperthermophiles in chimney walls are in the range of 10^5 per ml. Various studies showed that the microbial communities grew on the dissolved gases and metals in black smokers [4]. The walls of black smokers are porous, at least in their early stages, and are composed mainly of metal sulfides, anhydrite, and barite; in later stages, not further defined, veneer-like layers can cover the outside [7,8]. Temperature gradients of over 100°C per cm of wall material have been measured.

A newly formed black smoker will be sterile because the vent fluids have temperatures (up to 400°C) which are much higher than the upper temperature limit of life. Colonization of newly erupted black smoker walls, therefore, has to be from the outside; indirect data indicate that this can happen within a few days [7,9]. Colonization in our context is not meant as the extension of

Trends

Newly erupting black smokers, by their mechanism of formation, initially are sterile.

Hyperthermophiles are present in substantial amounts in 'matured' black smokers.

Most hyperthermophiles isolated from black smokers are highly mobile, exhibiting two different swimming styles, and are able to adhere to many abiotic and biotic surfaces.

Hyperthermophiles can survive for prolonged times at low temperatures and react within seconds to high temperatures.

Colonization of black smokers by hyperthermophiles should occur from the surrounding cold deep-sea water.

[1]University of Regensburg, Archaea-Centre, 93053 Regensburg, Germany

*Correspondence: reinhard.wirth@ur.de (R. Wirth).

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an already existing microbial community in a vent field to a nearby chimney. Such a secondary extension is argued to happen through random dispersion via bottom currents from neighboring subsurface habitats, because cracks in the rock formations close to black smokers will allow mixing of vent fluids with lower temperature habitat water. The primary colonization event covered in this opinion article refers to the establishment of life in/on a newly erupted chimney, which originally was sterile. Various prokaryotes have been identified in the walls of black smokers, many of which are hyperthermophiles.

Microorganisms Found in Black Smokers

Many studies sought the presence of microorganisms in black smokers; these used cultivation approaches, assays for the diversity of 16S rRNA genes, or a combination of both approaches. A few of these studies are listed below to exemplify the taxonomic width of (hyperthermophilic) microorganisms found in black smokers.

The microbial population from the northernmost black smoker field (73°N; between Greenland and Norway – Loki's Castle) revealed the following taxonomic classes to be most abundant by the numbers of 16S rRNA genes recovered: Epsilonproteobacteria: 36.1%; *Thermococcus*: 28.4%; Aquificae: 26.1%; Methanococci: 2.8%; Archaeoglobi: 1.8% [10].

An active black smoker chimney collected from the Dudley site at the Main Endeavour Vent Field (Juan de Fuca Ridge, close to Vancouver Island, Canada) resulted in ca. 50% of 16S rRNA sequences from Desulfurococcales; Thermococcales and deep-sea hydrothermal vent Euryarchaeota (DHVE) comprised ca. 20%, each [11].

A study on the distribution of archaea in a black smoker chimney collected at the PACMANUS site (Manus Basin near Papua New Guinea) differentiated subsamples into those being in direct contact with emanating hot fluids (samples I to IV), and samples V and VI derived from porous and hard layer structures of the chimney no longer flushed by vent fluids [12]. Subsamples I to IV resulted in mainly 62, 84, and 19 rRNA clone types representing Ignicoccales, *Thermococcales*, and DHVE respectively, whilst subsamples V and VI resulted in 101 rRNA clone types representing Halobacteriales. No data were given as to the actual temperature of the subsamples; successful enrichment cultures at 55, 75, and 90°C were obtained for subsamples I to IV, whilst enrichment cultures for subsamples V and VI were only successful at 30 and 45°C. Very interestingly, the data that more than 60% of all rRNA clones were derived from bacteria correlate very well with the data for Loki's Castle [10].

For black smokers, the general statement given in 2002 [13] still holds that archaea comprise ca. 40% of all microorganisms at the exterior parts and increase to ca. 90% in the inner regions; total cell numbers were reported to range from about 10^5 microbes per gram to 10^9 per gram with a maximum within 3 to 5 cm of the outer chimney wall.

During a study on the *in situ* temperatures of two deep-sea hydrothermal vents at Guaymas Basin, thermocouple arrays were placed over vents for various times [9]. Already after 4 days mineral deposits had formed on the thermocouples which were colonized by microorganisms as determined by 16S rRNA gene retrieval. It was argued that a temporal transition in the primary carbon sources used by the archaeal communities colonizing the thermocouples occurred from CO_2/H_2 methanogens, such as *Methanocaldococcus* after 4 days, to possible methylotrophic or acetoclastic methanogens after 2 months [9]. Other studies using 16S rRNA genes identified *Ignicoccus* and Nanoarchaeota as primary colonizers (3–4 days after deployment of the sampling units [7]), or Thermococcales and Archaeoglobales (5 days after deployment of growth chambers [14]), or Thermococcales (growth on aquarium wool for 2–11 days after deployment) [15].

Glossary

Adhesion: is an initial step in colonization and biofilm formation [56]; in most cases specific cell-surface appendages of microorganisms mediate adhesion to biotic and abiotic surfaces. One possibility how microorganisms measure their presence on a surface is the fact that membrane stress induced by interaction with the surface is sensed [57]. Besides other factors, the mechanical stiffness of a potential substrate can regulate adhesion [58].

Black smoker: the metals and sulfides dissolved in hydrothermal fluids precipitate when coming into contact with the low-temperature deep-sea water, forming chimneys of different size; see, for example, http://hydrothermalventszcrenshaw.weebly.com/black–white-smokers.html.

Hydrothermal vents: in this context, submarine fissures at a depth of a few meters to 5000 meters below surface, from which geothermally heated water issues. During its contact with magmatic heated rocks, normal sea water is transformed into hydrothermal fluids. Depending on the geological settings, the vents can lead to the formation of distinct structures, such as black and white smokers, snow-blower vents, beehive chimneys, or the heated water just is simmering out of the sea-floor.

Hydrothermal vent community: hydrothermal vents support the existence of life in sometimes extremely dense communities. Animals found there are, for example, different tube worms (e.g., *Riftia pachyptila*), mussels (e.g., *Calyptogena magnifica*), crabs (e.g., *Bythograea thermydron*), plume worms (e.g., *Alvinella pompejana*), dandelian siphonophores, amphipods, lobsters, shrimps, octopus, and even eel-like fishes – in total 500 + species.

Hydrothermal vent plumes: plumes formed by the mixing of hydrothermal fluids with cold oxidizing sea water; they contain, as potential energy sources for microbial life, H_2S, Fe, Mn, CH_4 and H_2.

TGFD: the temperature-gradient-forming device [21] can be attached to any light microscope, enabling microscopic observations of cells under anaerobic conditions at temperatures of up to 105°C for long-term experiments. An additional

A few studies combined molecular methods (16S rRNA genes or whole-cell hybridization) with culturing experiments, resulting in congruent data. Enrichment cultures from beehive structures resulted in mainly *Thermococcus* [16], whilst Thermococcales and Archaeoglobales were cultured from a 1.6 m long black smoker portion [17].

Microbial populations identified in **white smokers**, and in hydrothermal vent plumes via 16S rRNA genes, were determined to be different from those in black smokers (Box 2); these two habitats are not considered here. The so-called snow-blower vents are also not the topic of this opinion article, because in this case the microorganisms sprouting from cracks of newly formed basalt are seeded by subseafloor microbes [18].

Very recently a deep-sequencing approach examined the distribution of vent-typical and open-ocean-water-typical microorganisms by comparing ca. 30 million 16S rRNA reads from both locations [19]. The main result was that (supposed) endemic hydrothermal vent species were identified in the open ocean seed bank, explaining how species expected to be endemic to vent systems are able to colonize geographically distant hydrothermal habitats. Though it has been argued that these data reveal that everything is everywhere, the question remains whether the 16S rRNA reads represent living microbes [20] as other data show that hyperthermophiles can survive at least 9 months in cold surroundings [21]. In addition it has been calculated that microbes can persist over hundreds of kilometers from the smoker for prolonged times if transported in plumes [22].

A Possible Scenario for How Hyperthermophiles Colonize Newly Erupted Black Smokers

Since no experimental data exist for the mechanism of colonization of newly formed black smokers by microorganisms (see Outstanding Questions), one can only give a scenario as to how that might happen. For this, I synthesize various pieces of experimental data.

As mentioned at the beginning, colonization of newly erupted black smokers has to be by hyperthermophiles present in low-temperature sea water. Hyperthermophiles, indeed, are present in normal sea water, but only in minute numbers compared to mesophilic microorganisms (10^4–10^6 per liter at maximum [23]; a number of 0.001% seems more realistic [19]). Hyperthermophiles survive in low-temperature sea water for long times – pure cultures stored for over 1 year in a cold-room usually can be 'revived' to normal growth without problems. Most hyperthermophiles are anaerobes, but at 2°C their metabolism comes to a complete stop, resulting in their ability to survive in cold, oxic sea water [23].

Indeed, not only can hyperthermophiles survive for over 9 months in cold surroundings [21], more importantly these long-time, cold-stored microorganisms can react within 3–5 seconds to high temperatures by active swimming (see Video S1 in the supplemental material online). This means that cells coming into contact with the outside of newly formed black smokers by chance (via convections of cold sea water) are able to immediately sense the hot surface and react to it via motility, increasing their swimming speed with temperature [21]. I therefore argue that cells coming into contact with a 'naive' black smoker react in such a way that their movements will lead them, within seconds, to a temperature optimal for their life processes (chemoreceptor genes have been identified in most motile hyperthermophiles). It should be recalled that temperature gradients of > 100°C per cm of chimney walls can occur, and that hyperthermophiles are the fastest organisms on earth if speed is measured in bps (bodies per second [24]). With swimming speeds of up to 500 μm per second, a distance of 1 cm can be covered by them within 20 seconds, allowing escape from temperatures that are too high for them. In addition, hyperthermophiles show two different modes of swimming: the very fast mode – characterized by + /- linear tracks – is used to cover mm distances within seconds. If cells come into contact

advantage is the possibility to establish a temperature gradient of > 40°C at a distance of just 2 cm, thereby mimicking gradients found, for example, in the walls of black smokers. The required time for heating is a few seconds, enabling experiments aimed at mimicking colonization of black smokers.
White smoker: the hydrothermal fluids forming white smokers lack metals and/or sulfides; rather, they contain sulfates (anhydrites) or SiO_2. In general, they have lower temperatures than black smokers; for further details see, for example, http://www.ooi.washington.edu/files/5.axial_chadwick1.pdf.

Key Figure

Formation and Biology of Black Smokers

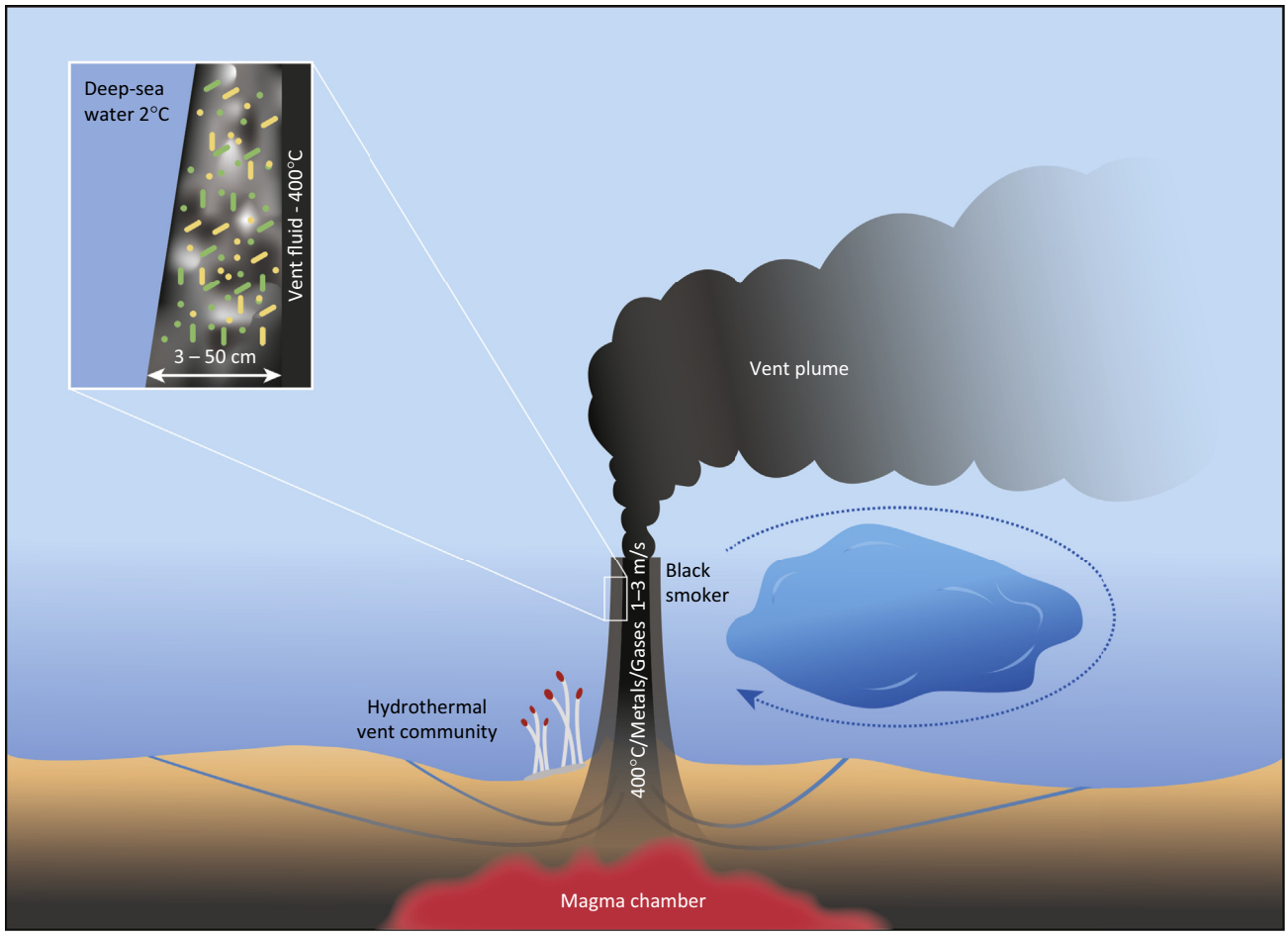

Figure 1. Black smokers are formed at locations of geothermal activity in the (deep-)sea by precipitation of metals and sulfides dissolved in vent fluids having temperatures of up to ~400°C. Seawater entering the sediment through small cracks is transformed into vent fluids by dissolving metals and sulfides from heated rocks overlying a high-reaching magma chamber and enriched with various gases. The walls of newly erupted black smokers are sterile during their formation due to the high temperatures, but 'mature' vent chimneys contain substantial amounts of hyperthermophiles. The mechanism by which newly erupted black smokers are colonized by hyperthermophiles is unknown. The chimney walls are porous and have a width of 3 to over 50 cm; temperature gradients of ~100°C/cm have been measured. Microorganisms in/on black smokers are the basis of a food chain for the hydrothermal vent community, which can exist in extremely dense populations of various animals, such as tube worms, clams, etc. Black smokers, vent plumes, and deep-sea water harbor distinct microbial populations. Deep-sea water contains very low numbers of hyperthermophiles, which might be transferred to the outside of black smokers by water convections (blue arrow) over long distances. This scheme is not drawn to scale: vent plumes can extend for hundreds of kilometers, black smokers can reach a height of > 40 m, tube worms of the hydrothermal vent community can be 2 m long, whilst hyperthermophiles (green and yellow symbols in chimney wall) measure a few micrometers.

with surfaces, an additional movement is observed: zigzag swimming which is much slower (ca. 10% of the fast movement; [24]). Such surface-scanning cells repeatedly attached to surfaces, some of them finally showing constant adhesion. In the following I present data supporting this scenario.

Box 1. A Closer Look at Black Smokers

The hydrothermal fluids forming black smokers have temperatures of up to 400°C, they are rich in dissolved transition metals such as Fe(II) and Mn(II), they contain high concentrations of CO_2 and H_2S, in addition to dissolved H_2 and varying amounts of CH_4 but no O_2, and they are acidic, with a pH of 2–4.5 ([3]; see also Table 1 in [4]). Black smoker chimneys have different colors, from grey to + /- black; the jets of superheated fluids they eject are of a + /- black color. A detailed description of the discovery of hydrothermal vents, together with data on the food chain found there, is given in

http://www.msi.harvard.edu/downloads/teacherworkshop/Readings/CSA_hydrothermal_ventsreview.pdf. See, for example, http://ocean.si.edu/ocean-videos/hydrothermal-vent-creatures for excellent video clips. Many more primary data on vent communities were reported (see e.g., [54] and [55] for a few of those). A common understanding is that chemotrophic microorganisms – often living as symbionts of hydrothermal vent animals – are at the basis of the food chain in these ecosystems, see, for example, http://www.indiana.edu/~g105lab/images/gaia_chapter_13/vent_communities.htm.

Box 2. Characterization of White Smokers, Snow-Blower Vents, Hydrothermal Vent Plumes, and Their Microorganisms

The first white smoker was discovered in 2000 [5]: the Lost City hydrothermal field is characterized by highly alkaline effluents (pH 9–11), temperatures of 'only' 200°C, and a chemical composition distinct from that of black smokers. The heated fluids form whitish carbonate–magnesium hydroxide chimneys. Vent fluids of white smokers in general have lower temperatures than black smokers, and they deposit + /- whitish barium, calcium, and silicon structures. The main groups of prokaryotes found in white smokers are the so-called Lost City Methanosarcinales (LCMS) and ANME1-Archaea, occurring in consortia with sulfate-reducing bacteria [4].

Snow-blower vents are characterized by white flocculent material emanating from the seafloor; they develop around low-temperature, diffuse flows, around lava from underwater volcanoes. The whitish, fluffy snow particles are argued to be of biological origin, formed by a subsurface microbial bloom. The dominant bacteria in fluids and white flocs were reported to be Epsilonproteobacteria, whilst Methanococcales were the dominant thermophilic archaea [18].

Hydrothermal vent plume fluids contain, as dominant microbial groups, those which are commonly found in oxygen-minimal zones, especially SUP05 (Gammaproteobacteria, closely related to symbionts of hydrothermal vent animals), ammonia-oxidizing archaea and SAR324 Deltaproteobacteria (uncultivated, putative sulfur-oxidizing bacteria; [2]).

Nature's Experiment on Colonization of Deep-Sea Hydrothermal Vents by Animal Larvae

In 2006 a catastrophic eruption, touching part of a deep-sea hydrothermal vent field (located 9°50′N on the East Pacific Rise), created a unique situation to study the colonization of vents by animal larvae [25]; unfortunately, no data for hyperthermophiles were collected. Data for the larvae had been obtained before the eruption, and a rapid-response cruise with the submersible Alvin to the vents enabled researchers to follow the recolonization (defined as larvae that had settled and metamorphosed) of the 'naturally cleared' vents. Some vents located just 6 km off the cleared vents were not touched by the eruption, and harbored larvae of various animal species. These nearby larvae were found in limited numbers on the post-eruption vents; very interestingly, however, larvae of *Ctenopelta porifera* did colonize the cleared vents – the only known population for this sea snail is located at 13°N, that is, more than 300 km away! It was concluded [26] that hydrodynamic jets that form along ridges can transport species over distances greater than 300 km, and probably even 1200 km apart [27]; for hydrothermal dissolved metals, transport over several thousand kilometers was shown [28]. The question as to how such 'naive vents' are colonized by animal larvae and by microorganisms is a crucial one for the establishment of the complex hydrothermal vent communities.

Motility of Microorganisms Found in Black Smokers

Most of the microorganisms identified in black smokers are motile; they therefore should be able to actively occupy specific niches in black smoker chimney walls, optimal for their physiological needs. For the main groups discussed above, the following number of species were reported to be motile. Aquificae: 27 of 30 [29]. Archaeoglobi: 5 of 8 [30]. DHVE: the only cultivated species,

Aciduliprofundum boonei [31], was reported to possess a single flagellum. Desulfurococcales: 8 of 17 [32]; the nets formed by three species might well act as adhesins. Epsilonproteobacteria: the three main mesophilic genera – *Wollinella, Helicobacter* and *Campylobacter* – are motile; for at least six species (mostly isolated from hydrothermal vents) of the thermophilic genera *Nautilia* and *Sulfurimonas*, motility has been reported [33–37]. Halobacteriales: 119 of 137 [38]. Ignicoccales: 0 of 4; the flagella-like fibers identified for them do not support motility – rather, they function as adhesins [39]. Methanococci: 6 of 6 [40]. Thermococcales: virtually all species of the order Thermococcales have been isolated from hydrothermal marine vents [41]; no reviewing data on their motility is available. Our own analyses, however, have shown that 12 of 15 species of the genus *Thermococcus* are motile [21].

It must be stressed here that microscopic observations of hyperthermophiles at room temperature, and under oxic conditions, can be very misleading: for example, none of 15 species of the genus *Thermococcus* was motile at room temperature [21]; however, 12 were motile at their optimal growth temperature. Analyses using our newly developed temperature-gradient-forming (swimming) device – **TGFD** [21] – allowing microscopic analyses at up to 105°C under anaerobic conditions, have shown that hyperthermophiles in general have a much higher swimming speed than mesophilic microorganisms [24] (Box 3).

Adhesive Properties of Hyperthermophiles

Many studies have shown that hyperthermophiles use a variety of surface appendages for **adhesion** to various materials. In this context the reader is referred to recent reviews on archaeal type IV pili [42] and on archaeal surface appendages in general [43,44].

In the case of the aap pilus (archaeal adhesive pilus) of *Sulfolobus acidocaldarius* the extraordinary stability was argued to reflect an adaptation to the harsh environments that *S. acidocaldarius* encounters [45]. Both the aap pili and the ups pili (UV-induced pili of *Sulfolobus*) of *S. acidocaldarius* facilitate biofilm formation; the UV-induced ups pili contribute to cell aggregation and conjugation.

For *Methanococcus maripaludis*, both flagella and pili are necessary for attachment to materials such as glass, silicon wafers, nickel, gold, and molybdenum; cells did not attach to mica [46].

In my laboratory, comparative analyses of the adhesion of different archaea to various materials via their cell-surface appendages were undertaken; we found that different archaea can adhere to different spectra of materials – see Table S1 in the supplemental material online. Very interestingly, adhesion by microorganisms onto similar materials has been observed, if these were placed close to active vents [47]. Our data were obtained by adding parts of the materials to be tested to serum bottles used for growth experiments; adhesion to surfaces was measured by staining adhering cells with DAPI. Our studies included *Pyrococcus furiosus*,

Box 3. Dispersion of Hyperthermophiles into Cold Sea Water

Fluids emanating from black smokers were reported to do so with velocities of 1–3 (sometimes even > 5) meters per second. If a hyperthermophile with a size of 1 μm would be translocated by this stream for just 1 second, this corresponds to 10^6 body sizes for a 'slow smoker'. If that hyperthermophile would be able to swim directed (= in a straight line, without tumbles) back to its original location at a speed of 100 μm/s it would take 10^4 seconds = 2.8 h for the cell to cover these 10^6 μm. The deep-sea water, with an average temperature of ca. 2°C, however, does suppress motility of hyperthermophiles; in addition, aerobic conditions also would prevent motility.

Colonization of newly erupted black smokers from the outside, therefore, has to be by hyperthermophiles present in deep-sea water in a kind of hibernating state. Such cells must be able to react very fast to temperatures indicating the potential presence of a black smoker, they must be able to avoid temperatures too high for their life (emanating fluids are up to 400°C), and they should be able to adhere to suitable surfaces. Colonization may occur within a few days [7,9,14–17].

Methanocaldococcus villosus, *Methanothermus sociabils*, and *Methanococcus voltae* (adhesion via flagella); *Ignicoccus hospitalis* (adhesion by fibers); *Methanothermobacter thermoautotrophicus* (adhesion by fimbriae). In the case of *Methanopyrus kandleri* no cell-surface appendages could be identified.

In addition, the flagella of *P. furiosus* are used not only for swimming but also for adhesion onto various materials, and for the formation of cell–cell contacts [48]; the same holds true for *M. villosus* [49]. Very interestingly, adhesion of hyperthermophiles was observed not only onto various abiotic surfaces, but also to biotic surfaces via the formation of bi-species biofilms composed of *P. furiosus* with various methanogenic archaea [50,51]. Not only are pili and flagella used by hyperthermophiles for adhesion; other cell appendages, such as fimbriae of *M. thermoautotrophicus* and fibers of *I. hospitalis*, enable these archaea to adhere onto various surfaces [39,52].

Concluding Remarks

In conclusion, I postulate the following scenario. Colonization of a newly erupted black smoker occurs by a first accidental contact of hyperthermophiles present in cold seawater. The cells react within seconds to the high temperature present at the surface, by very fast motility, and swim to a region whose temperature is optimal for them. Via their much slower zigzag mode of swimming, they scan for a place optimal for adhesion and use their motility organelle to establish contact. Their flagella allow them a first attachment and growth in mono- and at least bi-species biofilms there; additional adhesins well might contribute to permanent colonization. It has been argued very recently that life originated in a hydrothermal setting (by reconstructing the genome of the last universal common ancestor (LUCA) from 6.1 million protein coding genes [53]); the widespread ability for motility and adhesion between hyperthermophiles is not too surprising, then.

Outstanding Questions

No hard (= experimental) data exist for the mechanism of colonization of newly erupted, sterile black smokers by hyperthermophiles. Such experiments have to be done with newly forming vent chimneys. Is it possible to sample wall material (probably by coring) under strictly sterile conditions, without inclusion of 'background' seawater, from the top of a newly forming black smoker, to return repeatedly to the same black smoker within a few hours to days and take samples from the same spot of that vent chimney to ask for the presence of live hyperthermophiles and their cell surface appendages in these samples?

Can, alternatively, an experimental setup be established under laboratory conditions to mimic the extreme conditions characteristic of newly forming black smokers? Such conditions include: porous surfaces of materials constituting black smokers; steep temperature gradients and steep gradients of anoxic/oxic sea water over that material; the presence of various gases; rapid water fluxes; a possibility for *in situ* microscopic analyses, etc.

Supplemental Information

Supplemental information associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tim.2016.11.002.

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