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Hydrogen and thiosulfate limits for growth of a thermophilic, autotrophic *Desulfurobacterium* species from a deep-sea hydrothermal vent

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Summary

Hydrothermal fluids (341°C and 19°C) were collected < 1 m apart from a black smoker chimney and a tubeworm mound on the Boardwalk edifice at the Endeavour Segment in the northeastern Pacific Ocean to study anaerobic microbial growth in hydrothermal mineral deposits. Geochemical modelling of mixed vent fluid and seawater suggests the mixture was anoxic above 55°C and that low H_2 concentrations (79 μmol kg^{-1} in end-member hydrothermal fluid) limit anaerobic hydrogenotrophic growth above this temperature. A thermophilic, hydrogenotrophic sulfur reducer, *Desulfurobacterium* strain HR11, was isolated from the 19°C fluid raising questions about its H_2-dependent growth kinetics. Strain HR11 grew at 40–77°C (T_{opt} 72–75°C), pH 5–8.5 (pH_{opt} 6–7) and 1–5% (wt vol^{-1}) NaCl ($NaCl_{opt}$ 3–4%). The highest growth rates occurred when $S_2O_3^{2-}$ and S° were reduced to H_2S. Modest growth occurred by NO_3^- reduction. Monod constants for its growth were K_s of 30 μM for H_2 and K_s of 20 μM for $S_2O_3^{2-}$ with a μ_{max} of 2.0 h^{-1}. The minimum H_2 and $S_2O_3^{2-}$ concentrations for growth were 3 μM and 5 μM respectively. Possible sources of $S_2O_3^{2-}$ and S° are from abiotic dissolved sulfide and pyrite oxidation by O_2.

Introduction

Deep-sea hydrothermal vents are seafloor expressions of biogeochemical processes that occur deeper within the subseafloor (Deming and Baross, 1993; Orcutt *et al.*, 2011). Based on thermodynamic predictions of the energy available for redox reactions in mixtures of hydrothermal fluid and seawater, chemolithoautotrophy is generally dominated by aerobic H_2S oxidation at mesophilic growth temperatures (e.g. below 50°C) and by anaerobic H_2 oxidation at higher temperatures at most hydrothermal vents (McCollom and Shock, 1997; Amend *et al.*, 2011). The amount of H_2 available for growth in hydrothermal fluids varies significantly based on host rock composition and frequency of volcanic activity (for summaries see Von Damm, 1995; Amend *et al.*, 2011; Holden *et al.*, 2012). The *Aquificales*

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and the *Methanococcales* are among the more common H_2-oxidizing autotrophs found in hydrothermal vents (Huber and Holden, 2008). The *Aquificales* are strictly autotrophic and largely thermophilic H_2 oxidizers that use various sulfur compounds, NO_3^- and sometimes O_2 as electron acceptors (Huber and Eder, 2006). The *Methanococcales* are mesophilic-to-hyperthermophilic methanogens that are generally obligate hydrogenotrophs, although a few can also use formate (Whitman and Jeanthon, 2006).

In some terrestrial anoxic environments such as freshwater sediments and sewage treatment plants, CH_4 formation is inhibited when SO_4^{2-} concentrations are high (Lovley and Goodwin, 1988). Mesophilic sulfate-reducing bacteria (e.g. *Desulfovibrio*) have lower H_2 half-saturation constants (K_s) for H_2 uptake and growth and higher maximum H_2 utilization and growth rates than mesophilic methanogens (e.g. *Methanobacterium, Methanobrevibacter, Methanospirillum* and *Methanosarcina*) (Kristjansson *et al.*, 1982; Lovley *et al.*, 1982; Robinson and Tiedje, 1984; Karadagli and Rittmann, 2005). This enables sulfate reducers to inhibit methanogen growth by lowering the partial pressure of H_2 to concentrations below levels that methanogens can use for growth. This is in keeping with the traditional hierarchy of anaerobic metabolisms, in which methanogenesis occurs only when all other electron acceptors are absent (Lovley and Goodwin, 1988). Unlike hydrothermal systems, the H_2 in these terrestrial environments is derived from the microbial breakdown of organic matter, and the minimum thresholds for syntrophic microbial H_2 uptake are at nanomolar concentrations (Lovley and Goodwin, 1988). However, methanogens can coexist with sulfate-reducing bacteria in the presence of SO_4^{2-} where the outcome of competition is a function of the rate of H_2 supply, relative population sizes and SO_4^{2-} availability (Lovley *et al.*, 1982).

The purpose of this study was to assess the effects of H_2 and $S_2O_3^{2-}$ concentration on the growth of a thermophilic, autotrophic sulfur reducer from a marine environment, then compare its growth limitations with those of marine thermophilic methanogens. It might be assumed that sulfur-reducing bacteria would outcompete methanogens for H_2 in marine thermal systems, given the evidence from terrestrial systems. However, few measurements of H_2 growth kinetics have been made for autotrophic thermophiles. The minimum and Monod half-saturation H_2 values for the growth of deep-sea methanogens (*Methanocaldococcus*) at 70°C and 82°C were 17–23 μM and 67 μM respectively (Ver Eecke *et al.*, 2012). In this study, an obligately hydrogenotrophic, thermophilic bacterium, *Desulfurobacterium* strain HR11, a member of the *Aquificales* that reduces $S_2O_3^{2-}$, S° and NO_3^-, was isolated from 19°C fluid flowing from the top of the Boardwalk hydrothermal edifice along the Endeavour Segment in the northeastern Pacific Ocean. Its physiological characteristics and minimum K_s values for growth on H_2 and $S_2O_3^{2-}$ were measured and compared with those of high-temperature marine methanogens. The geochemistry of pure 341°C hydrothermal fluid collected within a metre of the 19°C fluid used to isolate strain HR11 (Fig. S1) was determined to provide an environmental context for the growth of microbes in that system.

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Results and discussion

Fluid chemistry and microbial redox reaction energies

Most of the calculated end-member chemical concentrations for the 341°C hydrothermal fluid emanating from the Boardwalk hydrothermal chimney (Table 1) fall within the range of previously measured values for Endeavour Segment hydrothermal fluids (Lilley *et al.*, 1993; 2003; Butterfield *et al.*, 1994). Hydrogen concentrations were low to normal relative to historical values for Endeavour (Lilley *et al.*, 1993; 2003; Butterfield *et al.*, 1994; Ver Eecke *et al.*, 2012). Hydrogen concentrations in most of the pure (zero-Mg^{2+}) hydrothermal fluids from the Endeavour Segment since 2008 have been below 100 μmol kg^{-1} (Ver Eecke *et al.*, 2012), which peaked in some vents at >1 mmol kg^{-1} in 1999 following seismic activity (Lilley *et al.*, 2003). For the Boardwalk edifice in 2011, diluting the 341°C end-member hydrothermal fluid with seawater to 40–75°C results in H_2 concentrations of 9–17 μM in the mixed fluid. Using geochemical mixing models, mixed fluids were predicted to be anoxic above 55°C, and their pH were calculated to be above pH 5 below 70°C (Fig. S2).

At 25–45°C, aerobic oxidation of S^{2-} and CH_4 was predicted to provide the largest amount of redox energy for autotrophic catabolism (up to 13.7 J kg^{-1} and 15.9 J kg^{-1} of mixed vent fluid respectively) (Fig. S2). They were both limited by the availability of O_2 in seawater. The energies for hydrogenotrophic sulfate reduction and methanogenesis increased with temperature due to the increased availability of H_2 (up to 0.8 J kg^{-1} and 0.4 J kg^{-1} mixed vent fluid, respectively). They were substantially lower than the reaction energy available for mesophilic aerobic S^{2-} and CH_4 oxidation (Fig. S2), as reported previously (Amend *et al.*, 2011).

Thiosulfate and sulfur are the preferred terminal electron acceptors for the growth of *Desulfurobacterium* strain HR11 (see below), but their concentrations in hydrothermal fluids are unknown. Thiosulfate is a key intermediate in the oxidation of HS^- to SO_4^{2-}, especially where O_2 concentrations are below saturation (Cline and Richards, 1969; Jørgensen, 1990). O_2 concentrations at 2200 m depth in the northeast Pacific Ocean near North America are low (~70 μmol kg^{-1}) due to an oxygen minimum zone in the region (Hartnett *et al.*, 1998). Thiosulfate also forms from pyrite from within hydrothermal chimney walls. Pyrite is abiotically oxidized by Fe^{3+}, which adsorbs to the pyrite and forms Fe^{2+} and $S_2O_3^{2-}$, although the $S_2O_3^{2-}$ is rapidly oxidized to SO_4^{2-} if additional Fe^{3+} is present (Luther, 1987; Moses *et al.*, 1987). Pyrite is also oxidized by O_2. The reaction rate is 10-fold slower than with Fe^{3+} as an oxidant, but $S_2O_3^{2-}$ is present in higher concentrations due to its slow oxidation rate with O_2 (Luther, 1987; Moses *et al.*, 1987).

Characteristics of strains HR11

Strain HR11 was isolated at 55°C from the 19°C hydrothermal fluid emitted from the Boardwalk edifice and produced H_2S in modified DSM 282 medium. Based on its 16S rRNA gene sequence, it is phylogenetically most closely related (>99% identity) to *Desulfurobacterium thermolithotrophum* (L'Haridon *et al.*, 1998) (Fig. S3). Electron microscopy revealed short oblong rods, 0.5 μm by 1–2 μm, with a typical Gram-negative bacterial cell envelope and lophotrichous flagellation with three flagella (Fig. S4). Growth

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was observed between 40°C and 77°C with an optimum of 72–75°C (Fig. 1A), between pH 5.0 and pH 8.5 with an optimum of pH 6.0–7.0 (Fig. 1B), and between 1% and 5% NaCl with an optimum of 3–4% (Fig. 1C). Metabolite measurements showed that the organism produced up to 6 mM H_2S. Strain HR11 is an obligate hydrogenotrophic autotroph that did not utilize yeast extract, maltose, tryptone, acetate or formate as an alternative source of carbon or electrons. In bottles, it grew at the same rate with elemental sulfur as the sole electron acceptor ($1.56 \pm 0.17\ h^{-1}$) as it did with $Na_2S_2O_3$ ($1.59 \pm 0.26\ h^{-1}$) and showed modest growth ($0.24 \pm 0.21\ h^{-1}$) when KNO_3 was the terminal electron acceptor. Strain HR11 did not grow when Na_2SO_3, Na_2SO_4, Fe(III)-citrate, Fe(III) (oxy)hydroxide or O_2 were supplied as the terminal electron acceptor. It did not grow on modified DSM 282 medium without the addition of an electron acceptor.

Monod kinetics for Desulfurobacterium strain HR11

Desulfurobacterium strain HR11 was grown in a gas flow-controlled bioreactor at 72°C to determine the effect of H_2 and $S_2O_3^{2-}$ concentration on growth. It had longer doubling times and lower maximum cell concentrations with decreasing H_2 and $S_2O_3^{2-}$ concentrations. The minimum H_2 concentration for growth was 3 μM and the K_s for growth on H_2 was 30 μM (Fig. 2A). When grown on excess H_2 (>100 μM), strain HR11 grew on as little as 5 μM $S_2O_3^{2-}$ and its K_s for growth was 20 μM (Fig. 2B). The maximum growth rate (μ_{max}) in the reactor was $2.0\ h^{-1}$. Ver Eecke and colleagues (2012) previously measured the minimum and K_s values of H_2 for the growth of three methanogens (*Methanocaldococcus* spp.) grown at 70°C and 82°C in the same reactor. All three organisms had minimum H_2 requirements of 17–23 μM, a K_s for H_2 of 67 μM and a μ_{max} of $0.8–1.2\ h^{-1}$. In this study, *Desulfurobacterium* strain HR11 had a lower minimum H_2 requirement, a lower H_2 K_s and a higher μ_{max} than those reported for *Methanocaldococcus*. The μ_{max}/K_s ratios for H_2 indicate that *Desulfurobacterium* strain HR11 has a growth advantage over *Methanocaldococcus* species ($0.067\ h^{-1}\ \mu M^{-1}$ versus $0.015\ h^{-1}\ \mu M^{-1}$).

For terrestrial mesophilic microbes, the Monod H_2 K_s is 2–4 μM for *Desulfovibrio* strain G11 and 6–7 μM for *Methanospirillum hungatei* JF-1 (Robinson and Tiedje, 1984). Similarly, the H_2 uptake K_s is 1–2 μM for five *Desulfovibrio* spp.; 3–7 μM for *Methanobrevibacter*, *Methanobacterium* and *Methanospirillum* species; and 13 μM for *Methanosarcina barkeri* strain MS (Kristjansson *et al.*, 1982; Robinson and Tiedje, 1984). These differences in substrate affinities confer a competitive advantage for sulfate-reducing bacteria over methanogens when SO_4^{2-} is not limiting. However, both groups of organisms can coexist in anoxic environments when both H_2 and SO_4^{2-} are plentiful (Lovley *et al.*, 1982). A global survey of low-temperature hydrothermal fluids with co-localized phylogenetic and chemical analyses shows that *Desulfurobacterium* and the *Methanococcales* are both present in vent environments with H_2 concentrations predicted to be above 17 μM at 72°C, and both are generally absent below this threshold (Table S1). This includes the 19°C hydrothermal fluid in this study where a thermophilic methanogen (*Methanothermococcus* strain BW11) was also isolated (Fig. S2). These data suggest that $S_2O_3^{2-}$ and S° are not at limiting concentrations in these hydrothermal systems and that generally there is sufficient H_2 flux in many vent systems to support both groups of organisms.

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The diversity of thermophilic anaerobes in hydrothermal vents is relatively low, making pure cultures of these organisms useful for modelling growth and competition in these systems. Thermophilic, autotrophic sulfur reducers such as *Desulfurobacterium* spp. and thermophilic methanogens such as *Methanothermococcus* and *Methanocaldococcus* spp. are common in vent systems; grow over the same temperatures, pHs and salinities; and compete for H_2, making them ideal candidates for environmental modelling. Although *Desulfurobacterium* appears to have a kinetic growth advantage over the *Methanococcales* as long as $S_2O_3^{2-}$ or S ° is present, the two functional groups appear to coexist where the flux of H_2 is sufficient. Important future research questions are how these organisms respond physiologically to H_2 limitation, whether spatial heterogeneity separates them *in situ*, and if they have physiological mechanisms to compete for resources.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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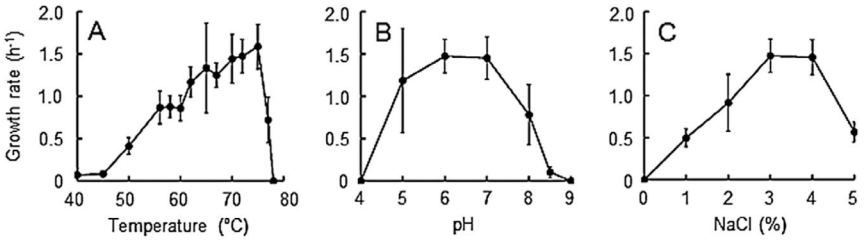

Fig. 1.

Growth rates for strain HR11 grown over its ranges of temperature (A), pH (B) and NaCl concentration (C). Strain HR11 was grown in 10 ml of modified DSM 282 medium in sealed Balch tubes, with 1 g l^{-1} $S_2O_3^{2-}$ as the electron acceptor and 2 atm 80:20 H_2:CO_2 headspace, and growth determined via cell counts on a Petroff–Hausser chamber. Error bars represent 95% confidence intervals.

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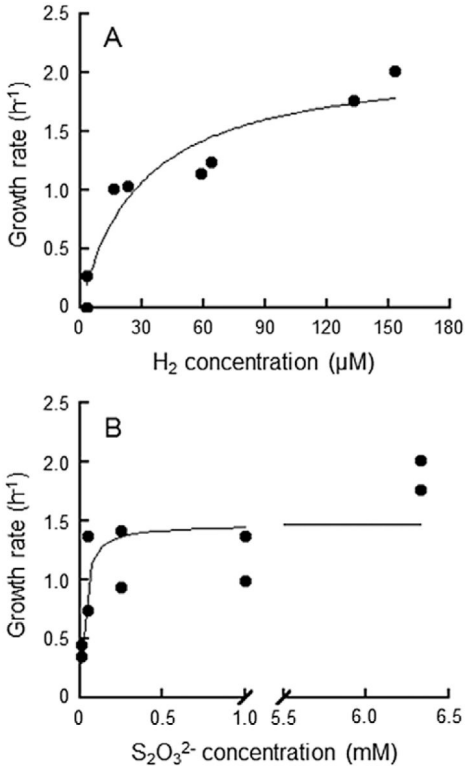

Fig. 2.

Growth rates for strain HR11 grown over its ranges of H_2 concentration (A) and initial $Na_2S_2O_3$ concentration (B). Strain HR11 was grown in 1.5 l of modified DSM 282 medium in a 2 l bioreactor, gassed with H_2, CO_2 and N_2 as required to achieve experimental H_2 concentrations. Growth was determined as for Balch tube experiments. The line is a Michaelis–Menten fit to the data.

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Table 1

Chemical composition of end-member hydrothermal vent fluid from the Boardwalk edifice extrapolated to zero-Mg^{2+} from this study and seawater for modelling purposes.

	Hydrothermal fluid	Seawater[a]
Temperature, max.	341°C	2°C
pH at 25°C	4.1	7.8
H_2 (μmol kg^{-1})	79	0
CH_4 (μmol kg^{-1})	2680	0
O_2 (μmol kg^{-1})	0	70
Na^+ (mmol kg^{-1})	506.9	441
K^+ (mmol kg^{-1})	36.2	9.8
NH_4^+ (μmol kg^{-1})	833	–
Mg^{2+} (mmol kg^{-1})	0.01	54.5
Ca^{2+} (mmol kg^{-1})	48.2	10.7
Fe^{2+} (μmol kg^{-1})	1300.4	0
Cl^- (mmol kg^{-1})	621.9	550
SO_4^{2-} (mmol kg^{-1})	1.7	27.9
HCO_3^- (mmol kg^{-1})	29.4	2.2
HS^- (mmol kg^{-1})	3.4	0
SiO_2 (mmol kg^{-1})	18.1	0.13

[a]Seawater composition from Amend and colleagues (2011), except the O_2 concentration, which is from Richard Thomson (Institute of Ocean Sciences, Fisheries and Oceans Canada, pers. comm.).