

Hydrogen syntrophy-driven methanogenesis by hyperthermophiles in hot subsurface environments

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Nearly all the annual global flux of CH₄ to the atmosphere comes from H₂ syntrophy. However, little is known about (hyper)thermophilic CH₄ production in high-temperature subsurface environments such as hydrothermal vents, oil and gas reservoirs, marine sediments, and terrestrial shale beds, which can be anoxic, organic-rich, and salty. H₂ syntrophy between hyperthermophilic, H₂-producing heterotrophs (e.g. *Thermococcus*) and H₂-consuming methanogens (e.g. *Methanothermococcus*) found in these environments could be a common mechanism for CH₄ production. H₂ was the primary limiting factor for the growth of methanogens in Axial Volcano diffuse vent fluid microcosms incubated at 55°C and 80°C. The addition of tryptone without H₂ to these microcosms demonstrated that methanogenic H₂ syntrophy was possible, involving primarily *Thermococcus*, *Methanocaldococcus*, and *Methanothermococcus*. *Thermococcus paralvinellae* produced H₂ when grown on a carbohydrate, peptides, or formate without S⁰ present. However, ~ 70 μM H₂ in the background led to growth inhibition, expression of formate hydrogenlyase genes, and formate secretion, with a modeled decrease in energy production due to diminished H⁺ translocation on the membrane. Among methanogens, the H₂ half-saturation constant for CH₄ production for *Methanocaldococcus jannaschii* and *Methanothermococcus thermolithotrophicum* was 27-37 μM with no growth below 3-9 μM H₂. Syntrophic growth of *T. paralvinellae* with either *M. jannaschii* or *Methanothermococcus* sp. BW11 ameliorated H₂-limited growth in both syntrophic pairs, and stimulated growth and H₂ production in *T. paralvinellae*. Both field and laboratory evidence demonstrate that (hyper)thermophiles cooperate with each other during periods of metabolic stress.