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Hydrogen utilization by *Methylocystis* sp. Strain SC2 expands the known metabolic versatility of type IIa methanothrophs

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Abstract:

Methane, a non-expensive natural substrate, is used by *Methylocystis* sp. As a sole source of carbon and energy. Here, we assessed whether *Methylocystis* sp. Strain SC2 is able to also utilize hydrogen as an energy source. The addition of 2 % H_2 to the culture headspace had the most significant positive effect on the growth yield under CH_4 (6 %) and O_2 (3 %) limited conditions. The SC2 biomass yield doubled from 6.41 (± 0.52) to 13.82 (± 0.69) mg cell dry weight per mmol CH_4, while CH_4 consumption was significantly reduced. Regardless of H_2 addition, CH_4 utilization was increasingly redirected from respiration to fermentation-based pathways with decreasing O_2/CH_4 mixing ratios. Theoretical thermodynamic calculations confirmed that hydrogen utilization under oxygen-limited conditions doubles the maximum biomass yield compared to fully aerobic conditions without H_2 addition. Hydrogen utilization was linked to significant changes in the SC2 proteome. In addition to hydrogenase accessory proteins, the production of Group 1d and Group 2b hydrogenases was significantly increased in both short- and long-term incubations. Both long-term incubation with H_2 (37 d) and treatments with chemical inhibitors revealed that SC2 growth under hydrogen-utilizing conditions does not require the activity of complex I. Apparently, strain SC2 has the metabolic capacity to channel hydrogen-derived electrons into the quinone pool, which provides a link between hydrogen oxidation and energy production. In summary, H_2 may be a promising alternative energy source in biotechnologically oriented methanotroph projects that aim to maximize biomass yield from CH_4, such as the production of high-quality feed protein.

Keywords: Knallgas bacteria, hydrogenase, methanotrophs, *Methylocystis*, proteomics, metabolic model