Inorganic Carbon Limitation and

Diversification of Hydrogenotrophic

Methanogens in the Samail

Ophiolite, **Oman**

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Serpentinization can generate fluids that are enriched in H₂ and depleted in inorganic carbon. Two metagenome assembled genomes (MAGs) affiliated with Methanobacterium were recovered from subsurface fracture fluids of the Samail Ophiolite, Oman. One MAG (Type I) was abundant in circumneutral waters (, while the other (Type II) was abundant in hydrogen rich, hyperalkaline waters. The Type I MAG couples H₂ oxidation to CO₂ reduction and methanogenesis. Surprisingly, the Type II MAG, which are derived from Type I lineage, lack two key H₂ oxidizing [NiFe]-hydrogenases (Frh and Mvh) and these functions appear to be replaced by formate dehydrogenases. This indicates the Type II Methanobacterium drives methanogenesis with formate and suggests that the availability of oxidants supplants that of reductants in shaping methanogen evolution in highly serpentinized waters. Single amplified genomes (SAGs) reveal ongoing strain-level diversification of the Type II MAGs, including at several loci of putative physiological and ecological importance. Results are discussed in relation to ongoing Methanobacterium evolution in response to inorganic carbon limitation.