LITHOTROPHIC NITRATE REDUCTION UNDER HIGH-PRESSURE CONDITIONS AT DEEP-SEA VENTS. D. I. Foustoukos[1], I. Pérez-Rodríguez[2], S. M. Sievert[3], and C. Vetriani[4], [1]Earth & Planets Laboratory, Carnegie Institution of Washington, Washington DC, USA (dfoustoukos@ciw.edu), [2]Department of Earth and Environmental Science, University of Pennsylvania, Philadelphia PA, USA (ilperez@sas.upenn.edu), [3]Biology Department, Woods Hole Oceanographic Institution, Woods Hole MA, USA (ssievert@whoi.edu), [4]Department of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ USA (vetriani@marine.rutgers.edu).

Introduction: To better constrain the extent and nature of the extreme biosphere at deep-sea vents, culture-based studies need to address the rates of chemosynthetic primary productivity at in-situ pressures, temperatures and substrate (e-donor/acceptor) conditions. A key parameter that has been poorly explored is the effect of pressure on the metabolic activities and function of deep-sea bacterial communities [1]. In a series of recent experimental and field studies, we assessed the effect of chemolithoautotrophic NO_3-reduction on the distribution of N compounds and on the adaptation strategies of microbial communities at the extreme conditions of deep-sea hydrothermal vents. We have succeeded in studying the evolution and metabolic activity of these microorganisms when grown as mixed (natural) or pure cultures under in-situ deep-sea vent pressure, temperature and nutrient-level conditions.

Culturing Mixed Communities: Vent fluids were collected from hydrothermal vents at the East Pacific Rise (2503 m): "*Crab Spa*" (9.8398° N, 104.2913° W) and "*Alvinella*" (9.8398° N, 104.2915° W) [2-4]. At these sites, diffuse flow fluids enriched in free-living microbes were emitted at 24 °C and 50 °C, respectively. By utilizing fluids collected and transferred onboard under seafloor pressures, we conducted high-pressure incubations (25 MPa) at 30 °C and 50 °C to constrain the function and metabolic rates of mesophilic and thermophilic chemosynthetic NO_3^--reducing microbial communities residing at *Crab Spa* and *Alvinella*, respectively [2]. To enhance the activity of nitrate respiring anaerobic bacteria, a NO_3^- (500 μM) and $H_{2(aq)}$ (130 μM) enriched medium was introduced under strictly anaerobic conditions. Dissolved HCO_3^- was used as carbon source. Two distinct sets of experiments were conducted for 356 (*Crab Spa*) and 50 hours (*Alvinella*).

Microbial growth was assessed by direct cell counts. Results revealed that the growth rates in fluid incubations at 50 °C were greater than fluid incubations at 30 °C (Fig. 1a). On the other hand, the rates of NO_3^- consumption were comparable between incubations at 30 °C and at 50 °C (Fig. 1b). The microbial activity observed in the high-pressure incubations is consistent with the growth and metabolic efficiencies reported in monocultures performed at ambient pressures [5]. However, the concentrations of e-donors/acceptors in the shipboard incubations approached natural diffuse vent fluid compositions [2, 3], and thus, they were significantly lower than those adopted in pure-culture experiments conducted under optimum growth conditions by utilizing substrate-enriched media (see in [5]).

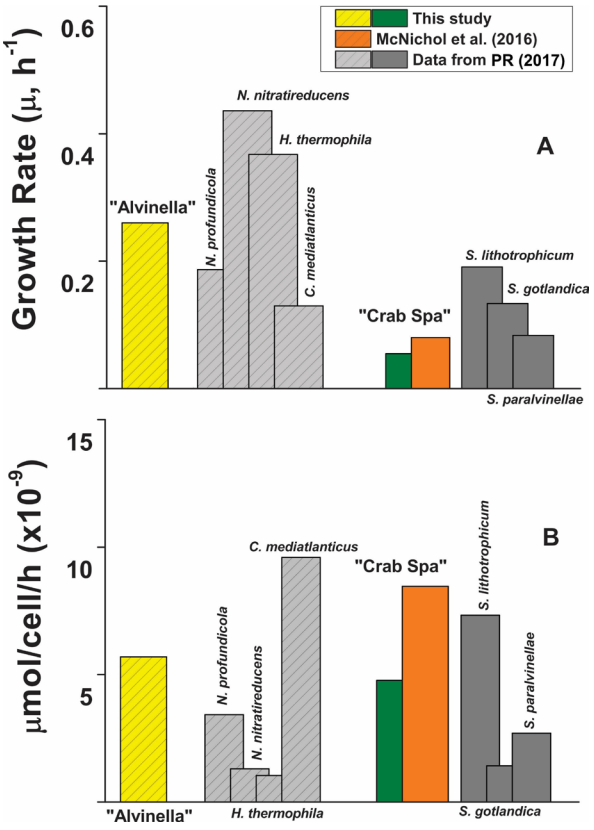

Figure 1. *(a) Growth rates and (b) cell-specific nitrate reduction rates attained during growth of NO_3-reducing chemosynthetic communities in shipboard high-pressure incubations. PR (2017) is [5].*

***Nautilia* strain PV-1:** A novel strain was isolated from the shipboard high-pressure enrichment cultures. A 16S rRNA gene-based phylogenetic analysis showed that strain PV-1 is an campylobacterium (aka epsilonproteobacterium) of the family *Nautiliaceae*. Strain PV-1 has been characterized as piezophilic, moderately thermophilic anaerobe that grows chemolithoautrophically with H_2 as electron donor and NO_3^- and S^0 as terminal electron acceptors, and utilize the reductive tricarboxylic acid cycle (rTCA) for CO_2 fixation [6] . The attained doubling time of ~16 min at 20 MPa, 55 °C is the shortest generation time of all known piezophilic microorganisms [7].

PV-1 attains higher growth rates than other DNRA-metabolizing campylobacteria even at ambient pressure conditions (Fig. 2). However, the rates of nitrate reduction were comparable between strain PV-1 and the other isolates. Thus, the decoupling between growth efficiency and rates of nitrate reduction inferred from shipboard and laboratory cultures [2, 5] appears to be conserved in this piezophilic bacterium

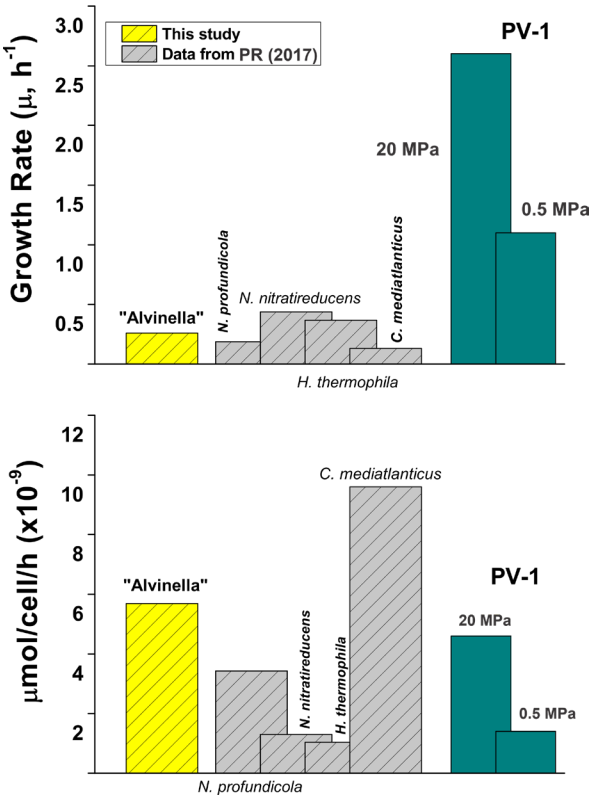

Figure 2. *Growth rates (top) and cell-specific nitrate reduction rates (bottom) of PV-1 strain compared to shipboard incubations and studies of DNRA per-forming bacteria. PR (2017) is [5].*

To understand the eco-physiological response of PV-1 to its natural environment, it is important to constrain the energy demands of growth and the rates of $NO_3^-/H_{2(aq)}$ utilization. In a series of high-pressure continuous cultures, we evaluated the $H_{2(aq)}$ requirements for the growth of PV-1 at low and high pressure conditions (Fig. 3). It became apparent that the availability of $H_{2(aq)}$ at different "dilution rates" (flow rate / culture volume) governed biomass yields. Data indicate that energetic demands were increased at seafloor pressures. The saturation constant K_s that describes substrate limitations [8] ranged between 1.5 mM and 0.75 mM at 20 and 0.5 MPa, respectively. As far as it concerns the effect of $H_{2(aq)}$ on microbial growth, the piezophilic behavior of this strain is accompanied with increased energy requirements at high hydrostatic pressures. This is consistent with what has been observed in another set of high-pressure cultures involving *Thiomicrospira thermophila* strain EPR 85, where pressure stresses at 10 MPa resulted in an increase of bioenergetical demands for electron acceptor ($O_{2(aq)}$) [9].

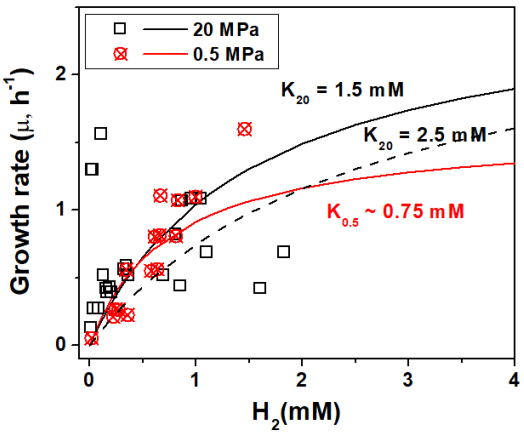

Figure 3. *The effect of $H_{2(aq)}$ availability on the growth of the chemosynthetic DNRA performing PV-1 strain. High pressure triggers an increase for energy demands.*

The strong effect of $H_{2(aq)}$ on the biomass yield of PV-1 indicates that direct application of the activities of pure cultures attained at optimum conditions may not be realistic for describing natural microbial communities. This becomes apparent when, to understand the apparent discrepancy between the growth rates of PV-1 and *Alvinella* microbial community (Fig.2) we need to account for the low $H_{2(aq)}$ concentrations attained in the shipboard incubations (<130 μM). Our results, further suggest that the deep-sea vent *Campylobacterota* adapted to similar optimal growth temperature conditions tend to display similar baseline metabolic rates regardless of their piezo-philic or -tolerant physiology [9].

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