



# Characterization of Two Hydrogen-Oxidizing *Hydrogenovibrio* Strains From Kermadec Volcanic Island Arc Hydrothermal Vents

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The genus *Hydrogenovibrio* consists of chemolithotrophic sulfur- and hydrogen-oxidizing bacteria that are found in diverse marine environments including hydrothermal vents where they can reach high cell numbers. Although several vent *Hydrogenovibrio* genomes encode for [NiFe]-hydrogenases (enzymes catalyzing the reversible reaction of hydrogen into protons and electrons), different attempts to grow these strains on hydrogen failed for a long time. Not long ago it was shown that some *Hydrogenovibrio* strains from hydrothermal vents are indeed able to oxidize hydrogen, which broadens their physiological spectrum in a competitive environment for energy sources. We here identify two active hydrogen consuming bacteria of the *Hydrogenovibrio* genus with different hydrogenase genes from vents in the South Pacific Ocean. Based on our results, hydrogen consuming *Hydrogenovibrio* species seem to be much more widespread in the oceans than expected.

**Keywords:** *Hydrogenovibrio*, [NiFe]-hydrogenase, geographic range, microbial hydrogen oxidation, Kermadec Arc

## INTRODUCTION

*Hydrogenovibrio* species are common in hydrothermal vent environments (Brazelton and Baross, 2010; Böhnke et al., 2019). They were originally described as *Thiomicrospira* species and based on physiology, morphology and phylogeny only recently reclassified as *Hydrogenovibrio* (Boden et al., 2017). Initially they were described as chemolithotrophic sulfur-oxidizers capable of using hydrogen sulfide, thiosulfate, tetrathionate and sulfur under aerobic and/or microaerobic conditions (e.g., Ruby and Jannasch, 1982; Jannasch et al., 1985; Brinkhoff and Muyzer, 1997; Takai et al., 2004). Mostly, they were shown to be autotrophic CO<sub>2</sub> fixers, but some were posited to be chemolithomixotrophs (Takai et al., 2004). Their ability to utilize alternative inorganic electron donors was expanded by hydrogen oxidation and iron oxidation in the last few years (Hansen and Perner, 2015; Barco et al., 2017). Recent incubation experiments with hydrothermal fluids demonstrate that when spiked with hydrogen or iron their numbers as well as biomass synthesis can increase considerably in some hydrothermal fluids (Böhnke et al., 2019). Consequently, they appear to be important at hydrothermal vents for carbon turnover.

The first *Hydrogenovibrio* species from a vent for which a hydrogenase (enzyme converting H<sub>2</sub> < - > 2H<sup>+</sup> + 2e<sup>-</sup>) was discovered on its genome was *H. crunogenus* XCL-2 (previously *Thiomicrospira crunogena* XCL-2) (Scott et al., 2006), although repetitive experiments under different conditions could not detect hydrogen consumption in cultivation experiments (Hansen and Perner, 2016a,b). Hydrogenases from other hydrothermal vent *Hydrogenovibrio* species were

later identified and respective hydrogen consumption ability verified (Hansen and Perner, 2016a). The hydrogenases of the actively consuming hydrogen oxidizing *Hydrogenovibrio* vent species were identified as [NiFe]-hydrogenases of the subgroup 1, associated with hydrogen uptake (Vignais and Billoud, 2007). Two major subgroups among the *Hydrogenovibrio* were recognized: those forming cluster I with MA2-6 and SP-41 (from the Mid-Atlantic Ridge, MAR) (Brinkhoff and Muyzer, 1997; Hansen and Perner, 2015) and *H. marinus* (from the water column) (Nishihara et al., 1991), other *Gammaproteobacteria*, *Alpha*- and *Zetaproteobacteria* (Hansen and Perner, 2016a). The cluster II combines the *H. crunogenus* hydrogenases encoded on the genomes of MA-3 (Wirsén et al., 1998), SP-41 (Hansen and Perner, 2015) from the MAR and TH-55 (Jannasch et al., 1985) and L-12 (Ruby and Jannasch, 1982) from the East Pacific Vents (Hansen and Perner, 2016a). According to Greening et al. (2016), cluster I hydrogenases are associated with group 1d and cluster II with group 1b. Just recently a new *Hydrogenovibrio thermophilus* strain from the Southwest Indian Ridge (SWIR) was isolated, namely S5 which encodes a hydrogenase associated with cluster I and which can consume hydrogen (Jiang et al., 2017).

To date no hydrothermal vent *Hydrogenovibrio* species have been enriched from vents in the Southern Pacific that encode a hydrogenase of cluster I or II and/or exhibit hydrogen consumption ability. Here, we expand the geographic range of hydrogen oxidizing *Hydrogenovibrio crunogenus* species, so far only found along mid-ocean ridges (MOR), to an island arc venting system.

## MATERIALS AND METHODS

### Sample Collection

A hydrothermal fluid (45 ROV 5F/6F KIPS A/B; 70°C, water depth 1318 m; -34°52.73383'S 179°04.26386'E) was taken with the remotely operative vehicle ROV Quest (MARUM, University Bremen) during the HYDROTHERMADEC cruise (SO253, December 2016/January 2017) with the RV Sonne. The hydrothermal fluid was retrieved with the pumped flow-through system KIPS (Kiel Pumping System) (Garbe-Schönberg et al., 2006) from Brothers lower cone in the Kermadec region. For further details on sampling see Perner et al. (2009). The sample was immediately processed after retrieval on board.

### Enrichment, Cultivation and Isolations Attempts

Initially an enrichment culture was grown by inoculating artificial seawater medium (MJ medium, 10 ml) and T-ASW medium (10 ml) under an atmosphere of H<sub>2</sub>:CO<sub>2</sub>:O<sub>2</sub> (79:20:1) (Westfalen AG, Münster, Germany) at standard pressure with 1 ml of the diffuse fluid sample. MJ was prepared as described before without yeast extract and trypticase peptone, but with addition of 10 ml vitamin solution (Balch et al., 1979). The reduction state of the MJ medium was monitored with resazurin (0.5 mg l<sup>-1</sup>). We prepared the T-ASW medium like described previously (Dobrinski et al., 2005) but with raised Ni and Fe concentrations (0.003 mM and 0.03 mM final concentration, same as in MJ

medium). After incubation at 28°C for 1 week a color change in the MJ medium (from blue to colorless), which accompanies the consumption of oxygen, could be observed. Serum bottles with 50 ml of MJ and T-ASW medium were then inoculated with 2 mL of the pre-cultures and supplemented with the gas mixture. The enrichment culture was routinely cultivated on the respective media as described above.

To obtain a pure culture, we used the PALM MicroTweezers microscope (Carl Zeiss AG, Oberkochen, Germany) for picking single cells. Additionally we made six-fold dilution series with T-ASW and MJ medium to 10<sup>-10</sup>. Since both methods were not successful for isolating the strains in MJ medium, we additionally plated the cultures on MJ agar plates.

### Hydrogen Consumption Measurements

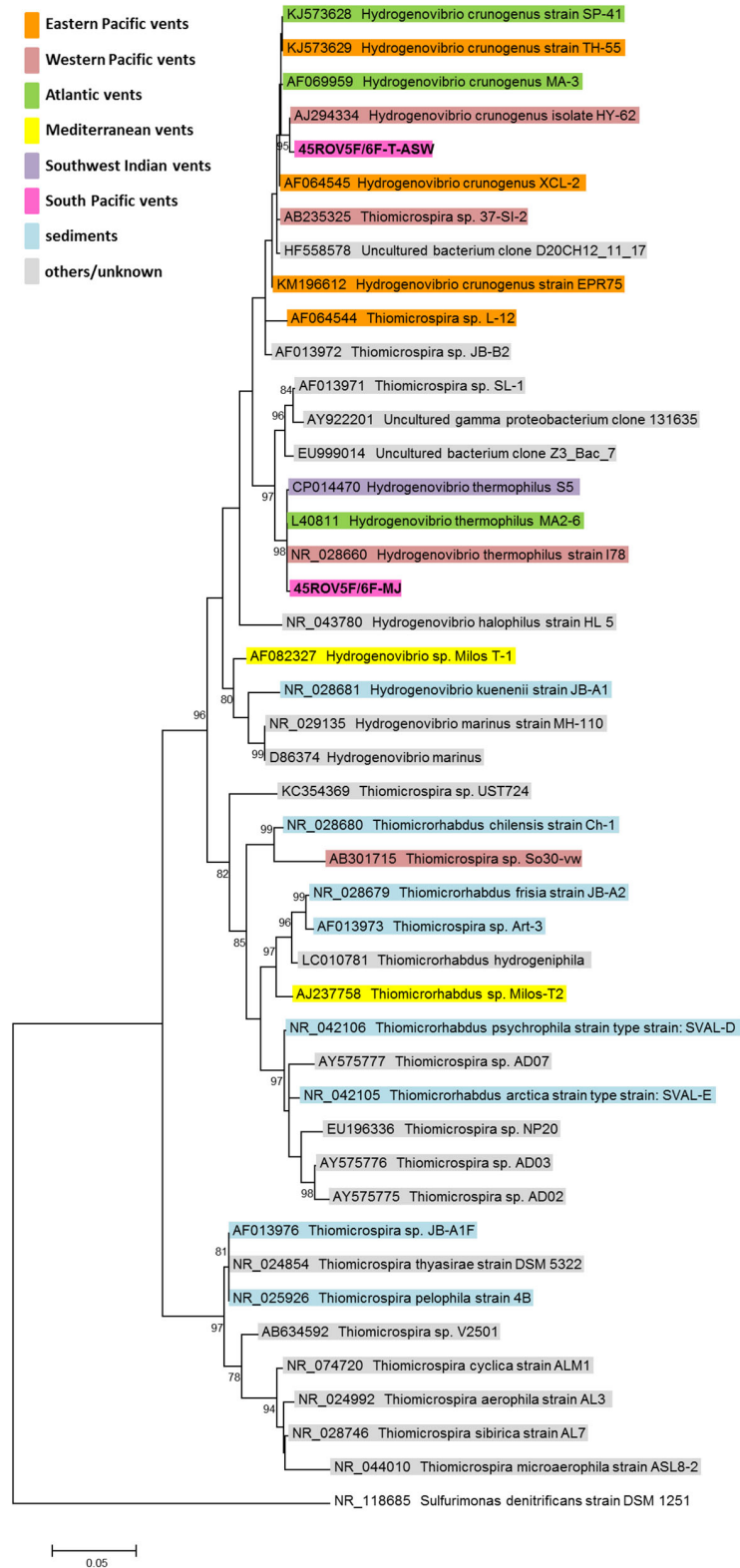
The cultures were grown on the respective medium (MJ or T-ASW) in serum bottles with an H<sub>2</sub>:CO<sub>2</sub>:O<sub>2</sub>:He (2:20:1:77) (Westfalen AG, Münster, Germany) atmosphere at standard pressure. The experiment was set up in triplicate and sterilized medium was used as a control. The concentration of hydrogen was measured using gas chromatography and cell numbers were determined using an Olympus BX41 (Olympus, K.K., Tokyo, Japan) microscope as described before (Hansen and Perner, 2015).

### DNA and RNA Extraction, cDNA Generation and Amplification of 16S rRNA and *hynL* Gene Fragments

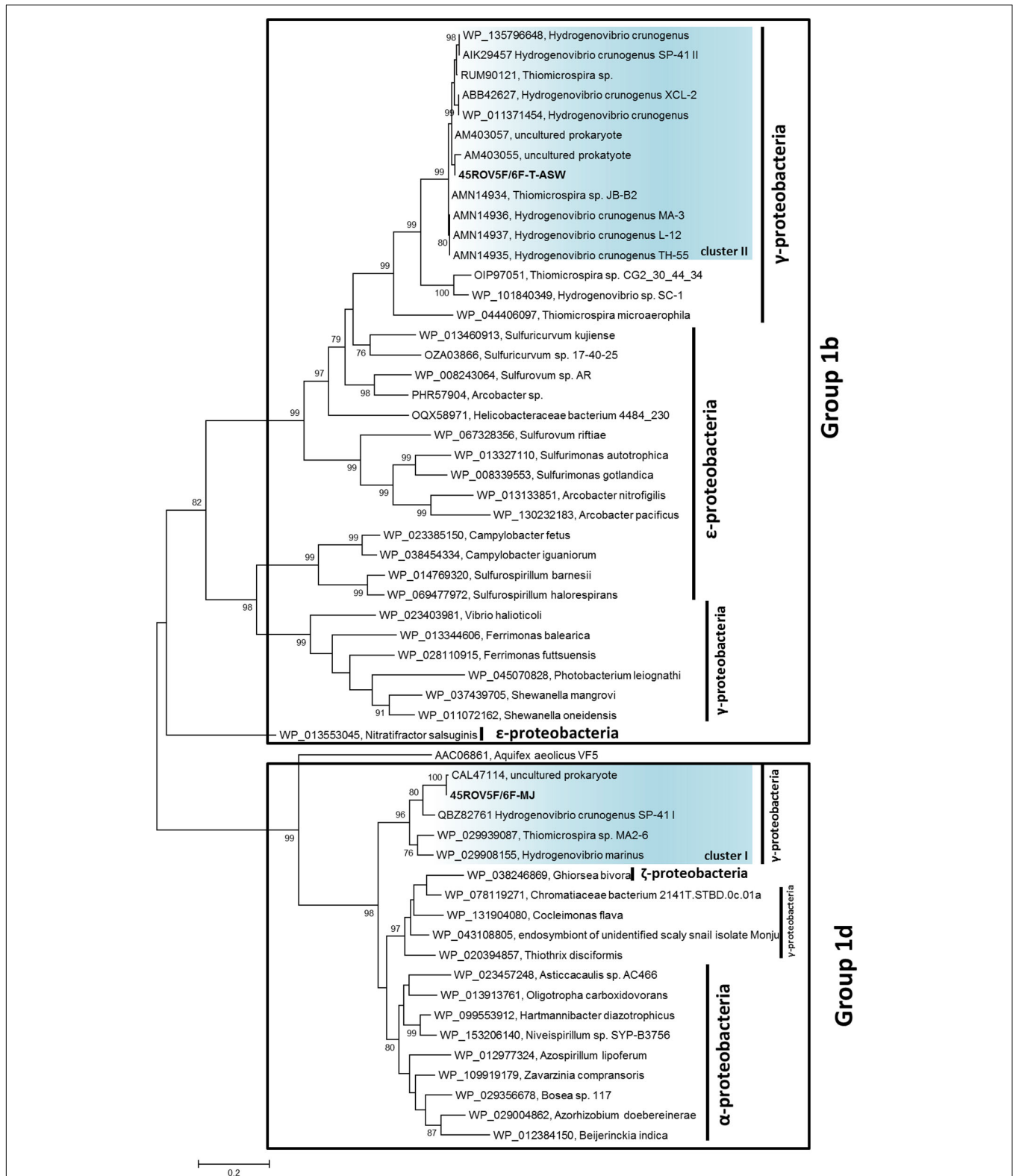
At the end of the hydrogen consumption experiments the active organisms were determined by simultaneous DNA and RNA extraction using TRIzol™ Reagent (Fisher Scientific, Schwerte, Germany) in combination with a phenol-chloroform extraction method and the Direct-zol™ RNA Miniprep Kit (Zymo Research, Irvine, United States). Complementary DNA synthesis was performed using the SuperScript™ VILO™ cDNA Synthesis Kit (Fisher Scientific, Schwerte, Germany). Polymerase chain reaction and sequencing of 16S rRNA and *hynL* genes were performed (see **Supplementary Figure 1**; Hansen and Perner, 2015, 2016a). The 16S rRNA and *hynL* gene sequences were deposited under the NCBI accession numbers MN923280, MN923281, MN935466, and MN935467.

## RESULTS AND DISCUSSION

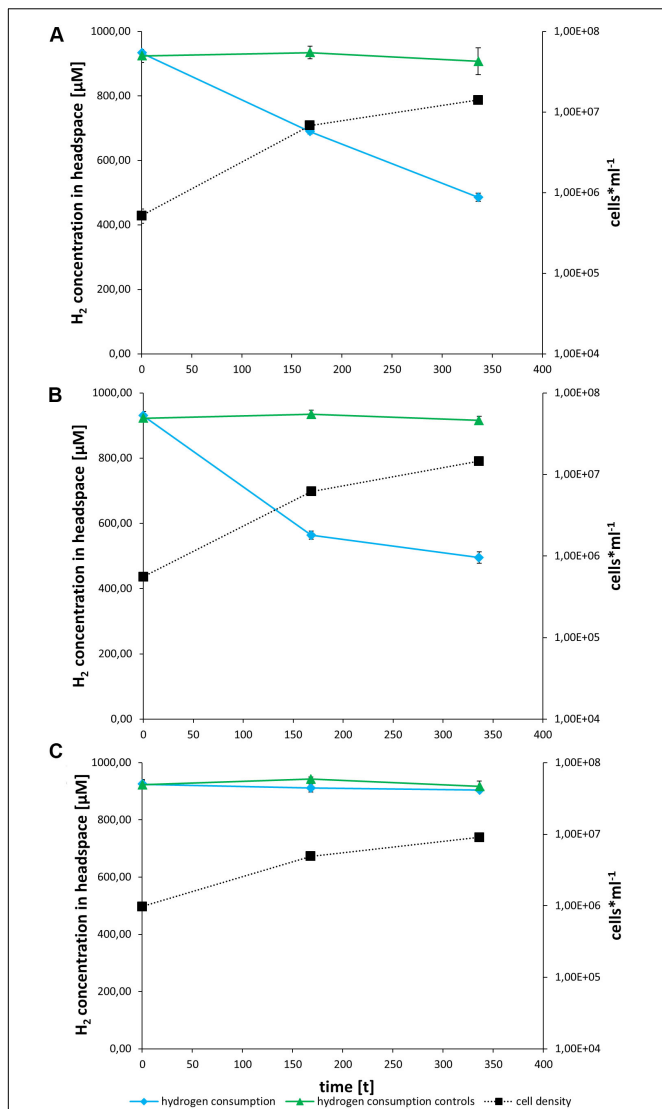
We used hydrothermal fluids (45ROV5F/6F) from the Brothers volcano on the Kermadec island arc (New Zealand) to enrich for *Hydrogenovibrio* species. Two types of artificial seawater media were used: (i) T-ASW medium and (ii) MJ medium (cf. Sako et al., 1996; Dobrinski et al., 2005; Hansen and Perner, 2015) – for differences of media compositions see Hansen and Perner (2016b). Since we aimed at culturing hydrogen oxidizers, the medium was supplemented with hydrogen gas (79H<sub>2</sub>:20CO<sub>2</sub>:1O<sub>2</sub>) and kept at room temperature. From these enrichments, two new hydrogen consuming *Hydrogenovibrio* species were identified: *Hydrogenovibrio* sp. 45ROV5F/6F-TASW and *Hydrogenovibrio* sp. 45ROV5F/6F-MJ. According to 16S



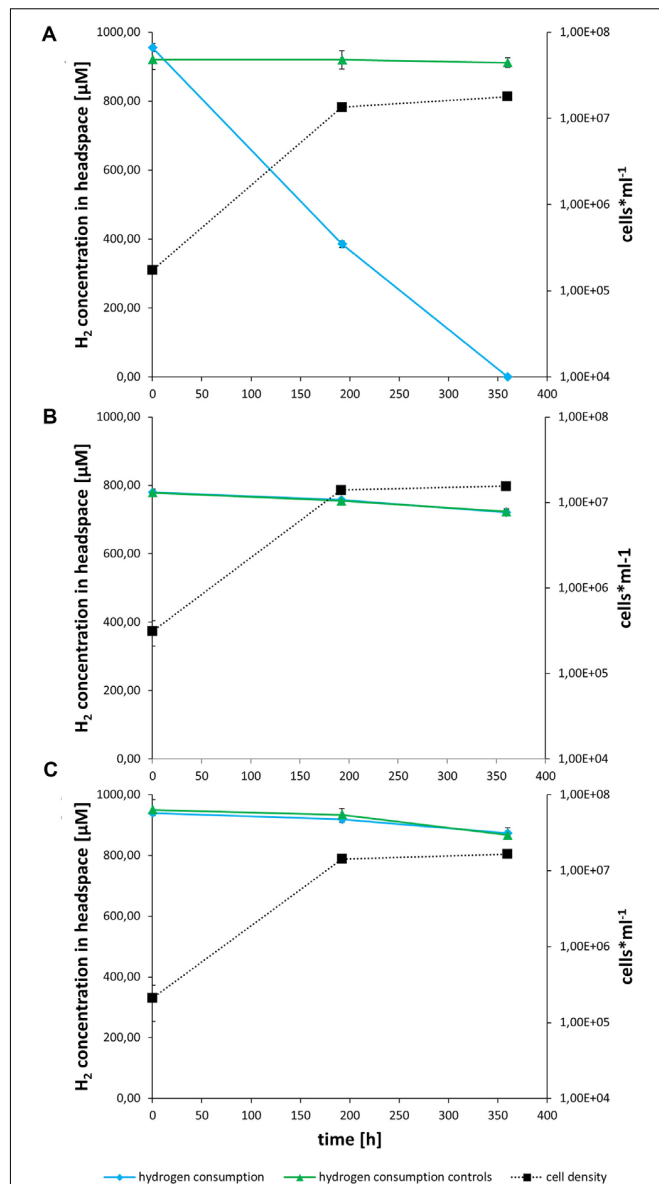
**FIGURE 1 |** Phylogenetic relationship of *Thiomicrospira* and *Hydrogenovibrio* species based on 16S rRNA genes. Sequences were aligned using the multiple sequence alignment web server T-Coffee (Notredame et al., 2000) with followed construction of the phylogenetic tree using Mega-X (Kumar et al., 2018) and 1000 bootstraps. The *Thiomicrospira* and *Hydrogenovibrio* species are color-coded according to their isolation source. The strains described here are written in bold. The scale bar represents the changes per nucleotide. Only bootstrap values higher than 75 are given.



**FIGURE 2 |** Phylogenetic relationship of *Thiomicrospira* and *Hydrogenovibrio* species based on *hynL* (large subunit of the [NiFe]-hydrogenase) genes. Sequences were aligned using the multiple sequence alignment web server T-Coffee (Notredame et al., 2000) with followed construction of the phylogenetic tree using Mega-X (Kumar et al., 2018) and 1000 bootstraps. The strains described here are written in bold. The scale bar represents the changes per amino acid. Only bootstrap values higher than 75 are given.



**FIGURE 3** | *In vivo* hydrogen consumption and growth of the T-ASW enrichment culture on MJ (A), MJ-T (B) and T-ASW (C) medium. Hydrogen consumption of the cultures is shown in blue with diamonds, controls containing only the respective medium in green with triangles and cell number of the cultures as dotted line in black with squares. The experiments were performed in triplicate, followed by polymerase chain reaction and sequencing of 16S rRNA and *hynL* genes (Hansen and Perner, 2015). Hydrogen consumption was determined by using gas chromatography and cell numbers were detected as described before (Hansen and Perner, 2015).



**FIGURE 4** | *In vivo* hydrogen consumption and growth of the MJ enrichment culture on MJ (A), MJ-T (B) and T-ASW (C) medium. Hydrogen consumption of the cultures is shown in blue with diamonds, controls containing only the respective medium in green with triangles and cell number of the cultures as dotted line in black with squares. The experiments were performed in triplicate, followed by polymerase chain reaction and sequencing of 16S rRNA and *hynL* genes (Hansen and Perner, 2015). Hydrogen consumption was determined by using gas chromatography and cell numbers were detected as described before (Hansen and Perner, 2015).

rRNA genes, the former was related to *H. crunogenus* species and the latter to *H. thermophilus* (Figure 1). Both strains encoded a hydrogen uptake group 1 [NiFe]-hydrogenase (Figure 2). The 45ROV5F/6F-TASW hydrogenase grouped in cluster II common for other *H. crunogenus* hydrogenases. 45ROV5F/6F-MJ's hydrogenase was related to the hydrogenase from MA2-6 in cluster I (Figure 2). It actually exhibited the highest similarity (99% AA identity) to an environmental hydrogenase sequence reported from the Lilliput venting field at 9°S on the MAR (Perner et al., 2007).

Both strains can consume hydrogen (Figures 3, 4). 45ROV5F/6F-TASW uses hydrogen in MJ-medium regardless of whether thiosulfate is present or not suggestive that in any case hydrogen oxidation is utilized as an energy source (Figure 3). In contrast, 45ROV5F/6F-MJ appears to use hydrogen only if hydrogen oxidation is the only available energy source, while hydrogen is not consumed if thiosulfate is present (Figure 4).

This indicates that hydrogen oxidation is not the preferred energy source under the provided incubation conditions when thiosulfate is present. Respective hydrogenase transcripts were identified in both strains when grown in hydrogen supplemented MJ-medium (**Supplementary Figure 1**). This makes it highly likely that the hydrogenases from cluster I and II are responsible for the hydrogen uptake in the cultures, as has been documented for other uptake hydrogenases from vent *Hydrogenovibrio* strains (Hansen and Perner, 2016a).

## Expanding the Geographic Range of Hydrogen Oxidizing Vent *Hydrogenovibrio* Species

We here expand the geographic range of hydrogen oxidizing hydrothermal vent *Hydrogenovibrio* species by two strains from the Southern Pacific. 45ROV5F/6F-TASW is also the first strain grouping with *Hydrogenovibrio crunogenus* exhibiting hydrogen uptake ability that is not from a MOR expanding this trait to an Island arc in the Southern Pacific and suggesting that dispersal limitation does not apply for this phenotype in this species. These hydrogenases form a monophyletic clade with those from *Epsilonproteobacteria* (**Figure 2**). This supports the previous assumption that hydrogenases in *H. crunogenus* species were taken up from *Epsilonproteobacteria* or were acquired from the same bacterium that *Epsilonproteobacteria* took up this trait from initially (Hansen and Perner, 2016a). Horizontal gene transfer appears to be a common feature in *Hydrogenovibrio* species (Petri et al., 2001; Scott et al., 2006, 2018).

Hydrogenases of cluster I form a monophyletic clade incorporating *H. marinus*, which was isolated from the water column (Nishihara et al., 1991) and S5 from a vent along the SWIR, MA2-6 from the MAR and 45ROV5F/6F-MJ from the Kermadec island arc (**Figure 2**). According to 16S rRNA genes the three vent *Hydrogenovibrio* species are all affiliated with the described species *H. thermophilus* (**Figure 1**). Different scenarios can explain the hydrogenase distribution in cluster I. *H. marinus* (i) took up its hydrogenase from a vent *Hydrogenovibrio*, (ii) the vent *Hydrogenovibrio* took it up from *H. marinus* or (iii) *H. marinus* was originally a vent-colonizing organism that emitted with the fluids into the open ocean from which it was essentially isolated. The latter may be supported by the fact that other (endemic) vent organisms have been detected in the open ocean (Gonnella et al., 2016).

It is interesting that all tested hydrogen oxidizing *H. crunogenus* species have the cluster II and all so far tested hydrogen oxidizing *H. thermophilus* related species have the cluster I hydrogenases regardless of biogeographic distribution. The only so far known exception is SP-41, which encodes both hydrogenases on its genome (Gonnella et al., 2019). Those from cluster II resemble hydrogenases from hydrothermal vent *Epsilonproteobacteria* indicating horizontal gene exchange in the respective environment across classes. In contrast, the cluster I hydrogenase clusters with hydrogenases from free-living and symbiotic species associated with hydrothermal vents across different classes including *Zetaproteobacteria* and *Gammaproteobacteria*. Hence, at least two events of

horizontal gene transfer in the two species groups resulted in the hydrogenase acquisition. Given that *H. marinus*' hydrogenase has proven to be extremely oxygen stable (Nishihara et al., 1997), it may transfer a major advantage in a thermally and chemically dynamic vent environment that is influenced by mixing processes of endmember fluids with oxygenated ambient seawater.

This is the first report of *Hydrogenovibrio* species isolated from deep-sea vents located in the Southern Pacific that express active hydrogenases and can consume hydrogen. We here expand the geographic range of the hydrogen-oxidizing *Hydrogenovibrio* species. *Hydrogenovibrio* strains can be abundant in hydrothermal vent habitats and they have been considerably enriched in incubation experiments where hydrogen was amended (Brazelton and Baross, 2010; Perner et al., 2011; Böhnke et al., 2019). For example, they were shown to be among the dominant species in incubations supplemented with hydrogen, where  $27 \pm 4 \text{ nmol H}_2 \text{ ml}^{-1} \text{ h}^{-1}$  was consumed and  $0.93 \pm 0.1 \text{ nmol CO}_2 \text{ ml}^{-1} \text{ h}^{-1}$  was fixed autotrophically. This suggests that in these incubations 15% of the energy gained by hydrogen oxidation could be used for biomass synthesis most likely by *Hydrogenovibrio* strains. *Hydrogenovibrio*'s ability to use hydrogen, additionally to reduced sulfur compounds, is a major advantage in these environments because it enhances their competitiveness and furthers their substrate spectrum.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the NCBI, MN923280, MN923281, MN935466, and MN935467.

## AUTHOR CONTRIBUTIONS

KS collected the samples, performed the experiments and molecular analyses. MP developed the idea and designed the experiments. KS and MP wrote the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.00295/full#supplementary-material>

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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