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# Detection of Diazotrophy in the Acetylene-Fermenting Anaerobe *Pelobacter* sp. Strain SFB93

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**ABSTRACT** Acetylene (C<sub>2</sub>H<sub>2</sub>) is a trace constituent of the present Earth's oxidizing atmosphere, reflecting a mixture of terrestrial and marine emissions from anthropogenic, biomass-burning, and unidentified biogenic sources. Fermentation of acetylene was serendipitously discovered during C<sub>2</sub>H<sub>2</sub> block assays of N<sub>2</sub>O reductase, and *Pelobacter acetylenicus* was shown to grow on C<sub>2</sub>H<sub>2</sub> via acetylene hydratase (AH). AH is a W-containing, catabolic, low-redox-potential enzyme that, unlike nitrogenase (N<sub>2</sub>ase), is specific for acetylene. Acetylene fermentation is a rare metabolic process that is well characterized only in *P. acetylenicus* DSM3246 and DSM3247 and *Pelobacter* sp. strain SFB93. To better understand the genetic controls for AH activity, we sequenced the genomes of the three acetylene-fermenting *Pelobacter* strains. Genome assembly and annotation produced three novel genomes containing gene sequences for AH, with two copies being present in SFB93. In addition, gene sequences for all five compulsory genes for iron-molybdenum N<sub>2</sub>ase were also present in the three genomes, indicating the cooccurrence of two acetylene transformation pathways. Nitrogen fixation growth assays showed that DSM3426 could ferment acetylene in the absence of ammonium, but no ethylene was produced. However, SFB93 degraded acetylene and, in the absence of ammonium, produced ethylene, indicating an active N<sub>2</sub>ase. Diazotrophic growth was observed under N<sub>2</sub> but not in experimental controls incubated under argon. SFB93 exhibits acetylene fermentation and nitrogen fixation, the only known biochemical mechanisms for acetylene transformation. Our results indicate complex interactions between N<sub>2</sub>ase and AH and suggest novel evolutionary pathways for these relic enzymes from early Earth to modern days.

**IMPORTANCE** Here we show that a single *Pelobacter* strain can grow via acetylene fermentation and carry out nitrogen fixation, using the only two enzymes known to transform acetylene. These findings provide new insights into acetylene transformations and adaptations for nutrient (C and N) and energy acquisition by microorganisms. Enhanced understanding of acetylene transformations (i.e., extent, occurrence, and rates) in modern environments is important for the use of acetylene as a potential biomarker for extraterrestrial life and for degradation of anthropogenic contaminants.

**KEYWORDS** acetylene hydratase, nitrogenase, acetylene fermentation, nitrogen fixation, *Pelobacter*, genomics, diazotrophy

Acetylene (C<sub>2</sub>H<sub>2</sub>) is a trace constituent (~20 to 40 parts per trillion [ppt]) of the present Earth's oxidizing atmosphere (1–3), reflecting a mixture of terrestrial and marine emissions from anthropogenic, biomass-burning, and unidentified biogenic

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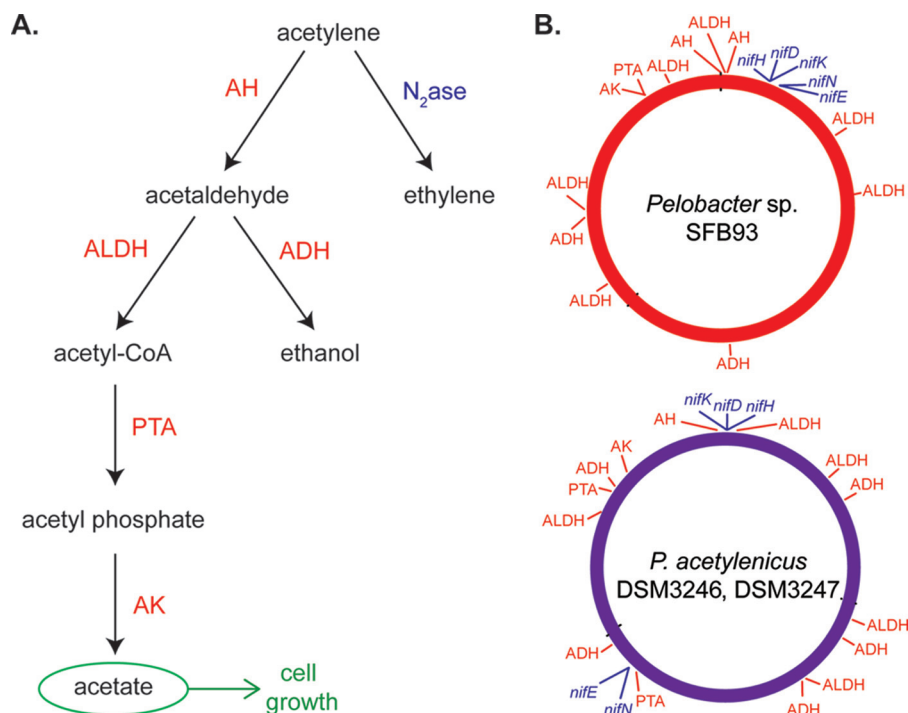
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**FIG 1** (A) Pathways of acetylene transformation via acetylene fermentation (left process) and  $N_2ase$  (right process). (B) BRIG plots showing the locations of genes in the acetylene fermentation pathway (red) and those encoding nitrogenase (blue) in the genomes of *Pelobacter* sp. SFB93 and *P. acetylenicus* DSM3246 and DSM3247, generated in this study. Genes in the acetylene fermentation pathway (red) include acetylene hydratase (AH), aldehyde dehydrogenase (ALDH), alcohol dehydrogenase (ADH), phosphate acetyltransferase (PTA), and acetate kinase (AK). AH catalyzes the reaction of  $C_2H_2 + H_2O \rightarrow CH_3CHO$ , providing free energy of  $\Delta G = -111.9$  kJ per mol (22). Acetaldehyde is disproportionated to acetate and ethanol by aldehyde dehydrogenase and alcohol dehydrogenase, respectively. Together, these enzymes catalyze the reaction of  $2CH_3CHO + H_2O \rightarrow CH_3CH_2OH + CH_3COO^- + H^+$ , yielding free energy of  $\Delta G = -17.3$  kJ per mol acetaldehyde (22). The nitrogenase genes *nifHDKEN* are highlighted (blue), and additional genes (*nifBX* and *fdxN*) are also found in all genomes (Fig. 3B; also see Table S3 in the supplemental material).

sources (4–7). Acetylene in the putative anaerobic atmosphere of early Earth (8, 9) might have reached an abundance of 5 ppm (10, 11) due to its formation from bombardment of simple molecules (e.g., methane, ammonia, hydrogen sulfide, and phosphine) with high-energy photons. Such reactive atmospheric chemistry derived from simple constituents formed the basis of the complex prebiotic chemistry thought to have led to the origin of life (12–14). In the outer solar system, acetylene has been detected in the atmosphere of Titan (15, 16), where it participates in the formation of complex organics (17, 18). It has been proposed that a form of extraterrestrial life on Titan could be sustained energetically by hydrogen's reductive splitting of acetylene to form methane at  $\sim 90$  K (19). The presence of acetylene catabolism on Earth and the potential for it to support life in our solar system raise the need to understand the mechanisms by which acetylene is biologically processed.

On Earth there are two known biochemical mechanisms whereby acetylene is transformed, namely, the low-electrochemical-potential and oxygen-sensitive enzymes nitrogenase ( $N_2ase$ ) and acetylene hydratase (AH); these enzymes are not structurally related. The well-studied  $N_2ase$ , which is possessed by an array of diazotrophic (nitrogen-fixing) prokaryotes, is promiscuous in the sense that it not only reduces dinitrogen to ammonia but also reduces other triple-bonded molecules, such as cyanide and acetylene. The latter forms the basis of the well-known bioassay in which acetylene serves as a proxy for dinitrogen and the resultant ethylene ( $C_2H_4$ ) (Fig. 1A) represents the amount of ammonia produced (20). In contrast, the much less studied AH initiates exergonic hydration of acetylene to acetaldehyde in a reaction catalyzed by its tungsten pyranopterin cofactor (21). Acetaldehyde undergoes further dismutation to ethanol and

acetate (22) (Fig. 1A). Unlike  $N_2$ ase, the AH enzyme, encoded by the *ahy* gene, is specific for acetylene. Characterization of AH has focused on the acetylene-fermenting enzymes from the anaerobic bacterium *Pelobacter acetylenicus* (23, 24). However, aerobic acetylene-degrading bacteria have been reported to use acetylene as their sole carbon and energy source (25–29). The aerobic acetylene-metabolizing enzymes have not been fully characterized, but experiments by Rosner et al. showed that aerobic AHs are structurally distinct, because of their lack of cross-reactivity with antibodies raised to the AH of *P. acetylenicus* (25), indicating that this is a heterogeneous group of enzymes.

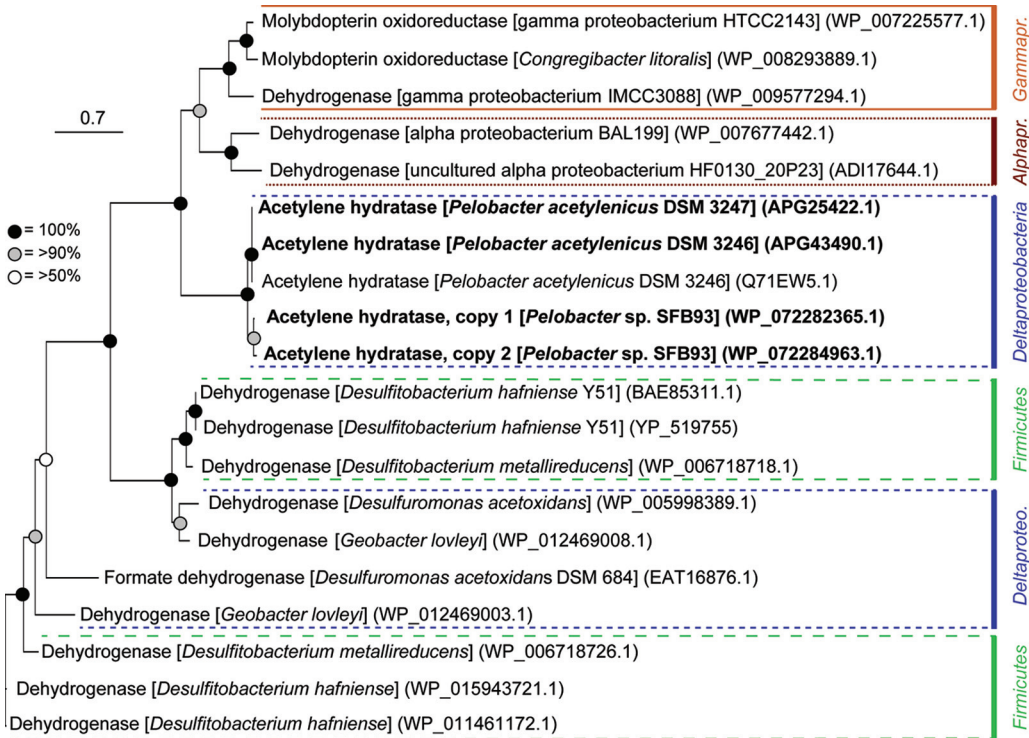
The specificity of AH for acetylene as a growth substrate has prompted speculation with respect to its early origin, when Earth's atmosphere was comparatively rich in this gas (30, 31). It was speculated by Postgate (32) that  $N_2$ ase may have coappeared to detoxify noxious substances such as cyanide, reducing  $N_2$  only when cyanide and ammonia became limiting as life emerged. We now report the unusual circumstance of gene sequences for both of these enzymes,  $N_2$ ase and AH, being present in the annotated genomes of two deposited type reference strains of *P. acetylenicus* (DSM3246 and DSM3247), as well as in *Pelobacter* strain SFB93, isolated from San Francisco Bay (30, 33) (Fig. 1B). We further present experimental evidence that *Pelobacter* strain SFB93 is capable of diazotrophic growth, during which both enzymes are active.

## RESULTS AND DISCUSSION

**Comparison of *Pelobacter* genomes.** Genome sequencing of the three *Pelobacter* strains produced complete genomes ranging in size from 3,175,390 bp for DSM3247 to 3,191,564 bp for DSM3246 (34) and 3,218,469 bp for strain SFB93 (35). Interestingly, DSM3246 also contained a 13,658-bp plasmid. Strain DSM3246 has four small-subunit (SSU) rRNA genes, whereas DSM3247 and SFB93 have three copies, but the two DSM strains share 100% sequence identity at the SSU rRNA gene sequence level. The SSU rRNA genes of SFB93 share 95.8 to 97.4% sequence identity with the two type strains, indicating that SFB93 is likely a new species within the *Pelobacter* genus (see Fig. S1 in the supplemental material). The divergence in SSU rRNA genes is also seen across the whole genome, with the levels of nucleotide identity between the three species being quite low except for the region containing the gene for AH (*ahy*) (Fig. S2). Average nucleotide identity (ANI) values for SFB93 versus DSM3246 and DSM3247 were 62.27% and 60.32%, respectively, suggesting that DNA-DNA hybridization would be below the 70% standard threshold for designating a new species.

**Acetylene fermentation and diazotrophy genes present in *Pelobacter* genomes.** The genomes of all three *Pelobacter* strains contain the genes for complete conversion of acetylene to ethanol and acetate, including genes for AH, aldehyde dehydrogenase, phosphate acetyltransferase, acetate kinase, and alcohol dehydrogenase (Fig. 1B; also see Table S1) (22). However, the genes are not located within a single locus but are scattered throughout the genomes. Most interestingly, SFB93 contains two copies of the *ahy* gene, located ~18,000 bp apart. The AH amino acid sequences from the three genomes are closely related to previously published AH sequences (Fig. 2; also see Table S1) (33). The two *ahy* copies in the *Pelobacter* SFB93 genome are most closely related to each other (96.9% amino acid sequence similarity) but demonstrate only 88.4 and 90.4% amino acid sequence similarity to the AHs of strains DSM3246 and DSM3247, respectively (Table S2). No genes related to *ahy* are found in the existing genomes of other *Pelobacter* strains.

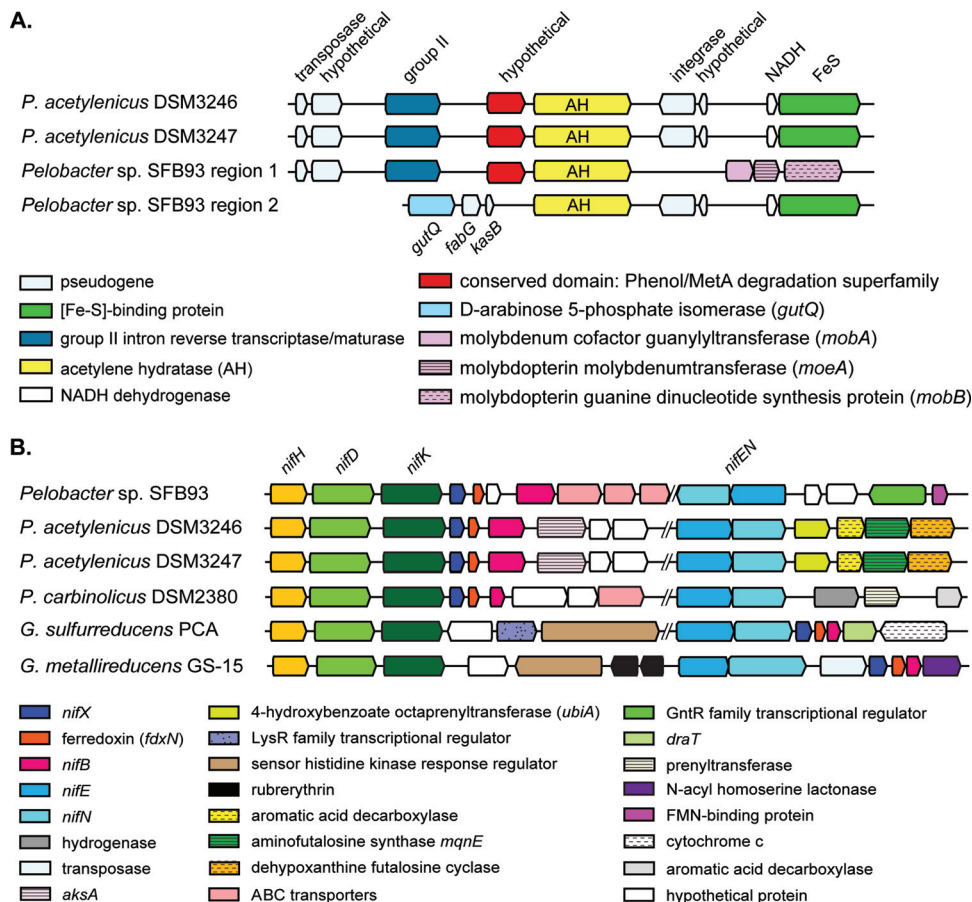
Phylogenetic reconstruction of AH and closely related molybdopterin oxidoreductase and dehydrogenase sequences suggests that AH evolved from an ancestral dehydrogenase (Fig. 2). The phylogenies of these amino acid sequences do not reflect phylogeny at the SSU rRNA gene level. In addition, the *ahy* genes are flanked by group II intron reverse transcriptases/maturases and integrases (Fig. 3). These observations suggest the potential for *ahy* genes to undergo horizontal gene transfer (HGT) into or out of the *Pelobacter* genomes or movement within the genomes. Currently, the National Center for Biotechnology Information (NCBI) has over 1,600 keyword hits for



**FIG 2** Phylogenetic tree of acetylene hydratase and related dehydrogenase and oxidoreductase amino acid sequences. All AH sequences are from organisms known to couple anaerobic growth to acetylene fermentation. Sequences from this study are indicated in bold. Bootstrap values (100 replicates) are indicated by node markers. The scale bar indicates 0.7 changes per amino acid.

AH, but it is not clear whether those annotations indicate functional genes or represent accurately annotated proteins. The only demonstrated anaerobic AH activity occurs in *P. acetylenicus* DSM3246 and DSM3247 and in *Pelobacter* strain SFB93. These observations imply either that there is an abundance of genes poorly annotated as *ahy* or that the genes code for the structurally distinct AHs from aerobic acetylene-metabolizing bacteria observed by Rosner et al. (25).

The genes essential for encoding an iron-molybdenum  $N_2$ ase (*nifHDKEN*) were found in the genomes of SFB93, DSM3246, and DSM3247 (Fig. 1B). Interestingly, *nifHDK* genes are located adjacent to *ahy* in all three of our sequenced *Pelobacter* genomes (~166 kb for SFB93 and ~40 kb for both DSM3246 and DSM3247). Nitrogenase genes in SFB93 are closely grouped (*nifHDKXB* genes are ~36,000 bp from *nifEN* genes), compared to DSM3246 and DSM3247 (*nifHDKXB* genes are ~1 to 2 Mbp from *nifEN* genes) (Fig. 3B; also see Table S4). The *nifHDKEN* genes from SFB93 are similar to those from *Pelobacter carbinolicus*, although the *nifEN* genes in SFB93 have undergone an inversion. In contrast, the *nifXBRS* genes in SFB93 are most similar to the *nif* genes in the genomes of other members of the *Desulfuromonadales* (Table S3 and Fig. S3). Although nitrogen fixation genes are known to occur on single or dispersed operons (36), the relatively small gap in SFB93 is more similar to the reference genomes of *Geobacter sulfurreducens* PCA (GenBank accession no. [NC\\_002939](#)) and *Geobacter metallireducens* GS-15 (GenBank accession no. [NC\\_007517](#)), in which all *nif* genes are closely grouped (*nifHDK* genes are ~8,000 bp and ~5,000 bp, respectively, from *nifEN* genes) (Fig. 3B; also see Table S4). The *Pelobacter* genus is within the order *Desulfuromonadales* (Fig. S2), and the *nifH* genes from our genomes are most closely related to *nif* genes from members of that order (Fig. S3). To date, *G. sulfurreducens* and *G. metallireducens* are the only organisms in that order that have been definitively shown to fix nitrogen (37, 38) (Fig. S3). Although the reference genomes of *P. carbinolicus* DSM2380 (GenBank accession no. [NC\\_007498](#)) (Fig. 3B) and *Pelobacter propionicus*

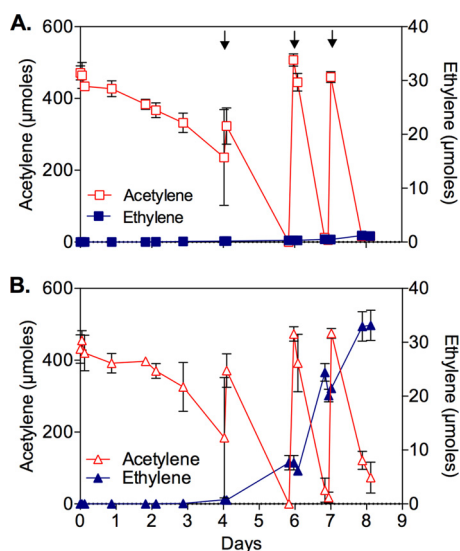


**FIG 3** Syntenic map of acetylene hydratase (*ahy*) and flanking genes (A) and nitrogenase genes (B) in *Pelobacter* sp. SFB93 and *P. acetylenicus* DSM3246 and DSM3247. *Pelobacter* sp. SFB93 contains two *ahy* genes in two regions, separated by ~18,000 bp. *Geobacter sulfurreducens* PCA and *G. metallireducens* GS-15, known nitrogen-fixing organisms, are included for reference in panel B. Nitrogenase genes are separated by gaps (indicated by double slashes) of different lengths, ranging from ~36,000 bp to 1.2 Mbp, between *nifHDKXB* and *nifEN* in SFB93 and DSM3247, respectively (see Table S4 in the supplemental material).

DSM2379 (GenBank accession no. [CP000482](https://www.ncbi.nlm.nih.gov/nuccore/CP000482)) contain annotated *nif* genes, to date there are no reports of those organisms being tested for nitrogen fixation.

**Nitrogen fixation and acetylene fermentation in *Pelobacter* sp. SFB93.** To ascertain whether the annotated *nif* genes for nitrogen fixation were expressed *in vivo* and were functional, we carried out acetylene reduction assays with strains DSM3246 and SFB93. Washed cells of acetoin-grown *P. acetylenicus* DSM3246 and pyruvate-grown SFB93 were tested for nitrogen fixation. Based on their growth preferences, different substrates were used to grow the organisms; strain DSM3246 grows best using acetoin but cannot utilize pyruvate (22), and strain SFB93 grows best on pyruvate but does not grow on acetoin (data not shown). *P. acetylenicus* DSM3246 cells consumed acetylene when incubated with or without ammonium present, but in neither case was any ethylene detected (Fig. S4). These results show that, under these conditions, strain DSM3246 lacks the capacity for nitrogen fixation, despite having a full complement of *nif* genes; based on these results, it is not clear whether the *nif* genes are functional. DSM3247 was not tested for diazotrophic growth, due to its high level of genetic similarity to DSM3246.

SFB93 cells incubated with or without ammonium ions demonstrated steady consumption of acetylene, which necessitated headspace replenishment after 4 days of incubation (Fig. 4). A buildup of ethylene ( $33 \pm 3 \mu\text{mol}$ ) occurred only in the absence of ammonium, however, and there was no significant accumulation ( $1.0 \pm 0.5 \mu\text{mol}$ ) in the presence of ammonium. Because ammonium represses the expression of  $\text{N}_2\text{ase}$



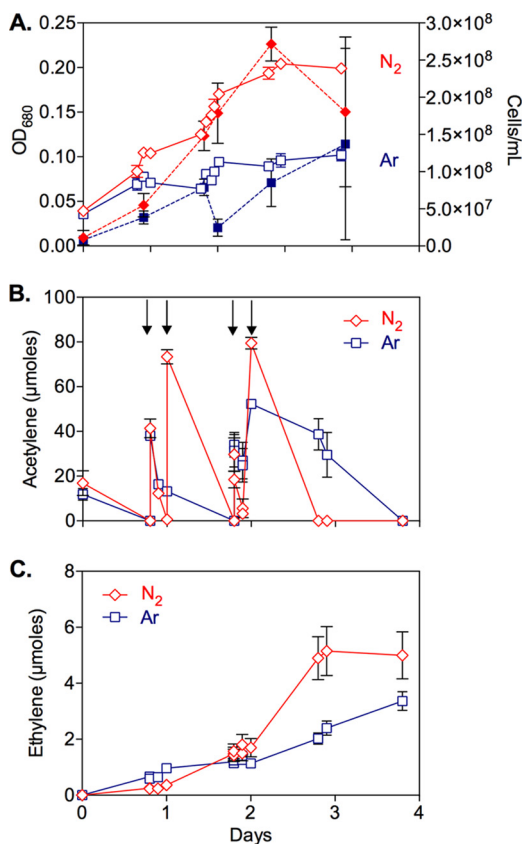
**FIG 4** Consumption of acetylene (red) and production of ethylene (blue) by washed cell suspensions of pyruvate-grown *Pelobacter* strain SFB93 incubated under N<sub>2</sub>. The starting cell density was  $\sim 4.5 \times 10^8$  cells/ml. (A) Cells incubated with 2 mM NH<sub>4</sub>Cl. (B) Cells incubated without NH<sub>4</sub>Cl. Symbols represent the means of three different cell suspensions, and bars indicate  $\pm 1$  standard deviation. Arrows indicate the times at which acetylene was added again to the gas phases of all samples.

(39), these results clearly demonstrate that SFB93 possesses an active N<sub>2</sub>ase. The cumulative amounts of acetylene consumed ( $\sim 1,386 \pm 34 \mu\text{mol}$  without ammonium and  $1,425 \pm 52 \mu\text{mol}$  with ammonium) exceeded by  $\sim 43$ -fold the amount of ethylene recovered under ammonium-depleted conditions. This further illustrates that the observed acetylene consumption was primarily a function of AH rather than N<sub>2</sub>ase.

A diazotrophic growth experiment was conducted with strain SFB93, in which acetylene was employed as the sole carbon and energy source in lieu of pyruvate (Fig. 5). Growth, measured as either optical density at 680 nm (OD<sub>680</sub>) or cell counts, was far more extensive in an atmosphere of N<sub>2</sub>, compared to one of argon (Fig. 5A). Acetylene uptake (Fig. 5B) and ethylene formation (Fig. 5C) were noted under both conditions but were always more extensive in the N<sub>2</sub> atmosphere, clearly indicating that N<sub>2</sub> was being fixed and used to meet cellular growth requirements. The small amount of growth observed in the Ar-incubated samples was attributable to NH<sub>4</sub><sup>+</sup> carryover from the inoculum, as well as to cell scavenging of nitrogen contained in the amino acid-reducing agent employed (cysteine) in the medium. It is relevant to note that, because acetylene is a competitive substrate that inhibits nitrogen fixation, diazotrophic growth under N<sub>2</sub> required the removal of acetylene through AH activity; this allowed electrons to be shifted from reduction of C<sub>2</sub>H<sub>2</sub> to C<sub>2</sub>H<sub>4</sub> to reduction of N<sub>2</sub> to NH<sub>3</sub>.

**Implications.** Our study showed not only that three *Pelobacter* strains contain multiple genes for acetylene transformation but also that a single microbe, SFB93, has the ability to transform acetylene via two separate reactions, catalyzed by the enzymes AH and N<sub>2</sub>ase. The presence of both enzymatic pathways in a single anaerobic organism likely represents a competitive advantage, with AH providing a mechanism for the organism to obtain carbon and energy and N<sub>2</sub>ase providing a mechanism to obtain nitrogen. In addition, SFB93 may benefit by having N<sub>2</sub>ase available for detoxification of other triple-bonded carbon compounds (30). Understanding acetylene transformations in modern environments (i.e., extent, occurrence, and rates) may allow better interpretation of acetylene as a potential biomarker for extraterrestrial life.

Given the very low concentrations of acetylene in the natural environment (e.g., parts per billion in seawater [5]) and the presence of flanking HGT elements, it is surprising that *ahy* has persisted in these organisms. Acetylene fermentation is thought



**FIG 5** Diazotrophic growth of *Pelobacter* strain SFB93 under N<sub>2</sub> (red) versus Ar (blue), with acetylene as the carbon and energy source. (A) Growth measured by optical density (solid lines) and cell counts (dashed lines). (B) Consumption of acetylene, which, after depletion, was added again to the gas phase at four times, as indicated by the arrows. (C) Ethylene accumulation. Symbols represent the means of three separate cultures, and bars indicate  $\pm 1$  standard deviation.

to be a rare route of metabolism but is not constrained to a single habitat type, with activity being found under fresh water and seawater conditions (33). Indeed, it was during the routine application of acetylene as a bioassay tool to quantify nitrogen transformations by diverse soils and sediments that the unexpected consumption of acetylene was often noted (40–44). The variety of habitats reported in those early serendipitous observations suggests a broad occurrence of *ahy*-like genes in nature. HGT may also be functioning to maintain metabolic versatility for the rare encounters of these organisms with acetylene. It is possible that anthropogenic inputs of acetylene, e.g., from pollutant degradation (45–48) or motor vehicles (49), are continuing to select for this metabolic pathway. Abiotic dehalogenation of trichloroethylene (TCE), a ubiquitous groundwater pollutant, with reduced iron minerals produces acetylene (45–48), thereby yielding an anthropogenic source for acetylene fermenters. In fact, Miller et al. (33) demonstrated acetylene fermentation in a TCE-contaminated subsurface aquifer in New Jersey. Further, TCE bioremediation efforts are aimed at stimulating complete dechlorination, resulting in the accumulation of ethylene (C<sub>2</sub>H<sub>4</sub>) as the desired innocuous end product (50, 51). Recent laboratory studies have shown that acetylene-fermenting organisms can be used to overcome acetylene-linked inhibition of TCE bioremediation (52). Considering that *Pelobacter* N<sub>2</sub>ase reduction of *in situ* acetylene may be a source of ethylene, production of any ethylene arising from N<sub>2</sub>ase activity versus dehalogenation could complicate interpretation of the use of ethylene buildup to assess the efficacy of TCE dehalogenation. This work highlights the need to examine acetylene transformations in more depth and to understand the interactions between acetylene and nitrogen cycling through coexpression of AH and N<sub>2</sub>ase.



## MATERIALS AND METHODS

**Growth of *Pelobacter* strains.** *Pelobacter* strain SFB93 was grown in the bicarbonate-buffered anaerobic bay water (ABW) medium described by Miller et al. (33). *Pelobacter acetylenicus* strains DSM3246 and DSM3247 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (Braunschweig, Germany). Freshwater strain DSM3246 was grown in DSMZ medium 298 using acetoin ( $\text{CH}_3\text{COCHOHCH}_3$ ) (0.01 mM). Estuarine strain DSM3247 was grown in DSMZ medium 293 using either acetoin or  $\text{C}_2\text{H}_2$  (1.5%).

**Genome sequencing and annotation.** Cultures of DSM3246, DSM3247, and SFB93 were grown to high density and then pelleted by centrifugation. DNA was extracted from multiple pellets using the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol, with DNA shearing being limited by mixing through inversion instead of vortex-mixing. The DNA concentrations and purity of the extracts were then measured with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the extracts were visualized on a 1% agarose gel. DNA was concentrated from multiple extractions using either the PowerClean Pro kit (Mo Bio Laboratories, Carlsbad, CA, USA) or a Zymo genomic DNA spin concentrator (Zymo Research Corp., Irvine, CA, USA), according to the manufacturer's instructions. The pooled and concentrated DNA was quantified with a Qubit 3.0 fluorometer (Life Technologies) and visualized on a 1% agarose gel, to verify the recovery of high-molecular-weight DNA.

DNA extracts from DSM3246, DSM3247, and SFB93 were sent to the University of California, Davis, Genome Center (<http://genomecenter.ucdavis.edu>) for PacBio long-read sequencing (Pacific Biosciences of California, Inc., Menlo Park, CA, USA); sequencing on a PacBio RS instrument yielded 71,385, 62,650, and 63,968 sequence reads, respectively, for the strains. The sequence reads were subsequently assembled using the hierarchical genome assembly process (HGAP) developed by PacBio (53). The HGAP was performed through the PacBio SMRT portal using version RS\_HGAP\_Assembly.3. Annotation was performed with the prokaryotic genome annotation pipeline (PGAP) from the NCBI (54).

SSU rRNA, *nifH*, and *ahy* genes were extracted from genomes using Geneious R9.1.8 (Biomatters, Ltd.) (55). The SSU rRNA genes of DSM3246 and DSM3247 were identical, and one representative copy was imported in ARB (56) for phylogenetic analysis. The SSU rRNA genes of SFB93 were more variable; therefore, all 3 copies were included in the phylogenetic analysis. All sequences were aligned to the SILVA release 123 database (57) in ARB. To determine the relationship of SFB93 to published *Pelobacter* strains and its placement within the *Deltaproteobacteria*, a maximum likelihood phylogenetic tree of those strains and a selection of related *Deltaproteobacteria* was computed using RAxML (58) within ARB, with the best tree being selected based on 100 iterations. The nucleic acid sequences of *nifH* and *ahy* were translated to amino acid sequences from genomic nucleotide sequences with Geneious R9.1.8, using the genetic code for bacteria and archaea (11). Amino acid sequences were aligned using MUSCLE, and phylogenetic trees were constructed using PhyML with 100 bootstrap replicates within Geneious.

**Nitrogen fixation assays.** *P. acetylenicus* strain DSM3246 was grown in 100 ml of anaerobic DSMZ m298 medium that was modified by using a HEPES buffer (5.2 g/liter) instead of sodium bicarbonate and 5 mM acetoin instead of butanediol as the carbon source. Late-log-phase cells were harvested by centrifugation and brought back to 30-ml volumes with ammonium-free medium. Cell suspensions were dispensed (5 ml) into 13-ml serum bottles and sealed under a  $\text{N}_2$  headspace. Cultures were amended with 5 mM acetoin and injected with 0.1 ml  $\text{C}_2\text{H}_2$ . One-half of the prepared bottles were also supplemented with  $\text{NH}_4\text{Cl}$  to a final concentration of 2 mM. The starting cell density was  $5.2 \times 10^7$  cells/ml.

Strain SFB93 was grown in anaerobic bicarbonate-buffered CM medium, as described by Visscher and Taylor (59), which was modified by including 5.2 g/liter HEPES for additional buffering capacity. For washed cell experiments, cells were grown on 10 mM pyruvate, harvested at late log phase (4 days), concentrated by centrifugation under a  $\text{N}_2$  atmosphere, resuspended to an  $\text{OD}_{680}$  of  $\sim 0.2$  in 200 ml of ammonium-free CM medium, and subsequently divided into two portions (100 ml each), with one portion supplemented with 0.2 ml of 2 M  $\text{NH}_4\text{Cl}$ . Thirty milliliters of this cell suspension was then dispensed into 70-ml serum bottles, after which all bottles were injected with 10 ml of  $\text{C}_2\text{H}_2$ . All manipulations were performed in anaerobic chambers, as outlined previously (33). Headspace analyses of  $\text{C}_2\text{H}_2$  and  $\text{C}_2\text{H}_4$  were carried out by gas chromatography with flame ionization detection, as described previously (33). The Henry's law  $K_{\text{H}}$  values employed were  $9.78 \times 10^{-1}$  for  $\text{C}_2\text{H}_2$  and  $1.17 \times 10^{-1}$  for  $\text{C}_2\text{H}_4$ , as calculated previously by Miller et al. (60). Growth experiments were conducted in Balch tubes sealed under  $\text{N}_2$  or Ar (10), with ammonium-free CM medium and 1.0 ml of added  $\text{C}_2\text{H}_2$ . Growth was determined by measuring  $\text{OD}_{680}$  values spectrophotometrically, as well as by assessing cell densities. Cell densities were determined by direct cell counting using acridine orange epifluorescence microscopy, as described by Miller et al. (60).

**Accession number(s).** The genomes reported in this paper are available from the NCBI GenBank database under BioProject number [PRJNA319824](https://doi.org/10.1128/AEM.01198-17) and accession numbers [CP015455](https://doi.org/10.1128/AEM.01198-17) (*P. acetylenicus* DSM3246 genome), [CP015456](https://doi.org/10.1128/AEM.01198-17) (*P. acetylenicus* DSM3246 plasmid), [CP015518](https://doi.org/10.1128/AEM.01198-17) (*P. acetylenicus* DSM3247 genome), and [CP015519](https://doi.org/10.1128/AEM.01198-17) (*Pelobacter* sp. strain SFB93 genome). Full descriptions of the genomes are also available as genome announcements (34, 35). Data from nitrogen fixation assays are available (61).

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AEM.01198-17>.

**SUPPLEMENTAL FILE 1**, PDF file, 2.5 MB.

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Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. government.

We declare no competing financial interests.

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