Syntrophus Conductive Pili Demonstrate that Common Hydrogen-Donating Syntrophs can have a Direct Electron Transfer Option

David J.F. Walker[1,2], Kelly P. Nevin[1], Dawn E. Holmes[1,3], Amelia-Elena Rotaru[1,4], Joy E. Ward[1], Trevor L. Woodard[1], Jiaxin Zhu[5], Toshiyuki Ueki[1], Stephen S. Nonnenmann[2,5], Michael J. McInerney[6], and Derek R. Lovley[1,2*]

[1]Department of Microbiology, University of Massachusetts-Amherst, Amherst, MA, USA

[2]Institute for Applied Life Sciences, University of Massachusetts-Amherst

[3]Department of Physical and Biological Science, Western New England University, Springfield, MA, USA

[4]Department of Biology, University of Southern Denmark, Odense, Denmark

[5]Department of Mechanical and Industrial Engineering, University of Massachusetts-Amherst

[6]Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, USA

*Corresponding author: dlovley@microbio.umass.edu

Abstract

Syntrophic interspecies electron exchange is essential for the stable functioning of diverse anaerobic microbial communities. Hydrogen/formate interspecies electron transfer (HFIT), in which H_2 and/or formate function as diffusible electron carriers, has been considered to be the primary mechanism for electron sharing because most common syntrophs were thought to lack biochemical components, such as electrically conductive pili (e-pili), necessary for direct interspecies electron transfer (DIET). Here we report that *Syntrophus aciditrophicus*, one of the most intensively studied microbial models for HFIT, produces e-pili and can grow via DIET. Pilin genes likely to yield e-pili were found in other genera of hydrogen/formate-producing syntrophs. The finding that DIET is a likely option for diverse syntrophs that are abundant in many anaerobic environments necessitates a reexamination of the paradigm that HFIT is the predominant mechanism for syntrophic electron exchange within anaerobic microbial communities of biogeochemical and practical significance.

Introduction

H_2/formate interspecies electron transfer (HFIT) and direct interspecies electron transfer (DIET) are mechanisms for the electron-donating partner in syntrophic consortia to dispose of electrons released from the oxidation of key intermediates (organic acids, alcohols, aromatics) during the anaerobic degradation of complex organic matter [1-5]. The relative proportion of electron flux through DIET or HFIT can influence the speed of interspecies electron transfer, the stability of anaerobic microbial communities, and their ability to adapt to environmental change [4,6,7]. However, there are no accurate methods for measuring the rates that H_2 and formate are transferred between microbes or for quantifying interspecies electrical currents in complex

communities. Therefore, the importance of HFIT or DIET in microbial communities is typically inferred from the composition of the microbial community.

It has previously been considered that DIET is primarily restricted to environments in which *Geobacter* species are abundant [8-12], because *Geobacter* species are the only microbes in pure culture that have been definitively shown to function as electron-donating partners for DIET [9,13-17]. It is assumed that HFIT predominates where microorganisms closely related to traditional syntrophs known to grow via HFIT are most abundant. However, most cultures of electron-donating syntrophs were characterized prior to the concept of DIET. Their capacity for DIET has not been fully explored.

For example, *Syntrophus aciditrophicus* is one of the most intensively studied pure culture models for HFIT [18-21]. It was previously concluded that *S. aciditrophicus* would be unlikely to participate in DIET [21,22] because it lacks a gene homologous to the gene for the *Geobacter* pilin monomer that assembles into its electrically conductive pili (e-pili) [23], a conduit for extracellular electron transfer required for *Geobacter* species to function as electron-donating partners in DIET[17]. This gene would not be expected in *S. aciditrophicus* because *Geobacter* e-pili have evolved relatively recently and are primarily restricted to *Geobacter* species and close relatives [24]. However, it has recently been found that e-pili, phylogenetically distinct from *Geobacter* e-pili have independently evolved multiple times [25].

Here we report that *S. aciditrophicus* expresses e-pili and is capable of growing via DIET. These results, and analysis of the pilin genes of other common syntrophs, indicate that the capacity for DIET should be considered as an option for microorganisms known to grow via HFIT and suggest that DIET may be more wide spread than previously considered.

Results and Discussion

Transmission electron microscopy of *S. aciditrophicus* revealed filaments with a morphology typical of type IV pili (Fig. 1a). A complement of genes consistent with type IV pili assembly is present in the genome (Fig. 1b). One gene, SYN_00814 encodes a N-terminal domain characteristic of PilA, the pilin monomer for Type IVa pili (Fig. 1c). This includes a short signal peptide (13 amino acids) which is cleaved by PilD at the G|FTLIE recognition site and a highly conserved, hydrophobic, transmembrane domain. Additional genes for pilus assembly (*pilD, pilM, pilN, pilO, pilP, pilQ*) and transcriptional control of pilus expression (*pilS, pilR*) are also present in the genome (Figure 1b). The amino acid sequence of the putative PilA protein fits the empirical criteria [25] for a pilin monomer likely to yield e-pili: 1) aromatic amino acids are located in the key positions required for conductivity in *G. sulfurreducens* e-pili; 2) the abundance of aromatic amino acids (10.9 % of amino acids) is above the minimum threshold of 9 % found to be necessary for high e-pili conductivity; and 3) no large gaps (> 35 amino acids) that lack aromatic amino acids (Fig. 1c).

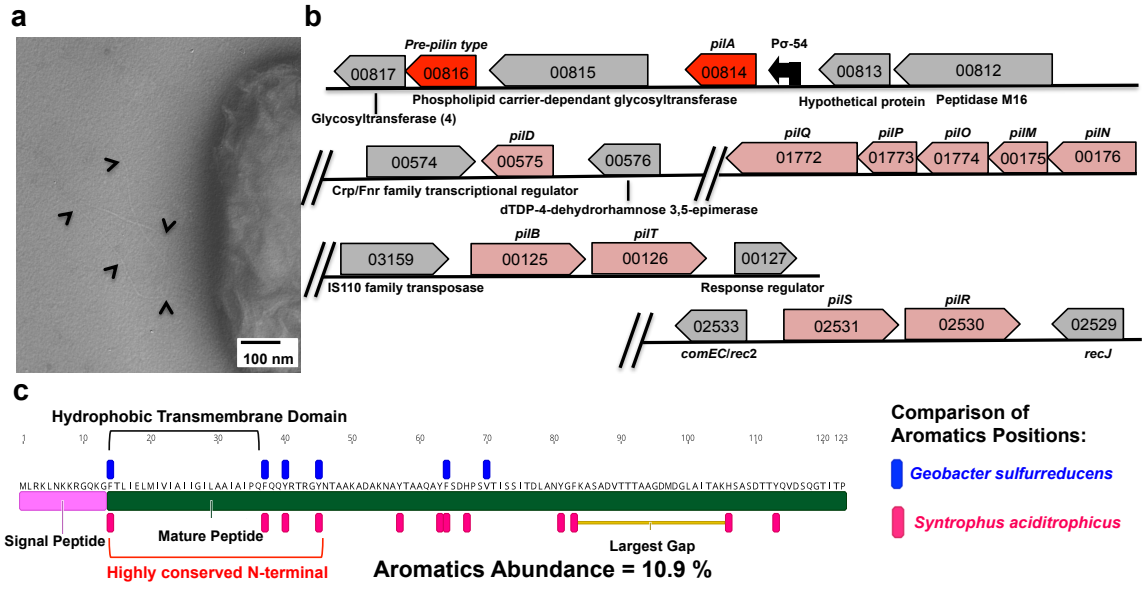


Fig.1. Pili expression and pili-related genes in *Syntrophus aciditrophicus.* (**a**) Transmission electron micrograph illustrating pili expression by *S. aciditrophicus* (pili highlighted with arrows). (**b**) Arrangement of genes associated with pili expression. (**c**) Key characteristics of the predicted amino acid sequence of the pilin monomer.

Low culture densities of *S. aciditrophicus* prevented harvesting sufficient quantities of pili to measure pili conductance on the electrode arrays previously employed for the study of other e-pili [25]. Therefore, the method initially employed to document the presence of e-pili in *G. sulfurreducens* [26] was adapted [27] as an alternative approach. Cultures of *S. aciditrophicus* were directly drop-cast on highly ordered pyrolytic graphite (HOPG), washed with deionized water, and examined with atomic force microscopy. Pili visualized with topographic imaging, (Fig. 2a), had a height/diameter of ca. 4 nm (Fig. 2b). Conductive imaging revealed that the pili were electrically conductive (Fig. 2b, c). Point-mode current-voltage (I-V) spectroscopy yielded an Ohmic-like response that was similar to *G. sulfurreducens* pili prepared in the same manner [27] (Fig. 2d) and in other similar studies of *G. sulfurreducens* pili [26]. As previously reported [27], the pili from *G. sulfurreducens* strain Aro-5, which lack key aromatic amino acids required for high conductivity [28-31], had much lower conductance (Fig. 2d).

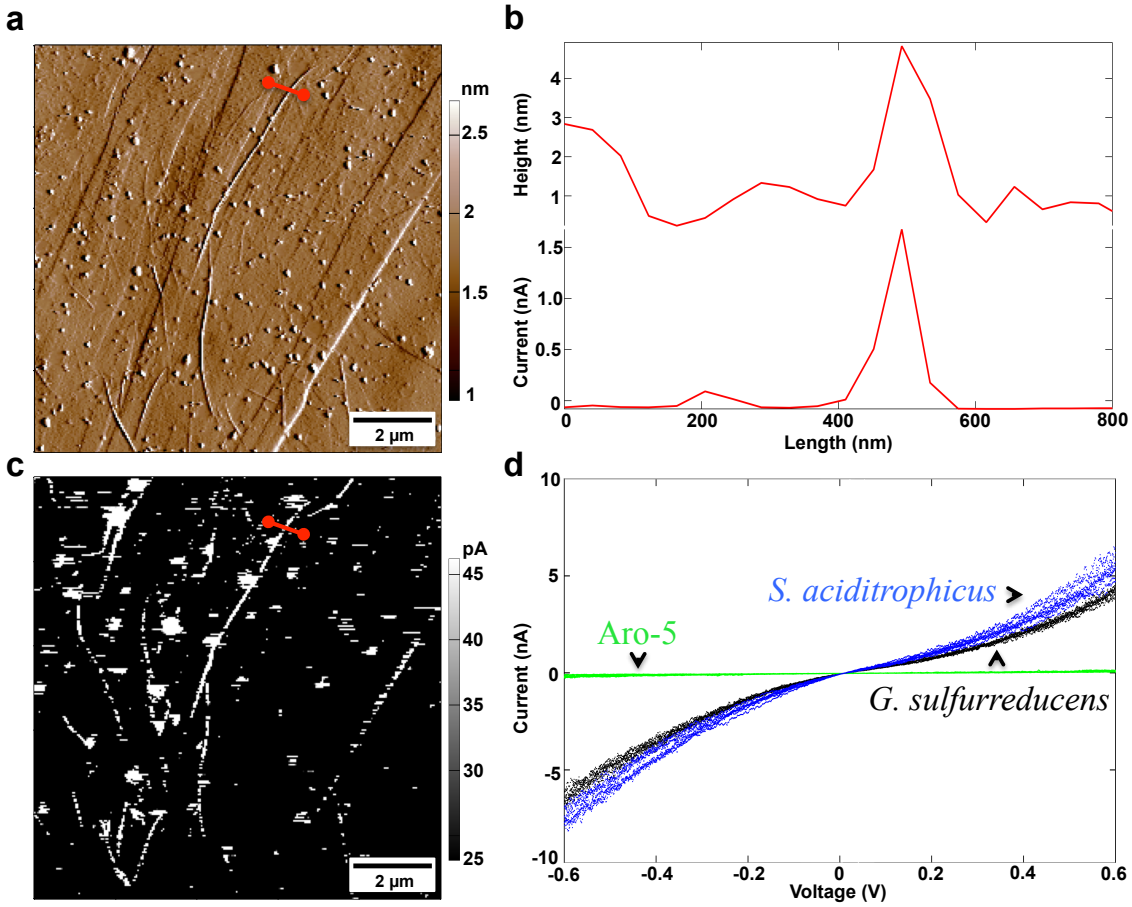
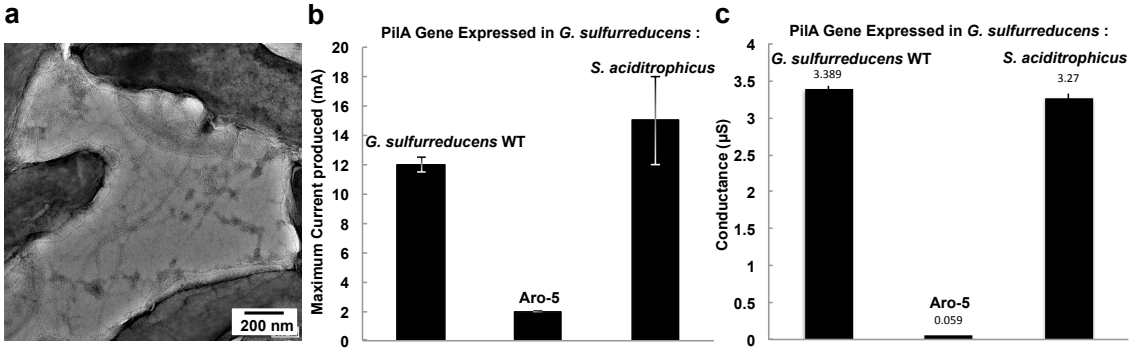


Fig. 2. Conducting tip atomic force microscopy demonstrated that *Syntrophus aciditrophicus* pili are electrically conductive. (**a**) Contact mode topographic imaging of pili on highly ordered pyrolytic (HOPG). Red line designates the cross section examined in (b). (**b**) Topographic analysis of the height/diameter of an individual pilus and corresponding current measurements (100 mV differential between the tip and the HOPG) across the same pilus cross section. (**c**) Current response of the pili shown in (a). (**d**) Current-voltage analysis of individual pili of *S. aciditrophicus* (blue data points), wild-type *G. sulfurreducens* (black data points) and the Aro-5 strain of *G. sulfurreducens* (green data points). Current-voltage scans are shown for one pilus of each type and are representative of analysis of 10 separate pili. *G. sulfurreducens* wild-type and strain Aro-5 data from reference 27.

106

107 The lack of tools for genetic manipulation of *S. aciditrophicus* limited further functional
108 analysis of the putative PilA gene predicted to yield its e-pili in the native organism. Therefore,
109 the gene was heterologously expressed in *G. sulfurreducens*, replacing the native *G.*
110 *sulfurreducens pilA* with an approach that has successfully yielded *G. sulfurreducens* strains that
111 express a diversity of both highly conductive and poorly conductive heterologous pili [25,32-35].
112 This new *G. sulfurreducens* strain, designated strain SP (for *Syntrophus* pili), expressed abundant
113 pili (Fig. 3a) and produced electrical current at densities comparable to the control strain
114 expressing the *G. sulfurreducens* wild-type *pilA* (Fig. 3b). Such high current densities are only
115 possible when *G. sulfurreducens* expresses e-pili [25,28]. As previously reported [25,28], *G.*
116 *sulfurreducens* strain Aro-5, with its poorly conductive pili, produced much lower currents (Fig.
117 3b). Networks of pili sheared from the electrode-grown biofilm of strain SP, purified, and drop
118 cast on electrode arrays as previously described [25], had a conductance comparable to *G.*
119 *sulfurreducens* wild-type pili (Fig. 3c). These results further demonstrated that the *S.*
120 *aciditrophicus* PilA gene yields a pilin monomer that can assemble into e-pili.



121

122 **Fig. 3. Functional analysis of *Syntrophus aciditrophicus* PilA gene via heterologous**
123 **expression in *Geobacter sulfurreducens*.** (**a**) Transmission electron micrograph of pili

124 expression in the strain of *G. sulfurreducens* in which the native *pilA* was replaced with the *S.*
125 *aciditrophicus pilA*. (**b**) Current production by *G. sulfurreducens* expressing its wild-type *pilA*,
126 the synthetic Aro-5 *pilA* designed to yield poorly conductive pili, or *S. aciditrophicus pilA*. (**c**)
127 Conductance of films of pili from strains of *G. sulfurreducens* expressing different *pilA*s. Error
128 bars represent the mean and standard deviation of triplicates.
129
130 The presence of e-pili in *S. aciditrophicus* suggested it might be capable of establishing
131 an electrical connection for DIET. To evaluate this, *S. aciditrophicus* was grown in co-culture
132 with *G. sulfurreducens*, the microbe most intensively studied as an electron-accepting partner for
133 DIET [4]. *G. sulfurreducens* can also function as a H_2- and formate-consuming partner for HFIT,
134 providing a positive control for establishing the *S. aciditrophicus/G. metallireducens* co-culture
135 if DIET was not possible [36]. The electron donor was benzoate, a substrate that *S. aciditrophicus*
136 can metabolize, but *G. sulfurreducens* cannot. The electron acceptor was fumarate, an electron
137 acceptor only *G. sulfurreducens* can utilize. In the presence of a H_2/formate-consuming partner
138 *S. aciditrophicus* metabolizes benzoate to acetate with the production of either H_2:
139 $C_7H_5O_2^- + 7\ H_2O \rightarrow 3CH_3COO^- + HCO_3^- + 3\ H^+ + 3\ H_2$
140 or formate:
141 $C_7H_5O_2^- + 2\ HCO_3^- + 4\ H2O \rightarrow 3\ CH_3COO^- + 3\ H^+ + 3\ HCOO^-$
142 With electron transfer via DIET the relevant reaction is:
143 $C_7H_5O_2^- + 7\ H_2O \rightarrow 3CH_3COO^- + HCO_3^- + 9\ H^+ + 6\ e^-$
144 In addition to oxidizing H_2 and formate, or consuming electrons released during DIET, *G.*
145 *sulfurreducens* can also metabolize acetate, making the overall reaction expected for the
146 oxidation of benzoate with the reduction of fumarate to succinate in the co-culture:

147 $C_7H_5O_2^- + 15\ ^-OOC\text{-}CH{=}CH\text{-}COO^- + 19\ H_2O \rightarrow 7\ HCO_3^- + 15\ ^-OOC\text{-}CH_2\text{-}CH_2\text{-}COO^- + 6\ H^+$

148 *S. aciditrophicus/G. sulfurreducens* co-cultures grew with repeated sub-culturing and,
149 within the experimental error, exhibited the expected stoichiometry of benzoate consumption and
150 succinate production (Fig. 4a). In these co-cultures HFIT, DIET, or a combination of the two,
151 were feasible. Therefore, to eliminate the possibility of HFIT, *S. aciditrophicus* was co-cultured
152 with the previously described strain of *G. sulfurreducens* [37], designated here as *G.*
153 *sulfurreducens*$_{HF}$, that can not utilize H_2 or formate because the genes for the uptake hydrogenase
154 and formate dehydrogenase were deleted. The *S. aciditrophicus/ G. sulfurreducens*$_{HF}$ co-culture
155 had a longer initial lag period, but then metabolized benzoate with the reduction of fumarate
156 almost as fast as the co-culture with wild-type *G. sulfurreducens* (Fig. 4b). These results
157 demonstrate that *S. aciditrophicus* can grow via DIET.

158 Although some co-cultures form visible aggregates during growth via DIET [13], others
159 produce small, relatively fragile aggregates [14]. There were no visible aggregates in the *S.*
160 *aciditrophicus/G. sulfurreducens* co-cultures but it appeared in scanning electron micrographs of
161 cells collected on filters that co-cultures grown under conditions in which only DIET was
162 possible have a greater tendency to form more small clumps than co-cultures grown under
163 conditions in which HFIT was also an option (Fig. 4 d,e).

164 Granular activated carbon (GAC) can greatly reduce the initial lag time in establishing
165 DIET-based co-cultures because both partners attach to GAC, which functions as an electrically
166 conductive conduit [14,38]. GAC significantly reduced the lag time of the *S. aciditrophicus/ G.*
167 *sulfurreducens*$_{HF}$ co-cultures (Fig. 4c). As previously observed for other co-cultures in which
168 GAC promoted DIET, cells from the *S. aciditrophicus/ G. sulfurreducens*$_{HF}$ co-culture heavily
169 colonized the GAC (Fig. 4e), consistent with a GAC conduit for DIET.

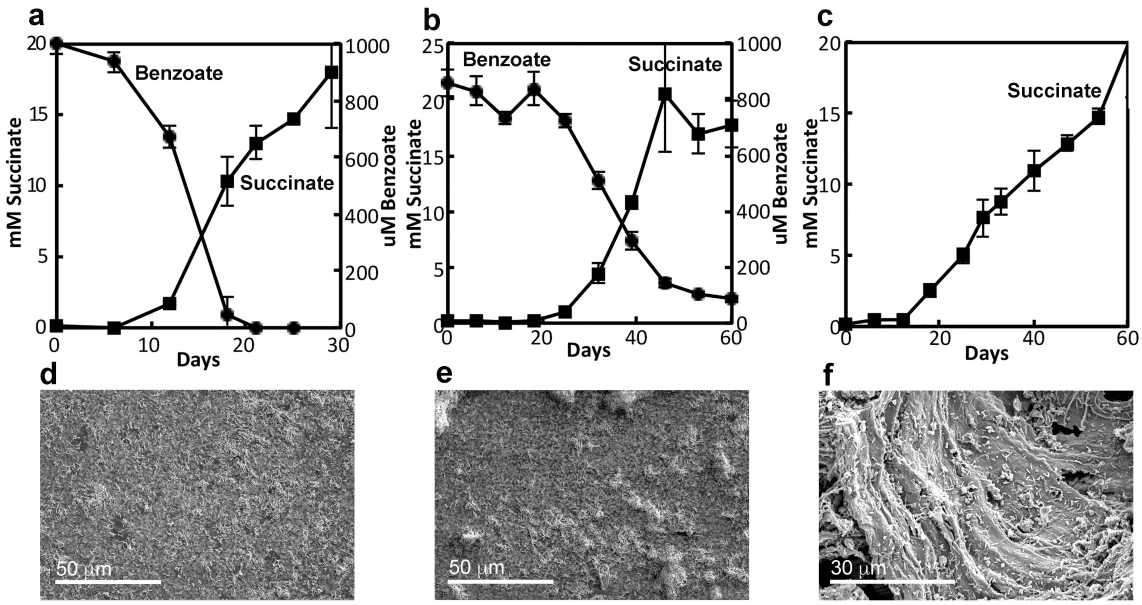


Figure 4. Co-cultures of *Syntrophus aciditrophicus* and *Geobacter sulfurreducens* grow via DIET. Metabolism with *S. aciditrophicus* co-cultured with (**a**) wild-type *G. sulfurreducens*, (**b**) *G. sulfurreducens* strain$_{HF}$, which is unable to use H_2 or formate, or (**c**) *G. sulfurreducens* strain$_{HF}$ with granular activated carbon (GAC) amendment. No acetate was detected in any of the co-cultures. GAC interfered with determination of benzoate, which is not shown for GAC-amended cultures. Scanning electron micrographs (SEM) of cells collected on filters from co-cultures with (**d**) *G. sulfurreducens* wild-type or (**e**) *G. sulfurreducens* strain$_{HF}$. (**f**) SEM of cells on GAC from co-culture of *S. aciditrophicus* with *G. sulfurreducens* strain$_{HF}$. Circles-benzoate; squares-succinate. Data are the mean and standard deviation of triplicate cultures.

In addition to e-pili, multi-heme outer-surface *c*-type cytochromes appear to play an important role in DIET in *Geobacter* species [4,5]. However, the S. *aciditrophicus* genome encodes only a few putative *c*-type cytochromes [21] and cytochromes were not readily apparent in heme-

stained preparations of cell protein (Supplementary Fig. 1). Not all microbes require cytochromes for effective electron transport to the outer cell surface [39]. More detailed examination of the role of e-pili and other *S. aciditrophicus* components in DIET will require the development of methods for genetic manipulation of this microorganism.

S. aciditrophicus is the first isolate outside the genus *Geobacter* found to grow via DIET and is the first syntroph shown to have the option to grow via HFIT or DIET. Other *Syntrophus* species also have pilin genes likely to yield e-pili, as do other diverse genera of syntrophic microorganisms known to grow via HFIT in defined co-cultures (Fig. 5). Establishing conditions that favor DIET often enrich for microbes in these genera [6]. For example, in enrichment cultures specifically designed to promote the metabolism of propionate or butyrate via DIET, *Smithella* (propionate enrichment) or *Syntrophomonas* (butyrate enrichment) species were the most abundant bacteria [40-42]. The greater energetic demands [43] required for synthesizing the abundant aromatic amino acids that are required for e-pili conductivity suggests that e-pili provide a strong selective advantage under some environmental conditions. Conferring the capacity for DIET is a likely explanation.

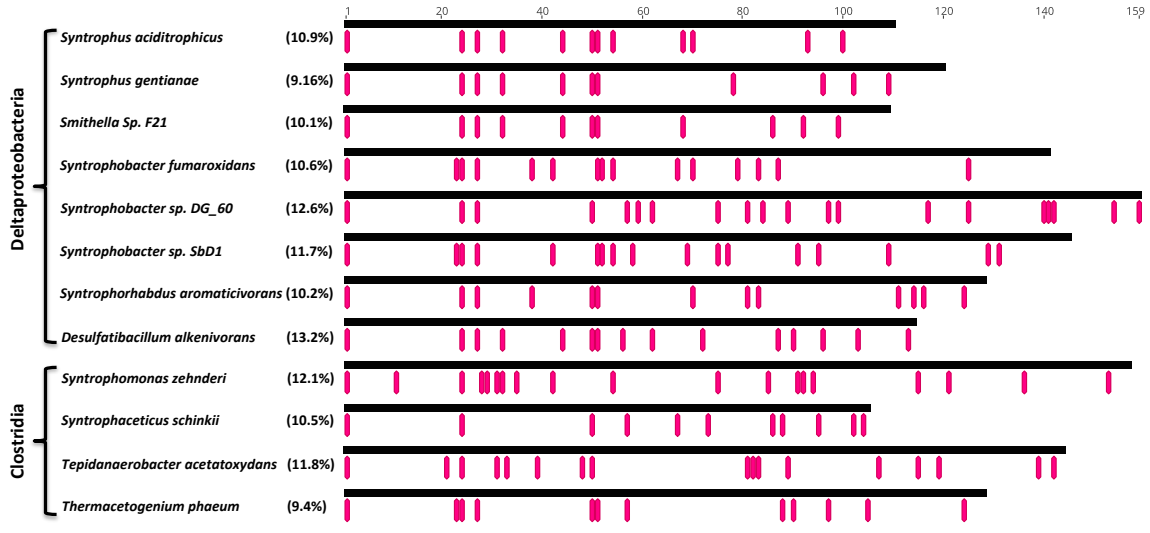


Figure 5. A diversity of syntrophs known to produce H_2 and/or formate as an interspecies electron carrier have pilin monomer genes likely to yield electrically conductive pili. The high abundance of aromatic amino acids as percentage of total amino acids is consistently greater than 9 % and the positioning of aromatic amino acids (red bars) are predictors of assembly into electrically conductive pili.

For decades, data on the functioning of complex anaerobic microbial communities has been interpreted through the lens of HFIT, but the available data does not rule out the possibility of DIET, which was not known to be an option at the time. For example, estimated H_2 turnover rates in anaerobic digesters, rice paddy soils, and aquatic sediments were consistently less than 10 % of the independently determined rate of methane production derived from the reduction of carbon dioxide to methane [44-46], a result that is consistent with DIET providing most of the electrons for carbon dioxide reduction. The mismatch between measured H_2 turnover rates and the concept of H_2 as a primary interspecies electron carrier was rationalized with the suggestion that there was a separate pool of H_2 within closely juxtapositioned assemblages of H_2 producers and H_2 consumers. However, the existence of two distinct pools of H_2 that did not equilibrate over time via diffusion, a seemingly physical impossibility, was never verified. Attempts to accurately measure formate fluxes have also been problematic [47] and there is no method for directly measuring DIET-based electron fluxes in complex communities.

A fresh perspective and new analytical tools will be required to resolve the relative importance of HFIT and DIET in diverse anaerobic microbial communities. Just as electron-accepting partners have different gene expression patterns depending on whether they are participating in HFIT or DIET [37,48], it may be possible to determine whether electron-donating

syntrophs are engaged in DIET or HFIT from metatranscriptomic analysis of anaerobic communities.

METHODS

Bacterial strains, plasmids and culture conditions

Syntrophus aciditrophicus, G. sulfurreducens wild-type, *G. sulfurreducens* strain Aro-5, and *G. sulfurreducens*$_{HF}$ were obtained from our laboratory culture collections. A strain of *G. sulfurreducens* expressing the PilA gene of *S. aciditrophicus* instead of the native *G. sulfurreducens* PilA gene was constructed as previously described [25]. *S. aciditrophicus* and *G. sulfurreducens* strains were routinely grown under strict anaerobic conditions at 30 °C in the previously described [49] defined, bicarbonate-buffered medium with N_2:CO_2 (80:20) as the gas phase. For *S. aciditrophicus* the medium was amended with crotonate (20 mM) and for *G. sulfurreducens* strains acetate (10 mM) was the electron donor and fumarate (40 mM) was the electron acceptor. The presence of *c*-type cytochromes in whole cell lysates was evaluated with heme-staining of proteins separated on denaturing gels as previously described [17]. *G. sulfurreducens* strains were grown with a graphite electrode as the electron acceptor as previously described [50].

Cocultures were established in 10 ml of culture medium [49] in anaerobic pressure tubes with benzoate (1 mM) as the electron donor and fumarate (40 mM) as the electron acceptor with cysteine (2 mM) and sulfide (1 mM) added as reducing agents. When noted cultures were amended with granular activated carbon (0.25 g; 8-20 mesh). Benzoate, acetate, and succinate were analyzed with high-performance liquid chromatography as previously described [16].

Previously describe methods were employed for transmission electron microscopy [21] and scanning electron microscopy [26].

Pili conductivity

A 100 µl sample of cultures was drop cast onto highly oriented pyrolytic graphite (HOPG). After 10 min the HOPG was washed twice with 100 µl of deionized water, blotted dry to remove excess water, and dried for 12 hours at 24 °C in a desiccator. Samples were equilibrated with atmospheric humidity for at least 2 hours and then examined with an Oxford Instruments Cypher ES Environmental AFM in ORCA electrical mode equipped with a Pt/Ir-coated Arrow-ContPT tip with a 0.2 N/m force constant (NanoWorld AG, Neuchâtel, Switzerland). Pili were located in contact mode, with a set point of 0.002 V and a scan rate of 1.5 Hz. For conductive imaging, the grounded tip, attached to a transimpedance amplifier, served as a translatable top electrode to locally detect the current response of the individual pili to a 100 mV bias applied to the HOPG substrate. Individual pili conductivity was further characterized by lightly pressing the AFM tip (set point 0.002 V) to the top of the pili and applying a quadruplicate amplitude of ±0.6 V voltage sweep at a frequency of 0.99 Hz, receiving ca. 8,000 points of reference per measurement. Pili expressed in *G. sulfurreducens* were further analyzed with four probe conductivity measurements on films of pili purified from the cells as previously described [25].

References

1 Stams, A. J. & Plugge, C. M. Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nature Rev. Microbiol.* **7**, 568-577 (2009).
2 McInerney, M. J., Sieber, J. R. & Gunsalus, R. P. Syntrophy in anaerobic global carbon cycles. *Curr Opin. Biotechnol.* **20**, 623-632 (2009).
3 Kouzuma, A., Kato, S. & Wantanabe, K. Microbial interspecies interactions: recent findings in syntrophic consortia. *Front. Microbiology* **6**, 477 (2015).
4 Lovley, D. R. Syntrophy goes electric: direct interspecies electron transfer. *Ann. Rev. Microbiol.* **71**, 643-664 (2017).

5 Lovley, D. R. Happy together: microbial communities that hook up to swap electrons. *ISME J.* **11**, 327-336 (2017).

6 Martins, G., Salvador, A. F., Pereira, L. & Alves, M. M. Methane production and conductive materials: a critical review. *Environ. Sci. Technol.* **52**, 10241-10253 (2018).

7 Park, J.-H., Kang, H.-J., Park, K.-H. & Park, H.-D. Direct interspecies electron transfer via conductive materials: A perspective for anaerobic digestion applications. *Bioresource Technol.* **254**, 300-311 (2018).

8 Morita, M. *et al.* Potential for direct interspecies electron transfer in methanogenic wastewater digester aggregates. *mBio* **2**, e00159-11 (2011).

9 Rotaru, A.-E. *et al.* A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane. *Energy Environ. Sci.* **7**, 408-415 (2014).

10 Shrestha, P. M. *et al.* Correlation between microbial community and granule conductivity in anaerobic bioreactors for brewery wastewater treatment. *Bioresource Tech.* **174**, 306-310 (2014).

11 Ha, P. T. *et al.* Syntrophic anaerobic photosynthesis via direct interspecies electron transfer. *Nature Communications* **7**, 13924 (2017).

12 Holmes, D. E. *et al.* Metatranscriptomic evidence for direct interspecies electron transfer between Geobacter and Methanothrix species in rice paddy soils. *Appl. Environ. Microbiol.* **83**, e00223-00217 (2017).

13 Summers, Z. M. *et al.* Direct exchange of electrons within aggregates of an evolved syntrophic co-culture of anaerobic bacteria. *Science* **330**, 1413-1415 (2010).

14 Rotaru, A.-E., Shrestha, P. M., Liu, F., Nevin, K. P. & Lovley, D. R. Direct interspecies electron transfer during syntrophic growth of Geobacter metallireducens and Methanosarcina barkeri on ethanol. *Appl. Environ. Microbiol.* **80**, 4599-4605 (2014).

15 Rotaru, A.-E., Woodard, T. L., Nevin, K. P. & Lovley, D. R. Link between capacity for current production and syntrophic growth in Geobacter species. *Front. Microbiol.* **6**, 744 (2015).

16 Wang, L.-Y., Nevin, K. P., Woodard, T. L., Mu, B.-Z. & Lovley, D. R. Expanding the diet for DIET: electron donors supporting direct interspecies electron transfer (DIET) in defined co-cultures. *Front. Microbiol.* **7**, 236 (2016).

17 Ueki, T. *et al.* Geobacter strains expressing poorly conductive pili reveal constraints on direct interspecies electron transfer mechanisms. *mBio* **9**, e01273-18 (2018).

18 Hopkins, B. T., McInerney, M. J. & Warikoo, V. Anaerobic syntrophic benzoate degradation: evidence for a threshold and isolation of a syntrophic benzoate degrader. *Appl. Environ. Microbiol.* **61**, 526-530 (1995).

19 Bradley, E. J., Bhupathiraju, V. K., Tanner, R. S., Woese, C. R. & McInerney, M. J. Syntrophus aciditrophicus sp. nov., a new anaerobic bacterium that degrades fatty acids and benzoate in syntrophic association with hydrogen-using microorganisms. *Arch. Microbiol.* **171**, 107-114 (1999).

20 McInerney, M. J. *et al.* The genome of Syntrophus aciditrophicus: life at the thermodynamic limit of microbial growth. *Proc Natl Acad Sci U S A* **104**, 7600-7605 (2007).

21 Sieber, J. R., Le, H. M. & McInerney, M. J. The importance of hydrogen and formate transfer for syntrophic fatty, aromatic and alicyclic metabolism. *Environ. Microbiol.* **16**, 177-188 (2014).

22 Sieber, J. R., McInerney, M. J. & Gunsalus, R. P. Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. *Ann. Rev. Microbiol.* **66**, 429-452 (2012).

23 Lovley, D. R. Electrically conductive pili: biological function and potential applications in electronics. *Curr. Opin. Electrochem.* **4**, 190-198 (2017).

24 Holmes, D. E., Dang, Y., Walker, D. J. F. & Lovley, D. R. The electrically conductive pili of Geobacter species are a recently evolved feature for extracellular electron transfer. *Microbial Genomics* **2**, doi: 10.1099/mgen.1090.000072 (2016).

25 Walker, D. J. F. *et al.* Electrically conductive pili from genes of phylogenetically diverse microorganisms. *ISME J.* **12**, 48-58 (2018).

26 Reguera, G. *et al.* Extracellular electron transfer via microbial nanowires. *Nature* **435**, 1098-1101 (2005).

27 Walker, D. J. F. *et al.* The archaellum of Methanospirillum hungatei is electrically conductive. *bioRixiv*, doi: https://doi.org/10.1101/458356 (2018).

28 Vargas, M. *et al.* Aromatic amino acids required for pili conductivity and long-range extracellular electron transport in Geobacter sulfurreducens *mBio* **4**, e00105-13. (2013).

29 Malvankar, N. S., Yalcin, S. E., Tuominen, M. T. & Lovley, D. R. Visualization of charge propagation along individual pili proteins using ambient electrostatic force microscopy. *Nature Nanotechnol.* **9**, 1012-1017 (2014).

30 Malvankar, N. S. *et al.* Structural basis for metallic-like conductivity in microbial nanowires. *mBio* **6**, e00084-15 (2015).

31 Adhikari, R. Y., Malvankar, N. S., Tuominen, M. T. & Lovley, D. R. Conductivity of individual Geobacter pili. *RSC Advances* **6**, 8354-8357 (2016).

32 Liu, X. *et al.* A Geobacter sulfurreducens strain expressing Pseudomonas aeruginosa type IV pili localizes OmcS on pili but Is deficient in Fe(III) oxide reduction and current production. *Appl. Environ. Microbiol.* **80**, 1219-1224 (2014).

33 Tan, Y. *et al.* The low conductivity of Geobacter uraniireducens pili suggests a diversity of extracellular electron transfer mechanisms in the genus Geobacter. *Front. Microbiol.* **7**, 980 (2016).

34 Tan, Y. *et al.* Synthetic biological protein nanowires with high conductivity. *Small* **12**, 4481–4485 (2016).

35 Tan, Y. *et al.* Expressing the Geobacter metallireducens PilA in Geobacter sulfurreducens yields pili with exceptional conductivity. *mBio* **8**, e02203-16 (2017).

36 Rotaru, A.-E. *et al.* Interspecies electron transfer via H2 and formate rather than direct electrical connections in co-cultures of Pelobacter carbinolicus and Geobacter sulfurreducens. *Appl. Environ. Microbiol.* **78**, 7645-7651 (2012).

37 Shrestha, P. M. *et al.* Transcriptomic and genetic analysis of direct interspecies electron transfer. *Appl. Environ. Microbiol.* **79**, 2397-2404 (2013).

38 Liu, F. *et al.* Promoting direct interspecies electron transfer with activated carbon. *Energy Environ. Sci.* **5**, 8982-8989 (2012).

39 Light, S. H. *et al.* A flavin-based extracellular electron transfer mechanism in diverse Gram-positive bacteria. *Nature* **562**, 140-144 (2018).

40 Zhao, Z. *et al.* Communities stimulated with ethanol to perform direct interspecies electron transfer for syntrophic metabolism of propionate and butyrate. *Wat. Res.* **102**, 475-484 (2016).

41 Zhang, W., Zhang, J. & Lu, Y. Stimulation of carbon nanomaterials on syntrophic oxidation of butyrate in sediment enrichments and a defined coculture. *Scientific Reports* **8**, 12185 (2018).

42 Zhao, Z., Li, Y., Yu, Q. & Zhang, Y. Ferroferric oxide triggered possible direct interspecies electron transfer between Syntrophomonas and Methanosaeta to enhance waste activated sludge anaerobic digestion. *Bioresource Technology* **250** (2018).

43 Akashi, H. & Gojobori, T. Metabolic efficiency and amino acid composition in the proteomes of Escherichia coli and Bacillus subtilis. *Proc Natl Acad Sci U S A* **99**, 3695–3700 (2002).

44 Conrad, R., Phelps, T. J. & Zeikus, J. G. Gas metabolism evidence in support of juxtaposition of hydrogen-producing and methanogenic bacteria in sewage sludge and lake sediments. *Appl. Environ. Microbiol.* **50**, 595-601 (1985).

45 Conrad, R. & Babbel, M. Effect of dilution on methanogenesis, hydrogen turnover and interspecies hydrogen transfer in anoxic paddy soil. *FEMS* **62**, 21-28 (1989).

46 Conrad, R., Mayer, H.-P. & Wust, M. Temporal change of gas metabolism by hydrogen-syntrophic methanogenic bacterial associations in anoxic paddy soil. *FEMS Microbiol. Lett.* **62**, 265-273 (1989).

47 Lovley, D. R. & Klug, M. J. Intermediary metabolism of organic carbon in the sediment of a eutrophic lake. *Appl. Environ. Microbiol* **43**, 552-560 (1982).

48 Holmes, D. E. *et al.* Electron and proton flux for carbon dioxide reduction in Methanosarcina barkeri during direct interspecies electron transfer *Front. Microbiol.* **(**in press) (2018).

49 Coppi, M. V., Leang, C., Sandler, S. J. & Lovley, D. R. Development of a genetic system for *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.* **67**, 3180-3187 (2001).

50 Nevin, K. P. *et al.* Anode biofilm transcriptomics reveals outer surface components essential for high current power production in *Geobacter sulfurreducens* fuel cells. *PLoS ONE* **4**, e5628 (2009).