August 24, 2012

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AEM Accepts, published online ahead of print on 24 August 2012
Appl. Environ. Microbiol. doi:10.1128/AEM.01946-12

Interspecies electron transfer via H_2 and formate rather than direct electrical connections in co-cultures of *Pelobacter carbinolicus* and *Geobacter sulfurreducens*

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Abstract

Direct interspecies electron transfer (DIET) is an alternative to interspecies H_2/formate transfer as a mechanism for microbial species to cooperatively exchange electrons during syntrophic metabolism. To understand what specific properties contribute to DIET, studies were conducted with *Pelobacter carbinolicus*, a close relative of *Geobacter metallireducens,* which is capable of DIET. *P. carbinolicus* grew in co-culture with *Geobacter sulfurreducens* with ethanol as electron donor and fumarate as electron acceptor, conditions under which *G. sulfurreducens* formed direct electrical connections with *G. metallireducens*. In contrast to the cell aggregation associated with DIET, *P. carbinolicus* and *G. sulfurreducens* did not aggregate. Attempts to initiate co-cultures with a genetically modified strain of *G. sulfurreducens* incapable of both H_2 and formate utilization were unsuccessful, whereas co-cultures readily grew with mutant strains capable of formate but not H_2 uptake, or vice-versa. The hydrogenase mutant of *G. sulfurreducens* compensated, in co-cultures, with significantly increased formate-dehydrogenase gene expression. In contrast, the transcript abundance of a hydrogenase gene was comparable in co-cultures with the formate dehydrogenase mutant of *G. sulfurreducens* or wild-type, suggesting that H_2 was the primary electron carrier in the wild-type co-cultures. Co-cultures were also initiated with strains of *G. sulfurreducens* that could not produce pili or OmcS, two essential components for DIET. The finding that *P. carbinolicus* exchanged electrons with *G. sulfurreducens* via interspecies transfer of H_2/formate rather than DIET demonstrates that not all microorganisms that can grow syntrophically are capable of DIET and that closely related microorganisms may use significantly different strategies for interspecies electron exchange.

Introduction

Since the discovery of the "S organism" (6) microbiologists have tried to understand the mechanisms of electron exchange between microorganisms syntrophically degrading organic compounds under anaerobic conditions. For example *Pelobacter carbinolicus*, which is a modern day analog for the S organism, can metabolize ethanol to acetate, H_2 and carbon dioxide only when a H_2-consuming partner, such as *Methanospirillum hungatei,* maintains low H_2 partial pressures (32). In some syntrophic cultures formate may be the electron carrier between species (24, 33, 35). Previous studies provided evidence for H_2 and formate transfer by evaluating H_2- and/or formate- utilizing microorganisms as electron accepting partners (24, 33, 35) , and as well by adding exogenous excess H_2 or formate to the co-cultures to disrupt the syntrophic metabolism, decoupling methanogenesis from utilization of the substrate (1, 2, 40). Thermodynamic calculations have demonstrated that a small window of opportunity exists for the syntrophic partners, where the concentration of H_2 or formate provides optimum conditions for both partners (33, 36). Other electron carriers that facilitate electron exchange between syntrophic partners include the humic substances analog anthraquinone-2,6-disulfonate (19, 21) and cysteine (15).Direct interspecies electron transfer (DIET), could be an efficient alternative strategy for microorganisms to cooperate in the anaerobic degradation of organic substrates (20, 27, 37). DIET was discovered in co-cultures of *G. metallireducens* and *G. sulfurreducens,* which grew with ethanol as the electron donor and fumarate as the electron acceptor (37). *G. sulfurreducens* can not metabolize ethanol, whereas *G. metallireducens* can not use fumarate as an electron acceptor. Adaptive evolution of the co-culture for enhanced ethanol metabolism was associated with the formation of large aggregates of the two species. Although *G. sulfurreducens* is capable of utilizing either H_2 or formate as an electron donor for fumarate reduction when acetate is available as a carbon source (9), cells within the aggregates were not

54 effective in H_2 or formate metabolism and co-cultures were readily initiated with a mutant strain
55 of *G. sulfurreducens* that was unable to use H_2 as an electron donor (37). These results
56 suggested that the co-culture was functioning via an alternative to interspecies H_2 or formate
57 transfer.

58 In the adapted co-cultures, *G. sulfurreducens* produced large quantities of the multiheme
59 c-type cytochrome OmcS (25, 37), which is localized (18) along the electrically conductive (23,
60 30) type IV pili of *G. sulfurreducens*. Increased OmcS expression was attributed to point
61 mutations that accumulated in the gene for the transcriptional regulator PilR (37). Deleting *pilR*
62 in *G. sulfurreducens* accelerated aggregate formation and adaption for rapid ethanol metabolism
63 (37). Deletion of genes required for OmcS or pili expression inhibited ethanol metabolism (37).
64 Furthermore, the aggregates were electrically conductive, likely due to the pili that have been
65 shown to provide long-range conductivity in *G. sulfurreducens* biofilms (23, 24). These results
66 suggested that electrons were directly transferred from *G. metallireducens* to *G. sulfurreducens*.

67 There was also substantial evidence for DIET within aggregates from an anaerobic
68 digester converting brewery waste to methane, in which *Geobacter* were abundant (27). The
69 mixed community aggregates exhibited metallic-like conductivity (27) similar to that of
70 *Geobacter* current-producing biofilms and the pili of *G. sulfurreducens* (23).

71 To better understand the mechanisms of DIET it is important to determine if other
72 microorganisms are capable of DIET and what features those microorganisms must have to
73 enable DIET. The potential for *P. carbinolicus* to participate in DIET was evaluated because
74 both *P. carbinolicus* and *G. metallireducens* appear to have evolved from a common ancestor
75 capable of extracellular electron transfer (7), but the two differ significantly in several aspects of

76 their basic physiology and mechanisms for extracellular electron transfer (7, 12, 31). Thus, it was
77 unknown whether the absence of previous evidence for DIET with *P. carbinolicus* could be
78 attributed to syntrophic growth being evaluated with an electron-accepting partner incapable of
79 DIET, or whether *P. carbinolicus* lacks key physiological features required for DIET. The
80 results indicate that *P. carbinolicus* is not capable of DIET and must rely on interspecies transfer
81 of H_2 or formate for electron exchange with *G. sulfurreducens*.

82 **Materials and Methods**

83 **Organisms, media and growth conditions**

84 All incubations of pure cultures and co-cultures were performed under strict anaerobic
85 culturing techniques as previously described (3). Cultures were incubated in 27 mL pressure
86 tubes or 160 mL serum bottles sealed with butyl rubber stoppers and filled with 10 or 50 mL of
87 medium. Increase in cultures turbidity was monitored at 600 nm by placing the culture tubes into
88 a Genesys 5 Spectrophotometer (Spectronics Instruments) with a path length of 1.5 cm.

89 *P. carbinolicus* (DSM 2380) was regularly transferred under fermentative conditions
90 with 10 mM acetoin as substrate, and 0.02 mM Na_2S as reductant, as previously described (12).
91 *G. sulfurreducens* PCA (ATCC 51573) and mutants of this microorganism which were tested for
92 the study (Δ*hybL,* Δ*fdnG*, a double mutant Δ*hybL*-Δ*fdnG,* Δ*omcS,* Δ*pilA*) were routinely cultured
93 in freshwater medium containing 1 mM cysteine as reductant, 10 or 15 mM acetate and 40 mM
94 fumarate as previously described (8). Newly constructed mutants of *G. sulfurreducens* were
95 tested for growth with H_2 (20 psi) or formate (40 mM and 10 mM) as the electron donor in
96 freshwater medium in the presence of 1 mM acetate as carbon source.

For co-cultures of *P. carbinolicus* and *G. sulfurreducens* 20 mM ethanol and 40 mM fumarate served as substrates for growth in a medium prepared as previously described (12). Co-cultures of *G. metallireducens* and the *G. sulfurreducens* strain deficient in formate dehydrogenase and hydrogenase activity were initiated using 2% inocula of each syntrophic partner added to a freshwater medium prepared as previously described (37) with fumarate and ethanol as substrates.

All co-cultures were regularly transferred (2% inocula) under strict anaerobic conditions at least six times prior to monitoring organic acids and ethanol over time. The only exception was a co-culture of *P. carbinolicus* with the *G. sulfurreducens* double mutant incapable of H_2 and formate utilization. This co-culture could not grow on ethanol, and was therefore analyzed during the initial transfer.

Construction of *G. sulfurreducens* mutants

The *fdnG* gene (GSU0777) was replaced with a kanamycin resistance gene, such that the coding region for amino acid residues from 62Asp to 951Pro was deleted. Double-crossover homologous recombination was carried out by electroporation (8) with the linear DNA fragment consisting of the kanamycin resistance gene flanked by ~0.7 kilobase pairs (kbp) DNA fragments containing the upstream and the downstream regions of *fdnG*. These flanking DNA fragments were amplified by PCR with primers *fdnG*-P1 (TCTCTAGAACGGCTTGGTGACGTAGTC, the *Xba*I site is underlined) and *fdnG*-P2 (TCGGATCCTTGGTATGGACGATCAG, the *BamH*I site is underlined) for the upstream region and *fdnG*-P3 (TCTAAGCTTCAACGTGCAGGGCAAGC, *Hind*III site is underlined) and *fdnG*-P4 (TCTCTCGAGACCACTTTCACGTAGCGGTC, *Xho*I site is underlined) for the downstream region. The kanamycin resistance gene was amplified by

PCR with Km-Fwd (GCATGAGAATTCCTGACGGAACAGCGGGAAGTCCAGC, *EcoR*I site is underlined) and Km-Rev (GCTATGAAGCTTTCATAGAAGGCGGCGGTGGAATCGAA, the *Hind*III site is underlined), and using pBBR1MCS-2 (17) as template. Gene replacement was confirmed by PCR analysis. The Δ*fdnG*-Δ*hybL* double mutant was constructed in a similar manner by deleting the *fdnG* gene from a previously characterized uptake hydrogenase mutant, Δ*hybL* (10).

Reverse transcription quantitative PCR

To quantify the abundance of hydrogenase and formate dehydrogenase transcripts in co-cultures of *P. carbinolicus* with the wild type strain of *G. sulfurreducens*, the hydrogenase deficient strain and the formate dehydrogenase deficient strain, four biological replicates of each late mid-exponential phase co-culture, 10 mL each, were treated with 2 mL RNA later (Ambion), mixed well and harvested at 4°C by centrifugation at 6000×g for 20 min. Tubes were opened and co-cultures were removed for further use for RNA extraction using Trizol (Invitrogen) with slight modification of manufacturers' protocol. Briefly, the cell pellets were mixed with 1 ml volume of TRIzol reagent and mixed homogenously. The mix was transferred to a 2 ml O-ring tube containing 0.5 g of 0.1 mm glass/zirconia beads and homogenized for 20 sec on a FastPrep Instrument (MoBio Laboratories) at 3 m/s. The tubes were then incubated at room temperature for 5 min before addition of 200 µl chloroform, vortexed for 15 sec and centrifuged at 12000 × g for 15 min at 4° C. The aqueous layer was then used for the RNA isolation. The RNA thus obtained was purified using MiniElute PCR Purification Kit (Qiagen) and further treated with rDNAse I (Ambion) to digest any traces of genomic DNA contamination. Final round of RNA purification was done on a MiniElute PCR Purification Kit (Qiagen) following the manufacturer's protocol. The quality and the quantity of pure RNA were accessed with the

Experion RNA standard sensitivity kit (Bio Rad). Furthermore, absence of genomic DNA contamination was verified by 16S rRNA gene PCR using 9F and 519R primer sets (34).

For whole transcriptome amplification (WTA) about 300 ng of total RNA were converted into WTA cDNA libraries and amplified by WTA PCR using reagents and protocols supplied with or recommended by Sigma. Briefly, 300 ng of total RNA was mixed with 2.5 µL WTA Library Synthesis Buffer and 2.5 µL WTA Library Stabilization Solution and the total volume was adjusted to 24 µL using nuclease-free water, the mixture was heated at 70°C for 5 min and immediately cooled. Library synthesis enzyme (1µL) was added, and WTA cDNA libraries were synthesized using the following thermocycler program: 24°C for 15 minutes, 42°C for 2 hours, and 95°C for 5 minutes. Aliquots were WTA PCR-amplified using JumpStart™ Taq DNA Polymerase (Sigma), WTA Amplification Master Mix and dNTP Mix following the manufacturers' protocol except total cycle was reduced to 15 cycles. The enriched product was then purified using PCR purification kit (Qiagen) and used as a template in qPCR experiment.

Real time PCR was carried out using ABI prism 7900 (Applied Biosystem). Primers designed for *G. sulfurreducens* (26) were used to target the *hybA, fdnG,* and the housekeeping gene *recA*: *fdnG*-F: 5'-ACTTCACCAAGGACGTCACC-'3, *fdnG*-R: 5'-TCCCTTCGTTGGTGTAGGAG-'3, *hybA*-F: 5'-CTACGGCGAGAAGGAAGTTG-'3, *hybA*-R: 5'-CCCCTTGTAGATGGTGTGCT-'3, *recA*-F: 5'-CACCGGCATAATCTCCAAGT-'3 and *recA*-R: 5'-ATCTTGCGGATATCGAGACG-'3. Reactions were performed in triplicate for each gene tested in a final volume of 20 µl containing 10 µl of Power Sybr Green PCR master mix, 0.6 mM of reverse and forward primers were made and 2 µl of enriched WTA product was added as template. The real time PCR was run for 50 cycles using 60°C as the annealing temperature using absolute quantification option.

165 **Microscopy**

166 To resolve if cells grew freely in the medium or if they were associated in aggregate
167 structures, cells were visualized by phase contrast microscopy on a Nikon Eclipse E600
168 microscope.

169 To resolve the cell abundance and overall distribution of the two microorganisms in the
170 co-cultures, cells were fixed (2% paraformaldehyde and 0.5% glutaraldehyde in 50 mM PIPES at
171 pH 7.2) for one hour at room temperature, a droplet was placed on a gelatin-coated slide and
172 dried at 46°C for 5 min, and was then dehydrated in 70% ethanol for 30 min at 4°C. Dehydrated
173 samples were hybridized as described (29) using the probes: PCARB1: 5'-
174 [cy3]GCCTATTCGACCACGATA-3', specific for *P. carbinolicus* (31), and GEO2: 5'-
175 [cy5]GAAGACAGGAGGCCCGAAA-3', specific for *G. sulfurreducens* (37). Samples were
176 visualized on a Leica TCS SP5 confocal fluorescence microscope using consecutive line
177 scanning to detect Cy3 and Cy5 fluorochromes.

178 **Identification of OmcS cytochrome content in co-cultures**

179 OmcS abundance was determined in *P.carbinolicus/G.sulfurreducens* and
180 *G.metallireducens/G.sulfurreducens* co-cultures versus *G. sulfurreducens* cells were grown on
181 fresh-water medium with 40 mM fumarate and 10 mM acetate as substrates (8). Cells were
182 retrieved during the late stages of mid-exponential growth, and the whole cells lysates obtained
183 (5µg), were separated by sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE)
184 followed by immunoblotting, and probing with an OmcS-specific antiserum as previously
185 described (37).

Analytical techniques

For determination of substrate depletion and production of metabolic products, samples were withdrawn with hypodermic needles and syringes under strict anaerobic conditions and passed through 0.2 µm Acrodisc filters. A minimum of three biological replicates was analyzed for each co-culture type. Volatile fatty acids were monitored by high performance liquid chromatography as previously described (28). Changes in ethanol concentration over time was monitored by gas chromatography as previously described (27).

193 **Results and Discussion**

194 **Syntrophic growth on ethanol**

195 When *P. carbinolicus* and *G. sulfurreducens* were simultaneously inoculated into a medium with
196 ethanol as the electron donor and fumarate as the electron acceptor, the co-culture grew with the
197 metabolism of ethanol and the reduction of fumarate to succinate (Fig. 1; Fig. 2a). In contrast to
198 the previously described co-cultures of *G. metallireducens* and *G. sulfurreducens*, which lagged
199 for several weeks before utilizing significant ethanol (37), growth and metabolism of the *P.*
200 *carbinolicus*/*G. sulfurreducens* co-cultures typically began within a day (Fig. 1). Furthermore,
201 the *P. carbinolicus* - *G. sulfurreducens* co-cultures metabolized most of the ethanol provided in
202 three days whereas even after months of adaptation for syntrophic growth, the *G.*
203 *metallireducens/G. sulfurreducens* co-cultures still required five days to metabolize 70% of the
204 added ethanol (37). Although *G. metallireducens/G. sulfurreducens* co-cultures formed large (>
205 1 mm) aggregates (37), the *P. carbinolicus/G. sulfurreducens* co-cultures did not aggregate even
206 after 400 consecutive transfers of the co-culture. The cells did not appear to form physical
207 associations, even at the level of individual cells (Fig. 3a). Contact between syntrophic partners
208 is considered to be a requirement for DIET, and although it may also facilitate interspecies H_2 or
209 formate transfer (5, 14, 39), long-term co-culture studies demonstrated that contact is not
210 necessary for the later (13). Examination of the co-culture with FISH probes specific for the two
211 species revealed that *G. sulfurreducens* was more abundant than *P. carbinolicus* (Fig. 3).

212 **Interspecies electron transfer via H_2 or formate**

213 In order to evaluate the possibility of interspecies H_2 or formate transfer, co-cultures were
214 initiated with one of the following strains of *G. sulfurreducens*: 1) a strain that could not

metabolize H_2 because the gene for the large subunit of the uptake hydrogenase (*hybL*) was deleted (10); 2) a strain that could not grow on formate because the gene for the catalytic subunit of formate dehydrogenase (*fdnG*) was deleted (Fig. 4b); or 3) a strain that could not grow on H_2 or formate because both *hybL* and *fdnG* were deleted (Fig. 4c). Co-cultures initiated with *G. sulfurreducens* strains that could metabolize only formate (Fig. 2b) or only H_2 (Fig. 2c) readily metabolized ethanol with the reduction of fumarate.

However, growth and ethanol metabolism did not proceed in co-cultures initiated with a strain of *G. sulfurreducens* that could not metabolize either H_2 or formate (Fig. 2d). These results indicate that either H_2 or formate can serve as electron carriers for interspecies electron transfer, and interspecies electron transfer via one of these two electron carriers was the only mechanism by which the co-culture could function. In contrast, *G. metallireducens* formed well-functioning syntrophic cultures with the *G. sulfurreducens* strain that could not utilize H_2 and formate, consistent with the concept of DIET in that co-culture system (Fig. 1SM).

In order to evaluate the potential contributions of H_2 and formate as electron carriers between *P. carbinolicus* and *G. sulfurreducens* the transcript abundance of an uptake hydrogenase subunit (*hybA*) and the large subunit of formate dehyrogenase (*fdnG*) were monitored (Fig. 5a). When H_2 uptake was not possible, *G. sulfurreducens* adapted with increased expression of *fdnG* ($P=0.009$). In contrast, when formate metabolism was inhibited, transcript abundance of *hybA* was not significantly different ($P=0.5$) than the wild type (Fig 5a). These results, and the fact that *hybA* transcripts were much more abundant than *fdnG* transcripts in wild-type, suggest that although the co-cultures could function via either interspecies H_2 or formate transfer, H_2 was the primary electron carrier between species in co-cultures with wild-type *G. sulfurreducens*.

In contrast to *G. metallireducens/G. sulfurreducens* co-cultures (Fig. S1), acetate accumulated over time in *P. carbinolicus/G. sulfurreducens* co-cultures (Fig. 1). The likely explanation for this difference is that the expression of citrate synthase in *G. sulfurreducens* is inhibited in the presence of H_2, preventing acetate metabolism (4, 38). Thus, the availability of H_2 in *P. carbinolicus/G. sulfurreducens* co-cultures would be expected to limit acetate metabolism of *G. sulfurreducens*, whereas no such inhibition of acetate metabolism is expected in *G. metallireducens/G. sulfurreducens* co-cultures because of the lack of H_2 production during DIET.

Pili and OmcS not required during H_2/formate electron transfer

Deleting the gene for PilA or OmcS in *G. sulfurreducens* did not prevent *P. carbinolicus* from forming effective co-cultures (Fig. 2e and 2f, respectively). This contrasts with the previous finding (37) that *G. metallireducens/G. sulfurreducens* co-cultures could not be established if either *pilA* or *omcS* was deleted from *G. sulfurreducens* (37). As previously reported (37), *G. sulfurreducens* expressed OmcS at high levels in *G. metallireducens/G. sulfurreducens* co-cultures, but OmcS was not detected in *P. carbinolicus/G. sulfurreducens* co-cultures (Fig. 5b). These results suggest that the model for DIET between *G. metallireducens* and *G. sulfurreducens*, in which OmcS and pili are important components of the electrical connection between the two species (20, 37), does not apply to the *P. carbinolicus*/*G. sulfurreducens* co-culture.

Implications

These findings demonstrate that not all microorganisms that can grow syntrophically via interspecies electron exchange are capable of DIET and that even closely related microorganisms may differ in their mode of syntrophic growth. The finding that *P. carbinolicus* was not able to

directly transfer electrons to another species capable of DIET is consistent with previous findings which suggest that *P. carbinolicus* is poorly suited for direct electron transfer to insoluble extracellular electron acceptors, such as electrodes (31) and Fe(III) oxide (12). The ability to growth syntrophically via interspecies hydrogen/formate transfer, but not DIET, may be common in laboratory co-cultures. For example a syntrophic co-culture of *Desulfovibrio vulgaris* and *Methanococcus maripaludis* did not form aggregates even after 300 generations (13), suggesting a lack of DIET in that system as well.

Although there is evidence for DIET in microbial aggregates from methanogenic wastewater digesters (27) the prevalence of DIET in natural environments and the factors that might favor DIET over interspecies H_2 and formate transfer are unknown. It may be that *G. metallireducens* interacts with *G. sulfurreducens* via DIET because it is well suited for extracellular electron transfer (22), but has limited ability to produce H_2 (11).

Metabolizing substrates with the release of electrons as H_2 or formate requires less coordination with syntrophic partners than DIET and may account for the ability of the *P. carbinolicus/G. sulfurreducens* co-cultures to initiate syntrophic growth much faster and to metabolize ethanol more rapidly than *G. metallireducens/G. sulfurreducens* co-cultures. Another consideration is that consortia cooperating via DIET must bear the additional energetic investment of producing the proteins necessary to establish the electrical connections required for DIET. However, the high abundance of *Geobacter* species in electrically conductive aggregates from methanogenic digesters (27) and the finding that addition of conductive/(semi)-conductive supplementary materials enhance DIET with increased rates of methanogenesis in sediments (16) and methanogenic digester aggregates (19), suggest that DIET can be more favorable than interspecies H_2/formate transfer in important methane-producing environments.

283 Genome-scale metabolic modeling might offer an approach for calculating the cost/benefit of the
284 different strategies for interspecies electron transfer under diverse environmental conditions as
285 evidenced by the ability of this approach to effectively predict the outcome of microbial
286 competition in different subsurface environments (41).

287 The physiological differences between microorganisms that are effective in DIET versus
288 those that rely on interspecies H_2/formate transfer are important considerations when attempting
289 to enrich and isolate syntrophic microorganisms capable of DIET. Common procedures for the
290 isolation of syntrophic microorganisms, such as the use of fermentable substrates (33) or co-
291 culturing with a H_2-consuming partner (24), may fail to recover organisms that specialize in
292 DIET. Thus, new approaches for isolation and study of syntrophic interactions are required to
293 better assess the diversity and environmental relevance of microorganisms capable of DIET.

294 **Acknowledgements**

295 We thank Trevor Woodard and Jaclyn Izbicki for facilitating HPLC, and GC analysis; Stefan
296 Hansen for developing the Chromatography Data Extractor software for HPLC data screening;
297 Muktak Aklujkar for helpful comments on the manuscript; and Dan Carlo Flores and Molie
298 Murnane for lab assistance. This research was supported by the Office of Science (BER), U. S.
299 Department of Energy, Award No. DE-SC0004485.

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434 **Figure legends**

435 **Figure 1.** Initial growth of co-cultures in ethanol-fumarate medium started with *P.*
436 *carbinolicus* and different strains of *G. sulfurreducens*. The results are the mean and standard
437 deviation of triplicate cultures.

438 **Figure 2.** Growth, ethanol metabolism, acetate accumulation, and succinate production
439 from fumarate reduction after more than five consecutive transfers of co-cultures of *P.*
440 *carbinolicus* with different strains of *G. sulfurreducens*. Also shown is the data from the initial
441 attempt to start a co-culture with a strain of *G. sulfurreducens* unable to utilize formate or H_2.
442 The results are the mean and standard deviation of triplicate cultures.

443 **Figure 3**

444 Phase contrast (a, b, c) and epifluorescence micrographs (d, e, f) of *P. carbinolicus* cells
445 in co-culture with *G. sulfurreducens* wild type cells (a, d) or the hydrogenase-deficient *G.*
446 *sulfurreducens* strain (b, e), or the strain deficient in formate dehydrogenase (c, f).
447 Epifluorescence of in situ hybridized cells with *P. carbinolicus* shown as green and *G.*
448 *sulfurreducens* shown as red. Scale is bar is 10μm.

449 **Figure 4**

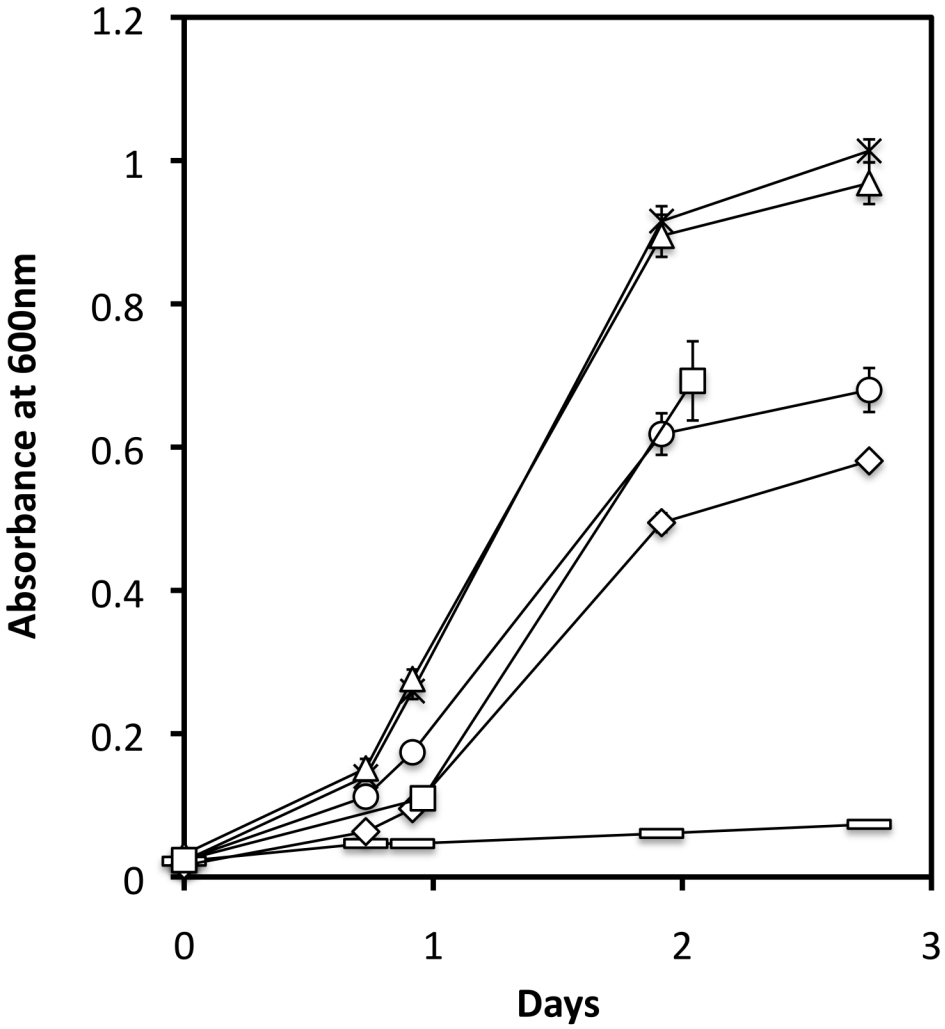
450 Growth on formate or H_2 in the presence of 1 mM acetate for *G. sulfurreducens* wild type
451 (a), a strain deficient in a formate dehydrogenase subunit (b) or a strain deficient in both a
452 formate dehydrogenase and an uptake hydrogenase subunit (c). In controls without added
453 hydrogen or formate the acetate added as a carbon source could also serve as electron donor to
454 support growth. Growth of the double mutant growth on 15 mM acetate is also shown (c) to

455 demonstrate that cells were viable, yet unable to grow on formate or H_2. The results are the
456 mean and standard deviation of triplicate cultures.

457 **Figure 5**

458 Molecular analysis of co-cultures. (a) Relative transcript abundance of formate
459 dehydrogenase (*fdnG*), hydrogenase (*hybA*) and the housekeeping gene, *RecA*, in *P.*
460 *carbinolicus*/*G. sulfurreducens* co-cultures as determined by RT-qPCR. Results are the mean and
461 standard deviation for triplicate cultures. (b) Western blot analysis of OmcS in equivalent cell
462 protein of *G. sulfureducens* grown with fumarate as the electron acceptor or ethanol-fumarate co-
463 cultures of *P. carbinolicus/G. sulfurreducens* or *G. metallireducens/G. sulfurreducens* co-
464 cultures.

465



Strain of *Geobacter*:

- Wild type
- Formate dehydrogenase-defficient mutant
- Hydrogenase-defficient mutant
- Formate dehydrogenase and hydrogenase double mutant
- PilA-defficient mutant
- OmcS-defficient mutant