



# Untangling *Geobacter sulfurreducens* Nanowires

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Ye et al. (1) present a model for how protein nanowires contribute to the current production of *Geobacter sulfurreducens* biofilms. They state that “In this study, we examined all three nanowires in the anode biofilm of *G. sulfurreducens* with the goal of defining both their structural and conductive contributions.” (1). However, Ye et al. (1) did not examine any nanowires in anode biofilms, and the only imaging they provided suggested that the strain of *G. sulfurreducens* that they studied did not express two of the three nanowire types under consideration. Furthermore, the authors misrepresent previously published studies of their own, as well as other investigators.

The three nanowires that Ye et al. (1) considered were electrically conductive pili (e-pili) and nanowires comprised of chains of multi-heme *c*-type cytochromes, either OmcS or OmcZ (1). Ye et al. (1) demonstrated that, as expected, their control strain of *G. sulfurreducens* expressed thin filaments (Fig. 1A). However, the resolution of their images was insufficient to determine whether the filaments were e-pili or cytochrome-based filaments. When Ye et al. (1) deleted the gene for PilB, no filaments were apparent (Fig. 1B). According to Ye et al. (1), deleting the PilB gene specifically eliminated the expression of e-pili. If this was true, then the filaments emanating from the control strain must have all been e-pili because they are all absent in the  $\Delta$ PilB strain. The lack of filaments emanating from the  $\Delta$ PilB strain could not be attributed to a lack of cytochrome expression because the same lab (2) previously demonstrated proper expression of outer-surface *c*-type cytochromes in their  $\Delta$ PilB strain under the same growth conditions (Fig. 1C). Thus, not only did Ye et al. (1) fail to document that their strain of *G. sulfurreducens* produced cytochrome-based filaments, but they also presented data that suggested that the outer-surface cytochromes that were expressed were not assembled into filaments.

Although OmcS nanowires emanating from cells grown under similar conditions were previously demonstrated in another strain of *G. sulfurreducens* (3), it is also known, as recently reviewed in detail in references (4, 5), that OmcS and OmcZ can be displayed on the outer cell surface without forming nanowires. Without first proving that their strain can express OmcS and OmcZ nanowires, Ye et al. (1) had no data to support their assertion that deleting genes for OmcS or OmcZ removed OmcS or OmcZ nanowires.

Previous studies concluded that e-pili plays an important role in long-range electron transport through current-producing *G. sulfurreducens* biofilms because strains expressing poorly conductive pili were defective in current production (6–10). Ye et al. (1) misrepresent these earlier studies by suggesting that expressing poorly conductive pili “is usually incurred at altering the extracellular cytochrome profile”. The reference they cite for this (8) stated the direct opposite conclusion and provided data that cytochromes were properly localized (Fig. 1D). Ye et al. (1) also ignored additional studies, including a previous publication by one of the authors (7), which demonstrated proper expression of outer surface cytochromes in strains expressing poorly conductive pili (Fig. 1E). The most recent study on this topic demonstrated that a strain of *G. sulfurreducens* that can express OmcS nanowires continued to produce OmcS nanowires in the same abundance when it was genetically modified to express poorly conductive pili (3). Ye et al. (1) needed to change their model

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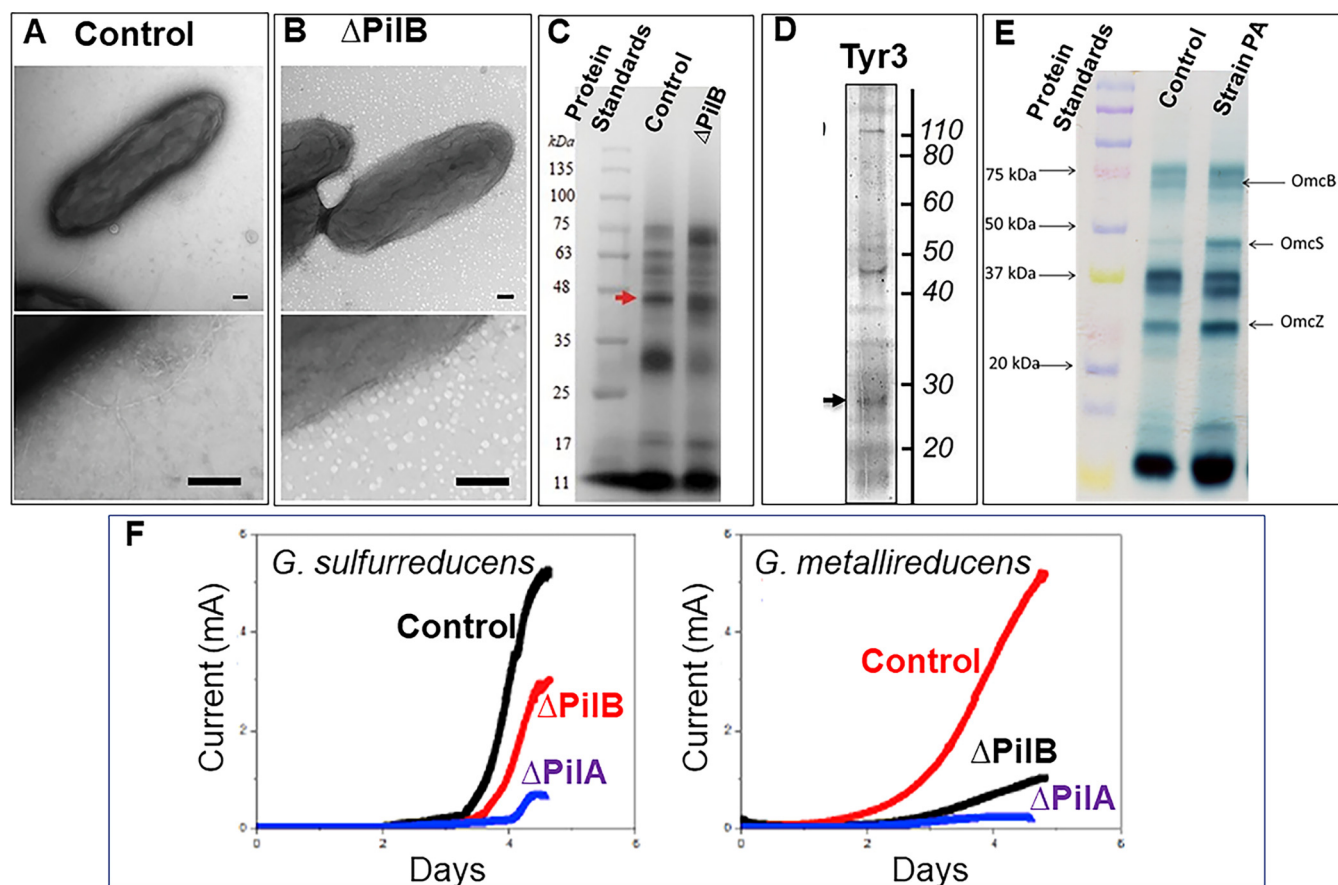
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**FIG 1** Data on the expression of nanowires and outer-surface cytochromes from previous studies. (A) Transmission electron micrographs of control strain of *G. sulfurreducens* from (1). Size bars are 100 nm. (B) Transmission electron micrographs of  $\Delta\PilB$  mutant of *G. sulfurreducens* from (1). Size bars are 100 nm. (C) Heme-stained SDS-PAGE of extracellular c-type cytochromes prepared from *G. sulfurreducens* control strain and  $\Delta\PilB$  mutant. The red arrow designates the band for OmcS. The gel image with an arrow is from reference (2) with permission. (D) Heme-stained proteins in the biofilm matrix from strain Tyr3, a mutant in which the tyrosines in the pilin monomer were replaced with alanine. The arrow designates the band for OmcZ. The gel image with an arrow is reprinted from reference (8) (publisher permission was not needed). (E) Heme stained SDS-PAGE of outer surface c-type cytochromes from *G. sulfurreducens* control strain and strain PA, a strain expressing poorly conductive pili. The gel image with band labeling is from reference (7) (publisher permission was not needed). (F) Current production of *G. sulfurreducens* and *G. metallireducens* control strains,  $\Delta\PilB$  strains, and  $\Delta\PilA$  strains. The data image is from reference (2) with permission. Descriptive labeling on original images was modified for consistency.

to account for Liu's prior results (7), as well as the results of other investigators, that have reported proper localization of outer surface cytochromes, but diminished current production in *G. sulfurreducens* strains expressing poorly conductive pili.

Liu et al. (2) previously reported that  $\Delta\PilB$  strains of *G. sulfurreducens* and *G. metallireducens* were defective in current production (Fig. 1F). Yet now, Ye et al. (1) claim that a  $\Delta\PilB$  strain produces ca. 90% as much current as wild-type. This reversal in the phenotype reported is remarkable because it has a major impact on the nanowire model. If, as the authors claim, deleting the gene for PilB specifically prevents e-pili expression, then the phenotype that their lab originally reported (2) refutes their claim that e-pili had a minor role in electron transfer through biofilms. An explanation for their change in the phenotype reported is required.

Ye et al. (1) presented a model in which OmcZ nanowires coursing throughout current-producing biofilms were the primary conduit for long-range electron transport. This conclusion was based on the finding that deleting the gene for OmcZ greatly diminished current production. Ye et al. (1) fail to note that this same phenotype was reported long ago (11) and that, based on those results, the localization of OmcZ was intensively investigated (12). Those studies demonstrated that OmcZ was specifically localized at the biofilm-anode interface and that OmcZ did not appear to be organized in filaments (12). Thus, the model of Ye et al. (1) was unfounded because they did not provide data to refute the earlier studies on

OmcZ localization, and as noted above, Ye et al. (1) did not provide evidence that their strain of *G. sulfurreducens* could express the OmcZ nanowires emanating from cells that their model required.

Word limit restrictions prevent full discussion of the failure of Ye et al. (1) to properly present all the data, including contradictory data from their prior studies, that refute their model. They misrepresent recent studies (13) that questioned the rationale behind generating  $\Delta$ PilB mutants by suggesting that those studies were conducted on a *G. sulfurreducens* strain not relevant to their strain without acknowledging that the prior studies also analyzed a  $\Delta$ PilB mutation made in the type strain background. Ye et al. (1) also fail to properly attribute previous key discoveries which they imply are their own, such as the fact that e-pili can also play a structural role in biofilm formation (14), as well as the major importance of OmcZ and lesser role of OmcS in high-density current production and biofilm growth on anodes (11).

## REFERENCES

1. Ye Y, Liu X, Nealon KH, Rensing C, Qin S, Zhou S. 2022. Dissecting the structural and conductive functions of nanowires in *Geobacter sulfurreducens* electroactive biofilms. *mBio* 13:e03822-21. <https://doi.org/10.1128/mbio.03822-21>.
2. Liu X, Zhuo S, Rensing C, Zhou J. 2018. Syntrophic growth with direct interspecies electron transfer between pili-free *Geobacter* species. *ISME J* 12:2142–2151. <https://doi.org/10.1038/s41396-018-0193-y>.
3. Liu X, Walker DJF, Nonnenmann S, Sun D, Lovley DR. 2021. Direct observation of electrically conductive pili emanating from *Geobacter sulfurreducens*. *mBio* 12:e02209-21. <https://doi.org/10.1128/mBio.02209-21>.
4. Lovley DR, Walker DJF. 2019. *Geobacter* protein nanowires. *Front Microbiol* 10:2078. <https://doi.org/10.3389/fmicb.2019.02078>.
5. Lovley DR, Holmes DE. 2022. Electromicrobiology: the ecophysiology of phylogenetically diverse electroactive microorganisms. *Nat Rev Microbiol* 20:5–19. <https://doi.org/10.1038/s41579-021-00597-6>.
6. Vargas M, Malvankar NS, Tremblay P-L, Leang C, Smith JA, Patel P, Snoeyenbos-West O, Snoeyenbos-West O, Nevin KP, Lovley DR. 2013. Aromatic amino acids required for pili conductivity and long-range extracellular electron transport in *Geobacter sulfurreducens*. *mBio* 4:e00105-13–e00113. <https://doi.org/10.1128/mBio.00105-13>.
7. Liu X, Tremblay P-L, Malvankar NS, Nevin KP, Lovley DR, Vargas M. 2014. 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. <https://doi.org/10.1128/AEM.02938-13>.
8. Steidl RJ, Lampa-Pastirk S, Reguera G. 2016. Mechanistic stratification in electroactive biofilms of *Geobacter sulfurreducens* mediated by pilus nanowires. *Nat Commun* 7:12217. <https://doi.org/10.1038/ncomms12217>.
9. Tan Y, Adhikari RY, Malvankar NS, Ward JE, Nevin KP, Woodard TL, Smith JA, Snoeyenbos-West OL, Franks AE, Tuominen MT, Lovley DR. 2016. The low conductivity of *Geobacter uraniireducens* pili suggests a diversity of extracellular electron transfer mechanisms in the genus *Geobacter*. *Front Microbiol* 7:980. <https://doi.org/10.3389/fmicb.2016.00980>.
10. Walker DJF, Adhikari RY, Holmes DE, Ward JE, Woodard TL, Nevin KP, Lovley DR. 2018. Electrically conductive pili from genes of phylogenetically diverse microorganisms. *ISME J* 12:48–58. <https://doi.org/10.1038/ismej.2017.141>.
11. Nevin KP, Kim B-C, Glaven RH, Johnson JP, Woodard TL, Methé BA, DiDonato RJ, Jr, Covalla SF, Franks AE, Liu A, Lovley DR. 2009. Anode biofilm transcriptomics reveals outer surface components essential for high current power production in *Geobacter sulfurreducens* fuel cells. *PLoS One* 4:e5628. <https://doi.org/10.1371/journal.pone.0005628>.
12. Inoue K, Leang C, Franks AE, Woodard TL, Nevin KP, Lovley DR. 2011. Specific localization of the c-type cytochrome OmcZ at the anode surface in current-producing biofilms of *Geobacter sulfurreducens*. *Environ Microbiol Rep* 3:211–217. <https://doi.org/10.1111/j.1758-2229.2010.00210.x>.
13. Ueki T, Walker DJF, Nevin KP, Ward JE, Woodard TL, Nonnenmann SS, Lovley DR. 2021. Generation of high current densities in *Geobacter sulfurreducens* lacking the putative gene for the PilB pilus assembly motor. *Microbiol Spectr* 9:e00877-21. <https://doi.org/10.1128/Spectrum.00877-21>.
14. Reguera G, Pollina RB, Nicoll JS, Lovley DR. 2007. Possible non-conductive role of *Geobacter sulfurreducens* pili nanowires in biofilm formation. *J Bacteriol* 189:2125–2127. <https://doi.org/10.1128/JB.01284-06>.