Electrically conductive *Geobacter* pili

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Abstract

A review of the recent publication by Gu et al. (*Nature*https://doi.org/10.1038/s41586-021-03857-w) reveals that important and well-known previously published data that directly refutes the primary conclusions of Gu. et al. were not discussed.

Main Text

The discovery of electrically conductive filaments emanating from the electroactive microbe *Geobacter sulfurreducens* [1]introduced new possibilities for microbial long-range electron transport of importance to global biogeochemical cycles, interspecies syntrophy, microbial conversion of wastes to useful energy sources, and the fabrication of sustainably produced electronic devices with novel functions[2-6]. The initial report of these filaments[1], as well as many subsequent studies (see[7,8] for recent reviews), have indicated that the most abundant conductive filaments are comprised of the pilin monomer protein PilA, also known as PilA-N. As detailed below, recent direct observation of filaments emanating from cells provides additional evidence that 90 % of *G. sulfurreducens'* extracellular filaments are comprised of PilA-N[9].

Gu et al.[10] state that these earlier conclusions were wrong. They claim that PilA-N combines with the protein PilA-C to form poorly conductive filaments. However, the 6.5 nm diameter PilA-N/PilA-C filaments that Gu et al. studied are artifacts produced by genetically modified cells. Filaments of this diameter have never been observed in wild-type *G. sulfurreducens* [7-9]. Not only do Gu et al. provide no evidence for these filaments in wild-type cells they also fail to discuss the previous contradictory finding that in wild-type cells PilA-C forms a PilA-C trimer that is associated with the inner membrane[11].

The foundation of the Gu et al. study is the initial result reported: "Purified filament preparations from wild-type cells grown under these nanowire-producing conditions did not show either PilA-N or PilA-C using immunoblotting". However, similar previous studies reported PilA-N in filament preparations from wild-type cells, as well as PilA-N antibody reacting with filaments[12-15]. In most studies[12-14] additional strong detergent or acidic conditions were required to recover PilA-N, indicating that PilA-N was a component of a highly stable filament and not a component of more easily disassociated filament types, such as cytochrome-based filaments or the proposed PilA-N/PilA-C filaments. Gu et al. fail to mention these previously published results that directly contradict their primary finding even though the corresponding author of Gu et al. was an author on papers reporting the recovery of PilA-N from filament preparations[13,15].

Gu et al. also neglected to mention additional lines of evidence for the presence of PilA-N in extracellular filaments. For example, *G. sulfurreducens* strains expressing PilA-N with peptide tags display filaments with those tags[16]. Decreasing the aromatic amino acid content of PilA-N variants expressed in *G. sulfurreducens* decreases filament conductivity and increasing the abundance of aromatic amino acids increases conductivity[9,17-21]. One of the most striking examples of such results is the finding that expression of the homologous PilA-N gene from *G. metallireducens* in *G. sulfurreducens* yields individual filaments with the same 3 nm diameter as the wild-type *G. sulfurreducens* filaments, but with a conductivity that is 5000-fold higher[21]. Changing the amino acid content of PilA-N would not influence the conductivity of filaments comprised of cytochromes or DNA that Gu et al. suggest predominate, but would be expected to tune the conductivity of filaments comprised of PilA-N.

Gu et al. state that *G. sulfurreducens* cannot produce filaments from PilA-N because the cells lack the twitching motility attributed to type IV pili in *Pseudomonas aeruginosa* . In making this claim Gu et al. fail to acknowledge the fact that *G. sulfurreducens* ' lack of twitching motility was previously described in the initial report on its electrically conductive pili[1]. Not all bacteria that have type IV pili exhibit twitching motility. The expectation that filaments comprised of PilA-N could only exist if they also conferred twitching motility is not justified.

The fact that deleting the gene for PilA-N impacts on the localization of potential filament-producing *c* -type cytochromes has been known for some time[22] and was the impetus for the construction of *G. sulfurreducens* strain Aro-5, a strain expressing a pilin with reduced aromatic amino acid content while properly localizing outer-surface cytochromes[17]. Strain Aro-5 expresses filaments with a morphology like that of wild-type cells, but the conductivity of individual filaments is orders of magnitude lower[19]. Again, the only logical explanation is that the filaments being investigated are comprised of PilA-N. The conductivity of cytochrome- or DNA-based filaments should not be influenced by a change in the PilA-N sequence.

Direct observation of cells demonstrated that 90% of the filaments emanating from *G. sulfurreducens* are not cytochrome-based filaments, rather they have a morphology and conductance like the filaments that *E. coli* displays when it heterologously expresses the *G. sulfurreducens* PilA-N[9]. The most likely explanation for these observations is that the filaments emanating from *G. sulfurreducens* are also comprised of PilA-N. In strain Aro-5, 90% of the filaments have this PilA-N filament morphology, but their conductance is more than 100-fold lower than the wild-type filaments[9]. This result is also consistent with the filaments being comprised of PilA-N and inconsistent with cytochrome- or DNA-based filaments.

Gu et al. and related studies[15,23] have argued that the 3 nm diameter filaments with the morphology and conductance expected for filaments comprised of PilA-N, which are readily observed emanating from cells[9], do not exist because they were not observed in cryo-EM analysis of *G. sulfurreducens* filament preparations. Capturing these filaments in cryo-EM preparations will be the key to unlocking their likely unique structure and mechanisms for long-range electron transport.

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Author Contributions

All authors contributed to data analysis and the writing of the manuscript.

Competing Interests

The authors declare no competing interests.