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Electrosynthesis of Organic Compounds from Carbon Dioxide Is Catalyzed by a Diversity of Acetogenic Microorganisms[▽]

Kelly P. Nevin,* Sarah A. Hensley, Ashley E. Franks, Zarath M. Summers, Jianhong Ou, Trevor L. Woodard, Oona L. Snoeyenbos-West, and Derek R. Lovley

Department of Microbiology, University of Massachusetts—Amherst, Amherst, Massachusetts

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Microbial electrosynthesis, a process in which microorganisms use electrons derived from electrodes to reduce carbon dioxide to multicarbon, extracellular organic compounds, is a potential strategy for capturing electrical energy in carbon-carbon bonds of readily stored and easily distributed products, such as transportation fuels. To date, only one organism, the acetogen *Sporomusa ovata*, has been shown to be capable of electrosynthesis. The purpose of this study was to determine if a wider range of microorganisms is capable of this process. Several other acetogenic bacteria, including two other *Sporomusa* species, *Clostridium ljungdahlii*, *Clostridium aceticum*, and *Moorella thermoacetica*, consumed current with the production of organic acids. In general acetate was the primary product, but 2-oxobutyrate and formate also were formed, with 2-oxobutyrate being the predominant identified product of electrosynthesis by *C. aceticum*. *S. sphaeroides*, *C. ljungdahlii*, and *M. thermoacetica* had high (>80%) efficiencies of electrons consumed and recovered in identified products. The acetogen *Acetobacterium woodii* was unable to consume current. These results expand the known range of microorganisms capable of electrosynthesis, providing multiple options for the further optimization of this process.

Microbial electrosynthesis, the process in which microorganisms use electrons derived from an electrode to reduce carbon dioxide to multicarbon, extracellular products (30), is a potential strategy for converting electrical energy harvested with renewable strategies, such as solar or wind, into forms that can be stored and distributed on demand within existing infrastructure (22, 30). Storage and distribution is a particular concern for solar energy, because it is a vast energy resource but harvests energy intermittently and not necessarily coincident with peak demand (19). The conversion of electrical energy to covalent chemical bonds may be one of the best storage and distribution options (19). Microbial electrosynthesis powered by solar energy is an artificial form of photosynthesis with the same net overall reaction as plant-based photosynthesis: carbon dioxide and water are converted to organic compounds and oxygen (30). Potential advantages of microbial electrosynthesis over biomass-based strategies for the production of fuels and chemicals include the 100-fold higher efficiency of photovoltaics in harvesting solar energy, eliminating the need for arable land, avoiding the environmental degradation associated with intensive agriculture, and the direct production of desired products (22, 24, 30). However, microbial electrosynthesis is a nascent concept, and much more information on the microbiology of this process is required.

Microbial electrosynthesis depends upon electrotrophy, the ability of some microorganisms to use electrons derived from an electrode as an electron donor for the reduction of a terminal electron acceptor (22). Although the ability of microorganisms to transfer electrons to electrodes has been studied for some time (9, 23), the capacity for electron transfer in the opposite direction, from electrodes to cells, has received less attention. *Geobacter* species are capable of using electrons derived from graphite electrodes for the reduction of a diversity of electron acceptors, including nitrate (12), fumarate (11, 12), U(VI) (13), and chlorinated solvents (37). *Anaeromyxobacter dehalogenans* also can reduce fumarate and reductively dehalogenate 2-chlorophenol (35). A wide diversity of undefined microbial consortia have been inferred to contain microorganisms capable of reducing these and other electron acceptors, including oxygen, with an electrode as the sole electron donor (22).

Mixed cultures produced methane from carbon dioxide with neutral red, reduced at an electrode surface, as the electron donor. *Methanobacterium palustre* has been reported to reduce carbon dioxide to methane with electrode-derived electrons (6), but there have been difficulties in confirming direct electron transfer in methanogens, because the low potentials required for methanogenesis also can produce significant hydrogen (22, 39).

The finding that an acetogenic microorganism, *Sporomusa ovata*, could use electrons derived from graphite electrodes for the reduction of carbon dioxide to acetate (30) provided the proof of concept that it is possible to convert carbon dioxide and water to extracellular, multicarbon products with electricity as the energy source. Biofilms of *S. ovata* growing on electrode surfaces produced acetate and small amounts of 2-oxobutyrate concomitantly with current consumption. Electron recovery in these products exceeded 85%, which is consistent with the reaction $2CO_2 + 2H_2O \rightarrow CH_3COOH + 2O_2$. The fact that carbon dioxide reduction to acetate in acetogens proceeds through acetyl-coenzyme A (CoA) (10) and that acetyl-CoA is a central intermediate for the production of a

* Corresponding author. Mailing address: 106B Morrill 4 North, University of Massachusetts, Microbiology, 639 North Pleasant St., Amherst, MA 01003. Phone: (413) 577-3103. Fax: (413) 577-4660. E-mail: knevin@microbio.umass.edu.

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diversity of useful organic products, including fuels (2), suggests that microbial electrosynthesis with *S. ovata* can be a strategy for storing electrical energy in chemical products (30).

The purpose of the study reported here was to screen a diversity of acetogenic bacteria available in culture to determine whether acetogens other than *S. ovata* were capable of electrosynthesis.

MATERIALS AND METHODS

Source of organisms and culture maintenance. *Sporomusa silvacetica* (DSM 10669), *Sporomusa sphaeroides* (DSM 2875), *Clostridium ljungdahlii* (DSM 13528), *Clostridium aceticum* (DSM 1496), *Moorella thermoacetica* (DSM 21394), and *Acetobacterium woodii* (DSM 1030) were obtained from the Deutsche Sammlung Mikroorganismen und Zellkulturen.

The cultures were routinely grown with H_2-CO_2 (80:20) at 30°C using a standard anaerobic technique unless otherwise noted. *Sporomusa* strains were cultured in DSM medium 311 omitting betaine, fructose, Casitone, and resazurin. *C. ljungdahlii* was cultured at 37°C in DSM medium 879 omitting fructose. *A. woodii* and *C. aceticum* were cultured in DSM medium 135 with resazurin and fructose omitted. *M. thermoacetica* was cultured at 37°C in DSM medium 60 omitting fructose and glucose and reducing the yeast extract to 1 g/liter.

Cathode biofilms. Each culture was grown on at least four cathodes in H-cell culturing systems as previously described (30). In these systems graphite stick cathodes and anodes (65 cm^2; Mersen, Greenville, MI) are suspended in two chambers, each containing 200 ml of media, that are separated with a Nafion 117 cation-exchange membrane (Electrolytica, Amherst, NY). The anode chamber was continually gassed with N_2-CO_2 (80:20). A potentiostat provides the energy to extract electrons from water at the anode and poise the cathode at −400 mV (versus a standard hydrogen electrode). This provides electrons at a sufficiently low potential for microbial electrosynthesis without the significant production of hydrogen (30). No organic products were produced in the absence of microorganisms. Hydrogen-grown cultures were inoculated into the cathode chamber, containing the medium appropriate for the organism described above, and the culture was bubbled with a hydrogen-containing gas mixture (N_2-CO_2-H_2; 83:10:7) as the electron donor to promote the growth of a biofilm on the cathode surface. As previously described (30), the medium was replaced several times to remove planktonic cells, and then the gas phase was switched to N_2-CO_2 (80:20). For those cultures capable of current consumption, current consumption was observed within 24 h, and at this point fresh medium maintained under N_2-CO_2 was continuously introduced (0.1 ml/min; dilution rate, 0.03 h^{-1}) with a peristaltic pump as previously described (29, 32).

Analytical methods. Acetate and other organic acids were measured via high-performance liquid chromatography (HPLC) (29). Organic acids were separated on an Aminex NPX-87H column with 8 mM H_2SO_4 as the eluent and detected at 210 nm with a detection limit of ca. 5 μM acetate, formate, and 2-oxobutryate. Hydrogen was measured with a Trace Analytical Model ta3000R gas analyzer (Ametel Process Instruments, Newark, DE).

Biofilms were visualized with confocal laser-scanning microscopy using LIVE/DEAD BacLight viability stain (29, 32). Samples of graphite electrode were prepared for scanning electron microscopy as previously described (1) using hexamethyldisilazane after ethanol dehydration to remove all remaining liquids from the sample. Scanning electron microscopy was performed with a Field Emission Gun SEM, model JEOL JSM 6320F. Protein was measured with the bicinchoninic acid method (Sigma, St. Louis, MO) as previously described (29).

RESULTS AND DISCUSSION

The previous finding that *Sporomusa ovata* was capable of electrosynthesis led to the evaluation of two additional species of *Sporomusa*, *S. sphaeroides* and *S. silvacetica*. Both *Sporomusa* species consumed current (Fig. 1) and formed thin biofilms on the cathode surface that were similar to those previously reported for *S. ovata* reducing carbon dioxide as the sole electron acceptor (30). Cells stained green with LIVE/DEAD stain, suggesting that they were metabolically active, even after extended incubation.

S. sphaeroides produced primarily acetate during current consumption (Fig. 1A). Of the electrons consumed, 84% ± 26% (means ± standard deviations; $n = 3$) were recovered in acetate. The rate at which *S. sphaeroides* consumed current was ca. 20-fold slower than that previously reported for *S. ovata*. *S. silvacetica* produced primarily acetate, with trace accumulations of 2-oxobutyrate (Fig. 1B). The recovery of electrons in acetate and 2-oxybutrate was only 48% ± 6%. This low rate of recovery is attributed to the production of other products that have yet to be identified, because peaks were observed in HPLC analysis that could not be attributed to any of a wide range of potential products/metabolites. Rates of current consumption for *S. silvacetica* were better than those of *S. sphaeroides* but still were only about 10% of those of *S. ovata*. These results demonstrate that the capacity for electrosynthesis can vary significantly within a single genus.

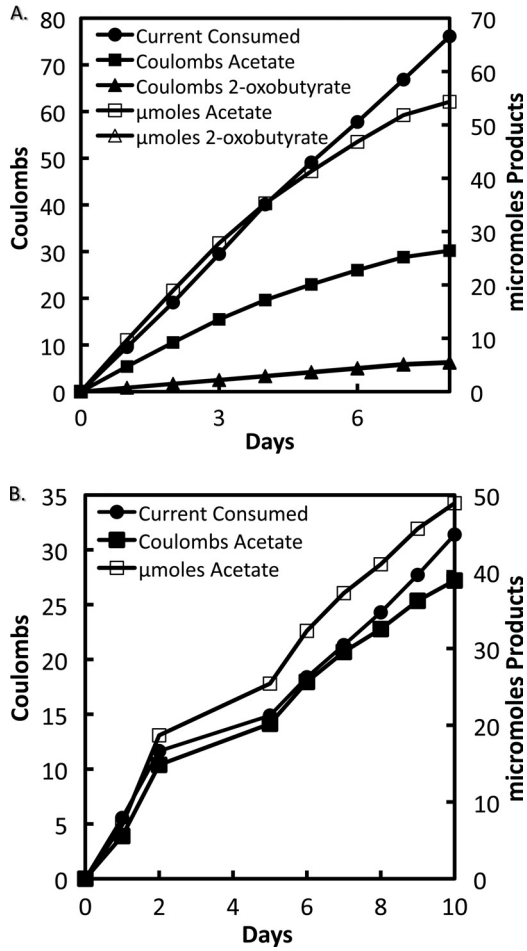

FIG. 1. Electron consumption and product formation over time with *Sporomusa sphaeroides* (A) and *Sporomusa silvacetica* (B). Results shown are from a representative example of three replicate cultures.

Although *Sporomusa* species are within the *Clostridium* phylum (5), they are Gram negative (28), as are the *Geobacter* (12, 13, 37) and *Anaeromyxobacter* (35) species that previously have been shown to accept electrons from electrodes. However, a diversity of Gram-positive microorganisms have the capacity to produce current in microbial fuel cells (26, 27, 31, 40), demonstrating that it is possible for Gram positives to establish electrical connections with electrodes. Therefore, the possibil-

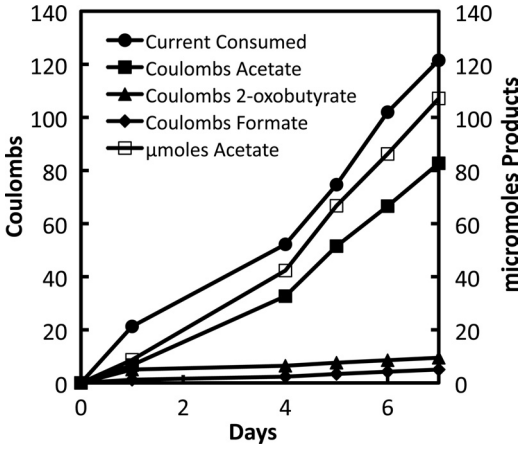

FIG. 2. *Clostridium ljungdahlii* electron consumption and product formation over time. Results shown are from a representative example of three replicate cultures.

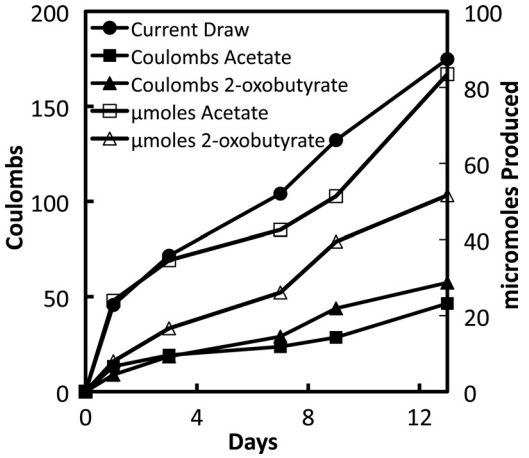

FIG. 4. *Clostridium aceticum* electron consumption and product formation over time. Results shown are from a representative example of two replicate cultures.

ity that Gram-positive acetogens reduce carbon dioxide with an electrode as the sole electron donor was evaluated.

Clostridium ljungdahlii consumed current with a concomitant accumulation of acetate and the minor production of formate and 2-oxobutyrate over time (Fig. 2). Electron recovery in these products accounted for 82% ± 10% (n = 3) of the electrons consumed, with 88% ± 2% of the electrons in these products appearing in acetate. Scanning electron microscopy (Fig. 3A) and confocal laser-scanning microscopy (Fig. 3B) revealed a thin layer of metabolically active cells on the cathode surface similarly to the cathode biofilms of the *Sporomusa* strains.

Clostridium aceticum consumed current more slowly than *C. ljungdahlii* (Fig. 4). Unlike any of the other cultures evaluated, 2-oxobutyrate was as important a product as acetate. The recovery of electrons consumed in acetate and 2-oxobutryate was low (53% ± 4%; n = 2). This poor recovery is attributed to the formation of other products that have yet to be identified. As with the other strains evaluated, only a thin biofilm developed on the cathode surface.

Moorella thermoacetica was able to consume current with the production of mainly acetate (Fig. 5). The electron recovery was 85% ± 7% (n = 3).

Acetobacterium woodii was the only acetogen tested that appeared unable to accept electrons from an electrode. Although *A. woodii* grew well in the cathode chamber when hydrogen was provided as an electron donor, more than 10 attempts to establish cultures with the cathode as the electron donor failed.

Mechanisms for electron transfer and energy conservation. Mechanisms for microbe-electrode interactions can best be critically evaluated with detailed genetic studies (4, 16, 30, 36),

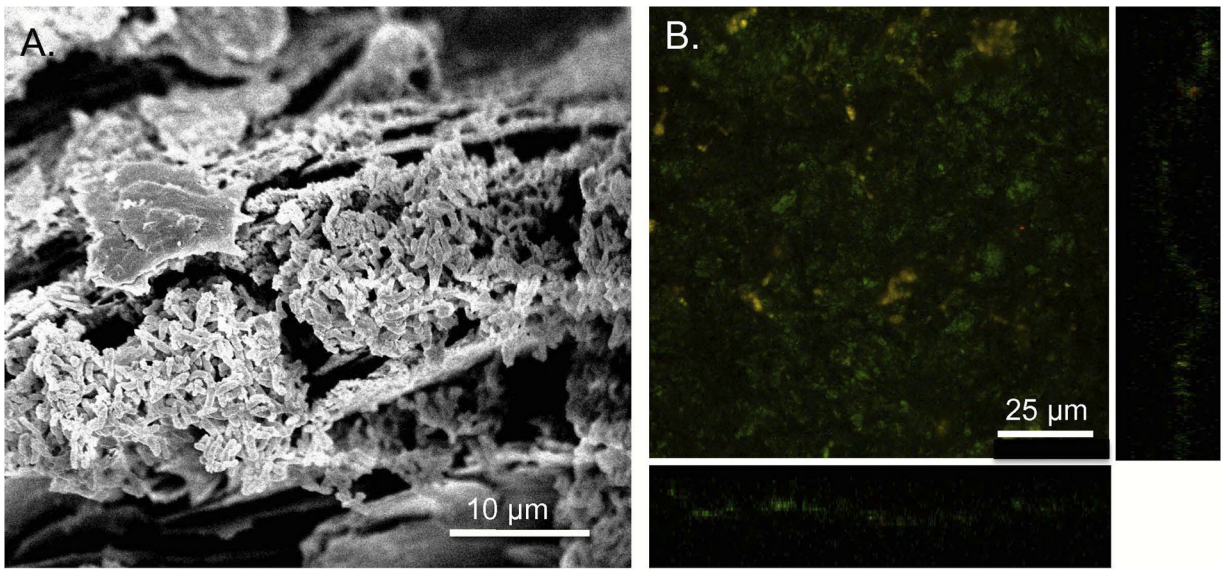

FIG. 3. Microscopy of *Clostridium ljungdahlii* after 14 days. Scanning electron micrograph (A) and top-down and side-view confocal laser scanning micrograph (B) of LIVE/DEAD BacLight viability-stained biofilm.

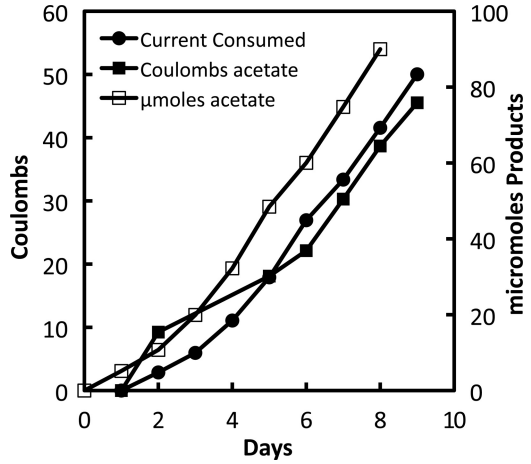

FIG. 5. *Moorella thermoacetica* electron consumption and product formation over time. Results shown are from a representative example of three replicate cultures.

which have not yet been carried out on any microorganisms capable of electrosynthesis. However, as was genetically verified for cathode electron transfer with *G. sulfurreducens* (12), it does appear that hydrogen is not an intermediary electron carrier between the cathode and the cells. As in previous studies (30), there was no accumulation of hydrogen (<10 ppm) with poised cathodes in the absence of microorganisms. Low, steady-state concentrations (10 to 100 ppm) of hydrogen were detected when cells were consuming current. This is attributed to the fact that metabolically active anaerobic microorganisms with hydrogenases produce hydrogen to levels that reflect the redox status of the cells (7, 21). These hydrogen levels were well below the >400 ppm that acetogenic microorganisms require for acetogenesis (8). Further evidence for a lack of hydrogen production was the finding that *A. woodii*, which was able to reduce carbon dioxide with hydrogen as the electron donor in the cathode chamber, did not metabolize once the hydrogen was removed.

Many potential mechanisms for microorganisms to accept electrons from cathodes have been proposed (17, 22, 34) based primarily on better established concepts for electron transfer in the reverse direction, i.e., from electrodes to cells. However, the only experimental study on the proteins that might be involved in electron transfer at the cathode (36) has indicated that mechanisms for electron transfer from the cathode to microorganisms is much different than electron transfer from microorganisms to an anode. We are currently developing genetic approaches to better evaluate electron transfer during electrosynthesis.

The inability of *A. woodii* to function on the cathode is consistent with a working model for how acetogenic microorganisms conserve energy with electrons directly derived from cathodes serving as the electron donor (22). In this model, the reduction of carbon dioxide to organic acids in the cytoplasm consumes protons, generating a proton gradient, and ATP is generated with proton-dependent ATPases. *A. woodii* would not be able to conserve energy in this manner because it contains sodium-dependent ATPases (14, 33).

Outlook for electrosynthesis. These results demonstrate that a wide diversity of microorganisms are capable of reducing carbon dioxide to organic acids with electrons derived from an electrode. Such proof-of-concept studies are needed, because microbial electrosynthesis has the potential to be an environmentally sustainable approach for the large-scale production of fuels and other chemicals from carbon dioxide (22, 30). However, substantial optimization will be required. The rates of electron transfer reported here are comparable to those in earlier studies on current production in microbial fuel cells fashioned from the same H-cell devices (3). Transforming microbial electrosynthesis to a practical process is likely to require a combination of improved reactor and material design to enhance electron transfer.

Electrodes are not natural extracellular interfaces for microorganisms (20). Adaptive evolution has proven to be an effective strategy for improving the rates of electron exchange between microorganisms and external electron acceptors (38, 41) and could be a strategy for improving the current-consuming capabilities of microbes capable of microbial electrosynthesis. Furthermore, sequencing the genomes of adapted strains can provide insights into the mechanisms of extracellular electron exchange (38).

Generating products other than acetate probably will require modifying metabolic pathways of electrosynthesis microorganisms. *C. ljungdahlii*, which, as shown here, is capable of electrosynthesis, already has been engineered to produce small amounts of butanol (18) and may be suitable for large-scale biofuel production (15). Genome-scale modeling and analysis can rapidly enhance the understanding of understudied microorganisms (25) and is likely to be key to optimizing microbial electrosynthesis.

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