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Isolation of Acetogenic Bacteria That Induce Biocorrosion by Utilizing Metallic Iron as the Sole Electron Donor

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Corrosion of iron occurring under anoxic conditions, which is termed microbiologically influenced corrosion (MIC) or biocorrosion, is mostly caused by microbial activities. Microbial activity that enhances corrosion via uptake of electrons from metallic iron [Fe(0)] has been regarded as one of the major causative factors. In addition to sulfate-reducing bacteria and methanogenic archaea in marine environments, acetogenic bacteria in freshwater environments have recently been suggested to cause MIC under anoxic conditions. However, no microorganisms that perform acetogenesis-dependent MIC have been isolated or had their MIC-inducing mechanisms characterized. Here, we enriched and isolated acetogenic bacteria that induce iron corrosion by utilizing Fe(0) as the sole electron donor under freshwater, sulfate-free, and anoxic conditions. The enriched communities produced significantly larger amounts of Fe(II) than the abiotic controls and produced acetate coupled with Fe(0) oxidation prior to CH_4 production. Microbial community analysis revealed that *Sporomusa* sp. and *Desulfovibrio* sp. dominated in the enrichments. Strain GT1, which is closely related to the acetogen *Sporomusa sphaeroides*, was eventually isolated from the enrichment. Strain GT1 grew acetogenetically with Fe(0) as the sole electron donor and enhanced iron corrosion, which is the first demonstration of MIC mediated by a pure culture of an acetogen. Other well-known acetogenic bacteria, including *Sporomusa ovata* and *Acetobacterium* spp., did not grow well on Fe(0). These results indicate that very few species of acetogens have specific mechanisms to efficiently utilize cathodic electrons derived from Fe(0) oxidation and induce iron corrosion.

Corrosion of iron structures causes great economic losses and environmental pollution. Iron corrosion is an electrochemical process involving oxidation of metallic iron [Fe(0)] to Fe(II) (anodic reaction; equation 1) and reduction of external electron acceptors (cathodic reaction):

$$Fe^{2+} + 2e^- \leftrightarrow Fe(0);\ E_0' = -0.47\ V \quad (1)$$

where −0.47 V references the standard hydrogen electrode (SHE). The cathodic reaction consists of oxygen reduction in the presence of air (equation 2), while it usually consists of proton reduction (H_2 evolution) under anoxic conditions (equation 3):

$$O_2 + 4e^- + 2H_2O \leftrightarrow 4OH^-;\ E_0' = +0.82\ V \quad (2)$$

$$2H^+ + 2e^- \leftrightarrow H_2;\ E_0' = -0.41\ V \quad (3)$$

where +0.82 V and −0.41 V reference the SHE.

The cathodic hydrogen evolution on iron surfaces is usually a particularly slow reaction under neutral pH conditions, because proton availability is limited and the reaction has low electrode potential and high overpotential. Hence, theoretically, iron corrosion in anoxic environments should not be a serious problem. However, iron corrosion in anoxic environments has been reported often and, in most cases, it is thought to be mediated by metabolic activities of microorganisms therein (1–3). Iron corrosion that occurs in this manner is termed microbiologically influenced corrosion (MIC) or biocorrosion. Diverse kinds of microorganisms, including sulfate-reducing bacteria (SRB), Fe(II) oxidizers, Fe(III) reducers, fermenting bacteria, and methanogens, have been reported as contributing to MIC (4–9). These microorganisms induce MIC in a number of ways, including formation of redox/chemical gradient on the iron surfaces, production of corrosive chemicals (e.g., H_2S and organic acids), degradation of protective coatings on iron surfaces, and acceleration of cathodic reactions (10).

Among such diverse MIC mechanisms, acceleration of cathodic reactions, often referred to as cathodic depolarization, was first proposed in the 1930s and has been considered one of the most prominent causes of MIC (3). Cathodic depolarization is induced by microorganisms utilizing electrons in Fe(0) as the electron donor, resulting in acceleration of the cathodic reaction and hence the overall corrosion process. Microorganisms consuming H_2 abiotically generated on iron surfaces (equation 3) coupled with sulfate reduction (equation 4) or methanogenesis (equation 5) have long been considered agents of cathodic depolarization (11, 12):

$$SO_4^{2-} + 9H^+ + 8e^- \leftrightarrow HS^- + 4H_2O;\ E_0' = -0.22\ V \quad (4)$$

$$HCO_3^- + 9H^+ + 8e^- \leftrightarrow CH_4 + 3H_2O;\ E_0' = -0.24\ V \quad (5)$$

where −0.22 V and −0.24 V reference the SHE.

However, recent studies demonstrated that only microorganisms with special mechanisms to take up electrons from Fe(0)

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have the ability to induce MIC. Dinh et al. reported that novel SRB and methanogens enriched and isolated with Fe(0) as the sole electron donor reduced sulfate and produced methane, respectively, at much higher rates than were seen with abiotic H_2 production from Fe(0), while authentic H_2 scavengers did not show such activities (13). After that, two different methanogenic strains with an increased ability to induce MIC compared to the closely related hydrogenotrophic strains were isolated (7, 14). Physiological and electrochemical analyses have disclosed that the MIC-inducible SRB strains appear to take up electrons directly from Fe(0) rather than consuming abiotically generated H_2, although the molecular mechanisms remain unknown (15–17). The MIC induced by direct consumption of cathodic electrons was specially termed electrical MIC (EMIC) and was considered the major causal factor of cathodic depolarization (15–17).

All EMIC-inducing microorganisms isolated so far were from marine environments (7, 13, 14). Although MIC is also problematic in freshwater and terrestrial soil environments (12, 18–21), knowledge on MIC-inducible freshwater microorganisms has been limited. In addition to methanogens, microorganisms that conserve energy through acetogenesis (equation 6) have been proposed to engage in EMIC in freshwater environments (22):

$$2HCO_3^- + 9H^+ + 8e^- \leftrightarrow CH_3COO^- + 4H_2O; \quad E_0' = -0.28\ V \quad (6)$$

where −0.28 V references the SHE.

Acetogenesis-dependent MIC was recently demonstrated to induce biocorrosion in sulfate-free freshwater environment under anoxic conditions (23). However, acetogenesis-dependent MIC has been observed in cultures of complex microbial communities. Pure cultures that perform acetogenesis-dependent MIC have not been isolated, and the mechanisms for acetogenesis-dependent MIC remain unknown.

In the present study, we enriched for MIC-inducing microorganisms by culturing rice paddy field soil microbial communities in a sulfate-free freshwater medium with Fe(0) granules as the sole electron donor. Further enrichment was made in the presence of methanogen inhibitor with the hope of observing MIC concomitantly with acetogenesis. We found that acetogenesis was coupled with MIC and eventually isolated the organisms responsible for acetogenesis-dependent MIC.

MATERIALS AND METHODS

Enrichment cultures of iron-corroding microbial communities. Microbial communities were enriched in serum bottles (68 ml in capacity) filled with 20 ml of a freshwater basal medium containing the following ingredients (per liter): 0.3 g of KH_2PO_4; 1 g of NH_4Cl; 0.1 g of $MgCl_2 \cdot 7H_2O$; 0.08 g of $CaCl_2 \cdot 7H_2O$; 0.6 g of NaCl; 2 g of $KHCO_3$; 9.52 g of HEPES; 0.03 g of $Na_2S \cdot 9H_2O$; 0.1 g yeast extract; and 10 ml each of trace metal solution and vitamin solution (24). The medium pH was adjusted to 7.0 and kept constant during incubation (pH 7.0 ± 0.2). For the enrichments of the iron-corroding and H_2-oxidizing microbial community, 1 g of iron granules (Alfa Aesar) (1 to 2 mm in diameter, 99.98% purity) and 200 kPa of H_2:CO_2 (80:20), respectively, were supplemented as the sole electron donor. Approximately 50 mg (wet weight) of rice paddy field soil was inoculated as a source of microorganisms. Unless stated otherwise, the cultivations were conducted at 30°C under an atmosphere of N_2:CO_2 (80:20) without shaking. When methanogenesis reached a plateau, 0.5 ml of culture solution was transferred to the fresh media. After five passages, the enrichment cultures were subjected to chemical and phylogenetic analyses.

Isolation of acetogenic bacteria. Cultures of Fe(0) and Fe(0)-plus-2-bromoethanesulfonate [Fe(0)+BES] enrichments were subjected to vigorous vortex mixing to detach microbial cells from iron granules. The culture solution was serially diluted with the freshwater basal medium and spread onto the same medium solidified with 0.6% (wt/vol) gellan gum. Lactate (10 mM) or ethanol (10 mM) or methanol (10 mM) or H_2:CO_2 (80:20) was supplemented as the carbon and energy source(s). All procedures were conducted in an anaerobic chamber filled with N_2 gas. The inoculated plates were incubated under anaerobic conditions using an AnaeroPack pouch bag (Mitsubishi Gas Chemical) with an AnaeroPack oxygen absorber at 30°C. Grown colonies were further purified by repetitive plating. The purity of isolates was confirmed by microscopic observation.

Pure culture experiments for iron corrosion. *Sporomusa sphaeroides* DSM2875, *Sporomusa ovate* DSM2662, *Acetobacterium carbinolicum* DSM2925, and *Acetobacterium woodii* DSM1030 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). These strains and isolated acetogens were routinely cultivated in the freshwater basal medium supplemented with 20 mM trimethylglycine under anaerobic conditions at 30°C. For the test of biocorrosion, all strains were cultivated in the freshwater basal medium supplemented with 1 g of iron granules as the sole electron donor.

Phylogenetic analyses. Microbial DNA was extracted with a Fast DNA Spin kit for soil (MP Biomedicals) according to the manufacturer's instructions. The whole lengths of 16S rRNA gene sequences of isolated strains were determined by direct sequencing of the DNA fragment amplified by PCR with primer pair 27F and 1492R as described previously (25). PCR amplification of 16S rRNA gene fragments for clone library analyses was conducted as described previously (26), with primer pair U515f and U1492r for bacteria and primer pair A25f and A958r for archaea (27). The sequences obtained were assigned to each phylotype with a cutoff value of 97% identity using FastGroup II software (28). Classification of phylotypes was performed using the Classifier program in the Ribosomal Database Project database (29). The sequence of each phylotype was compared with those in the GenBank nucleotide sequence database using the BLAST program (30) to infer the most closely related species.

Chemical analyses. The partial pressure of H_2 and CH_4 was determined using a gas chromatograph (GC-2014; Shimadzu) as described previously (31). The concentration of acetate was determined using a high-performance liquid chromatography system (D-2000 LaChrom Elite HPLC system; Hitachi) equipped with an Aminex HPX-87H ion exclusion column (Bio-Rad) and an L2400 UV detector (Hitachi). Ferrous iron in the whole cultures, including iron granules and microbiologically produced mineral particles, was dissolved by 0.67 M HCl (containing 0.67 M hexamethylenetetramine to avoid dissolution of metallic iron) and quantified colorimetrically using a ferrozine method as described elsewhere (32).

Nucleotide sequence accession numbers. The nucleotide sequence data reported in this study have been submitted to GenBank under accession no. AB854327 to AB854356 and AB981438.

RESULTS AND DISCUSSION

Enrichment of MIC-inducing microorganisms with Fe(0) as the sole electron donor. Iron-corroding microorganisms were enriched from rice paddy field soil using a sulfate-free freshwater medium supplemented with Fe(0) granules as the sole electron donor under an N_2/CO_2 gas atmosphere [here designated Fe(0) enrichments]. The medium contained only CO_2 and H^+ as available electron acceptors. After several weeks of cultivation, CH_4 generation was observed, the surface of the iron granules turned a dull-gray color, and grayish precipitate was formed (Fig. 1). CH_4 production and corrosion products were not observed in the abiotic controls (without inoculation of soil) and cultures without iron granules. These observations demonstrated that growth of microorganisms utilizing Fe(0) as the sole electron donor occurred in the Fe(0) enrichments and that most of the reducing equivalents for the CH_4 generation were derived from Fe(0) oxidation.

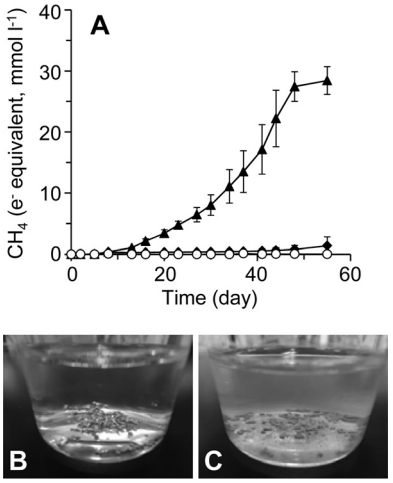

FIG 1 Corrosion and methanogenesis in the cultures of the soil microbial community with Fe(0) granules. (A) Generation of CH_4 was monitored during the incubation of a paddy soil microbial community in the presence (filled triangles) and absence (filled diamonds) of Fe(0) granules. The data of the abiotic controls (inoculated with sterilized soil) are also shown (open circles). Data are presented as the means of the results of three independent cultures, and error bars represent standard deviations. mmol l^{-1}, millimoles per liter. (B and C) The photographs were taken after 50 days of incubation of the abiotic control (B) and paddy soil microbial community (C) in the presence of Fe(0) granules.

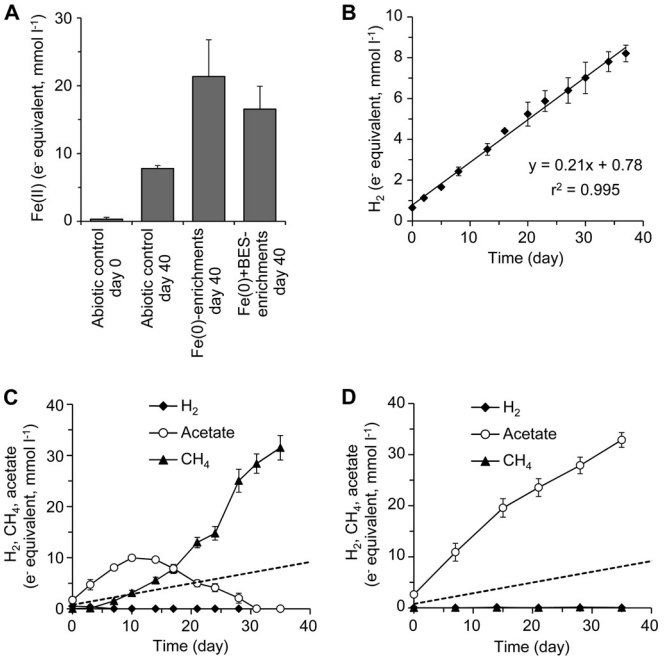

FIG 2 Metabolites generated in the enrichment cultures. (A) Generation of Fe(II) from Fe(0) granules by the enrichment cultures and in the abiotic controls. (B) Generation of H_2 in the abiotic controls with Fe(0) granules. The approximation curve estimated by the least-squares method is given. (C and D) Acetate, CH_4, and H_2 production in the Fe(0) (C) and Fe(0)+BES (D) enrichments. The dashed lines represent the abiotic corrosion rate calculated from the abiotic H_2 production rate shown in panel B. Data are presented as the means of the results of three independent cultures, and error bars represent standard deviations.

Metabolic profiles of the enrichment cultures. After five successive subcultures, the metabolic products in the Fe(0) enrichments were analyzed. For ease of comparison, the amounts of all metabolites were represented as electron equivalents in the unit of mmol e^- per liter of culture medium, using the respective half-reaction formulas (equations 1 to 6). To confirm the occurrence of MIC, the amounts of Fe(II) were measured after the cultivation of the Fe(0) enrichments (Fig. 2A). The amount of HCl-extractable Fe(II) in the Fe(0) enrichment cultures was significantly greater than that of the Fe(II) generation in the abiotic control, indicating that microorganisms in the Fe(0) enrichments have the ability to induce MIC. It should be noted that the value of Fe(II) generation in the Fe(0) enrichment cultures may be underestimated, since some grayish precipitate remained even after the acid treatments.

The amounts of H_2, CH_4, and organic compounds were measured to infer the microbial metabolisms causing MIC. Acetate was the only organic compound detected from the enrichment cultures throughout this study. Continuous generation of H_2 was observed during incubation of the abiotic control (Fig. 2B). Since the reducing equivalents for the H_2 generation should be derived from chemical oxidation of Fe(0), the abiotic corrosion rate under the experimental conditions was calculated to be 0.21 ± 0.01 mmol e^- equivalent liter^{-1} day^{-1}.

In contrast, instead of H_2 generation, production of CH_4 and acetate was observed in the Fe(0) enrichment cultures (Fig. 2C). The amount of acetate gradually increased and peaked at day 10, followed by a gradual decrease. CH_4 was produced after acetate accumulation. The acetate production rate during the first 10 days (0.83 ± 0.04 mmol e^- equivalent liter^{-1} day^{-1}) was nearly 4 times higher than the abiotic corrosion rate. These observations imply that acetate production with Fe(0) as the electron donor was the main metabolic process occurring in the Fe(0) enrichments during at least the first 10 days.

The Fe(0) enrichments were further enriched in the presence of a specific inhibitor of methanogens to confirm a role of acetogenesis for MIC [here designated Fe(0)+BES enrichments]. While methanogenesis was inhibited completely in the presence of 5 mM BES, the acetate concentration gradually increased (Fig. 2D). The acetate production rate (1.13 ± 0.12 mmol e^- equivalent liter^{-1} day^{-1}) was more than 5 times higher than the abiotic corrosion rate. The amount of Fe(II) generated by the Fe(0)+BES enrichments after 40 days of incubation was also significantly higher than that seen with the abiotic control (Fig. 2A). These results clearly demonstrated that acetogenesis with Fe(0) as the electron donor is the main causative factor for MIC observed in the enrichment cultures.

Microbial community analysis of the enrichment cultures. Microbial community analysis was conducted to identify the microbial species contributing to MIC. As a control, enrichment cultures with H_2 as the sole electron donor, designated H_2 enrichments, were separately constructed with microbes derived from rice paddy field soil, and consumption of H_2 and concomitant generation of both CH_4 and acetate were observed (see Fig. S1 in the supplemental material). The 16S rRNA gene fragments were amplified by PCR from genomic DNA extracted from the H_2, Fe(0), and Fe(0)+BES enrichments using universal primers targeting either the bacterial or archaeal counterparts, cloned, sequenced, and subjected to phylogenetic analysis. The summarized features of the bacterial and archaeal community analyses are presented in Fig. 3. All phylotypes detected from each enrichment culture are listed in Tables S1 and S2 in the supplemental material.

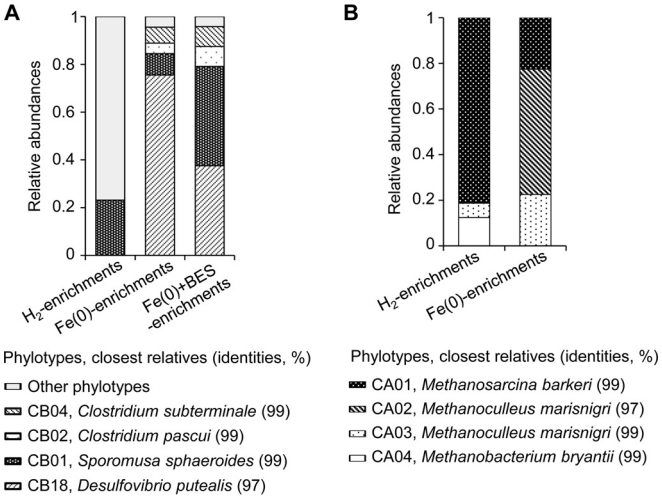

FIG 3 Phylogenetic distribution of bacterial (A) and archaeal (B) 16S rRNA gene clones recovered from the H_2, Fe(0), and Fe(0)+BES enrichments.

Relatively simple bacterial populations were found in the Fe(0) and Fe(0)+BES enrichments, in which most of the phylotypes are affiliated with either the phylum *Proteobacteria* or the phylum *Firmicutes*. All *Proteobacteria* clones recovered from the Fe(0) and Fe(0)+BES enrichments were classified into the CB18 phylotype, whose closest relative is *Desulfovibrio putealis* (97% identity) (Fig. 3A; see also Table S1 in the supplemental material). This phylotype was not detected from the H_2 enrichments. *D. putealis* has been reported to be a typical SRB and has not been examined for acetogenic activity (33). The enrichment cultures were sulfate free, and the major energy metabolism occurring in the Fe(0) and Fe(0)+BES enrichments was most likely Fe(0)-dependent acetogenesis. Some autotrophic SRB, including *Desulfovibrio* spp., harbor the enzymatic system for the Wood-Ljungdahl pathway, which is required for acetogenesis, as a carbon fixation pathway (34, 35). Also, *Desulfovibrio* spp. have often been detected as the major microbial constituent in sulfate-free environments with H_2 or cathodic electrodes as the sole electron donor (36–38). These reports support the possibility that the *D. putealis*-like phylotype contributes to the acetogenesis-dependent MIC observed in the Fe(0) and Fe(0)+BES enrichments.

The most abundant *Firmicutes* phylotype detected from the Fe(0) and Fe(0)+BES enrichments was the CB01 phylotype, which is closely related to *Sporomusa sphaeroides* (99% identity) (Fig. 3A; see also Table S1 in the supplemental material). Since *Sporomusa* spp. are typical acetogenic bacteria (39), the CB01 phylotype is also assumed to engage in acetogenesis-dependent MIC. The other major *Firmicutes* phylotypes detected from the Fe(0) and Fe(0)+BES enrichments, phylotypes CB02 and CB04, were affiliated with *Clostridium* cluster I and are closely related to *Clostridium pascui* (99% identity) and *Clostridium subterminale* (99% identity), respectively (Fig. 3A; see also Table S1). While *C. pascui* and *C. subterminale* are nonsaccharolytic, amino-acid-fermenting bacteria and their acetogenic activity has not been reported (40), *Clostridium* cluster I contains some acetogenic species such as *Clostridium magnum* and *Clostridium drakei* (41). Also, stable-isotope-labeling studies of H_2/$^{13}CO_2$-fed soil microbial communities demonstrated that *Clostridium* cluster I bacteria function as dominant acetogens in natural environments (42). While the *Clostridium*-like phylotypes detected in this study are assumed to grow on amino acids derived from supplemented yeast extract and/or produced by acetogenic bacteria, the possibility of their contribution to acetogenesis-dependent MIC cannot be excluded.

Only 4 archaeal phylotypes were recovered from the H_2 and Fe(0) enrichments (Fig. 3B; see also Table S2 in the supplemental material). The CA01 phylotype (99% identity to *Methanosarcina barkeri*) was the only phylotype classified as an aceticlastic methanogen. This phylotype dominated in the H_2 and Fe(0) enrichments and was the phylotype most likely to contribute to conversion of acetate into CH_4. In the Fe(0) enrichments, the ratio of hydrogenotrophic and aceticlastic methanogens was significantly higher than in the H_2 enrichments (Fig. 3B). The CA02 phylotype (97% identity to *Methanoculleus marinigri*) dominated in the Fe(0) enrichments, while this phylotype was not detected in the H_2 enrichments. This observation suggests that the CA02 phylotype contributed to methanogenesis-dependent MIC in the Fe(0) enrichments. Further studies needed to be done to confirm the assumption.

Isolation of acetogenic bacteria from the enrichment cultures. After enrichment, acetogenic bacteria were isolated from the enrichment cultures. Samples of the Fe(0) and Fe(0)+BES enrichments were serially diluted and inoculated onto the gellan gum-solidified freshwater basal medium supplemented with lactate, ethanol, methanol, or H_2:CO_2 as the carbon and energy sources. Colonies appeared within 1 week of incubation with all tested substrates and were transferred to the same medium for further purification. After purification, the partial 16S rRNA gene sequences of >20 strains were determined. Among the isolates, 3 strains had nearly identical sequences (>99.5% identity to each other), and their sequences are closely related to those of both *S. sphaeroides* DSM2875 and the CB01 phylotype that dominated in the Fe(0) and Fe(0)+BES enrichment (>99.5% identity). One of the isolates, designated strain GT1, was selected as a representative strain for further experiments. The 16S rRNA gene sequence of strain GT1, containing a continuous stretch of 1,446 nucleotides (nt), was determined. The sequence-similarity calculations indicated that strain GT1 is closely related to *S. sphaeroides* DSM2875 (99.7% identity).

Acetogenesis-dependent MIC by the isolated strain and authentic acetogens. The isolate (*Sporomusa* sp. GT1) and type strains of known acetogenic bacteria (*S. sphaeroides*, *S. ovata*, *A. carbinolicum*, and *A. woodii*) were cultivated in the freshwater basal medium supplemented with Fe(0) granules as the sole electron donor to evaluate their ability for acetogenesis-dependent MIC (Fig. 4). The acetate production rates of pure cultures of *Sporomusa* sp. GT1 (2.08 ± 0.23 mmol e^- equivalent liter^{-1} day^{-1}) and its closest relative, *S. sphaeroides* (1.71 ± 0.11 mmol e^- equivalent liter^{-1} day^{-1}), were nearly 10 and 8 times higher than the abiotic corrosion rate, respectively (Fig. 4A). The amounts of Fe(II) generated by *Sporomusa* sp. GT1 and *S. sphaeroides* were also significantly higher than that of the abiotic control (Fig. 4B). These results clearly demonstrated that *Sporomusa* sp. GT1 and *S. sphaeroides* have the ability to induce corrosion by utilizing Fe(0) as the electron donor for acetogenesis.

In contrast, the acetate production rates of pure cultures of the other acetogenic species (*S. ovata*, *A. carbinolicum*, and *A. woodii*) (0.27 to 0.48 mmol e^- equivalent liter^{-1} day^{-1}) were only slightly higher than the abiotic corrosion rate, and no significant differences were observed for Fe(II) generation compared to the abiotic

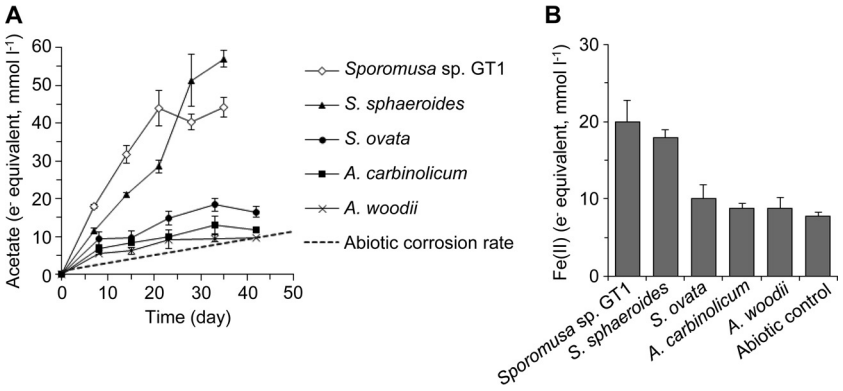

FIG 4 Acetate (A) and Fe(II) (B) production from Fe(0) granules by pure cultures of acetogenic bacteria. The dashed line in panel A represents the abiotic corrosion rate calculated from the abiotic H_2 production rate shown in Fig. 2B. Data are presented as the means of the results of three independent cultures, and error bars represent standard deviations.

control results (Fig. 4). Mand et al. reported that the type strain of *A. woodii*, a close relative of the dominant acetogens in their iron-corroding enrichment cultures, did not enhance corrosion by the pure culture (23). Mori et al. reported that 11 strains of H_2-consuming acetogens isolated from oil facilities, mostly affiliated with the genus *Acetobacterium*, did not show significant enhancement of iron corrosion (14). Taking the data together, consumption of H_2 derived from chemical oxidation of Fe(0) is not sufficient for acetogenesis-dependent MIC, as reported for sulfidogenesis- and methanogenesis-dependent MIC (7, 13–17).

It has been reported that typical H_2-consuming SRBs and methanogens closely related to MIC-inducing strains do not enhance iron corrosion (7, 13, 14), indicating that MIC-inducing microorganisms have specific mechanisms to efficiently take up cathodic electrons derived from Fe(0) oxidation. The proposed mechanism is direct uptake of electrons from solid compounds, including Fe(0). Venzlaff et al. and Lohner et al. reported that a MIC-inducing SRB (*Desulfobacterium corrodens*) and a methanogen (*Methanococcus maripaludis*), respectively, appear to have the ability to directly take up electrons from polarized electrodes in a manner independent of abiotical H_2 generation on the electrode surfaces (16, 43). However, the molecular mechanisms for direct electron uptake from solid compounds and its engagement in iron corrosion remained unclear. The present study demonstrated a similar phenomenon for acetogenesis-dependent MIC. Only limited strains of acetogens were MIC inducible, while other hydrogen-consuming acetogens were not. Corrosion-inducing acetogens found in this study should also have specific mechanisms for efficient uptake of electrons from insoluble materials such as Fe(0). Nevin et al. reported that some strains of genus *Sporomusa*, including the type strain of *S. sphaeroides*, generate acetate with poised cathodic electrodes as the sole electron donor (44), indicating the some *Sporomusa* spp. have the ability to effectively take up electrons from extracellular solid materials. Comparison of MIC-inducing and noninducing strains of *Sporomusa* spp. found in this study, with respect to genomics, transcriptomics, and electrochemistry, will shed light on the molecular mechanisms of electron transfer from solid compounds to acetogenic bacteria.

Ecological implications. From the physiological and evolutionary viewpoints, utilization of Fe(0) as an energy source is an enigmatic ability, since almost all Fe(0) has been recently introduced into environments by human activities. One plausible explanation is that the utilization of electrons in Fe(0) is due to the promiscuous usage of other metabolic systems that take up electrons from naturally occurring solid materials. Our group recently demonstrated that naturally occurring, (semi)conductive iron minerals serve as an electron source and sink for some soil microbial species (26, 45–47). Furthermore, a novel process of microbial syntrophic metabolism, namely, electric syntrophy, in which electrons released by one microorganism are transferred to another through electric current flowing through biological and mineralogical solid compounds, has recently been demonstrated (48, 49). Previous reports suggested that diverse kinds of microorganisms, including nitrate reducers, ferric iron reducers, methanogens, and dehalorespirators, participate in the electron-consuming part of electric syntrophy (48–52). The present report proposes a probability that electron uptake from such naturally occurring conductive materials is an unidentified strategy by which acetogenic bacteria thrive in natural environments.

Conclusion. This paper is the first to demonstrate acetogenesis-dependent MIC in pure cultures of acetogenic bacteria. The newly isolated acetogen *Sporomusa* sp. GT1 and its close relative *S. sphaeroides* enhanced iron corrosion by generating acetate with Fe(0) granules as the sole electron donor, while other acetogens did not. Further studies on the corrosion-inducing acetogens will shed light on the relevance of acetogenesis to iron corrosion in actual environments and the extracellular electron transfer mechanisms of acetogenic bacteria.

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