Full Paper

***Geobacter sulfurreducens* subsp. *ethanolicus*, subsp. nov., an ethanol-utilizing dissimilatory Fe(III)-reducing bacterium from a lotus field**

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An ethanol-utilizing Fe(III)-reducing bacterial strain, OSK2A^T, was isolated from a lotus field in Aichi, Japan. Phylogenetic analysis of the 16S rRNA gene sequences of OSK2A^T and related strains placed it within *Geobacter sulfurreducens* PCA^T. Strain OSK2A^T was shown to be a Gram-negative, motile, rod-shaped bacterium, strictly anaerobic, 0.76–1.65 µm long and 0.28–0.45 µm wide. Its growth occurred at 20–40°C, pH 6.0–8.1, and it tolerated up to 1% NaCl. The G+C content of the genomic DNA was 61.2 mol% and DNA-DNA hybridization value with *Geobacter sulfurreducens* PCA^T was 60.7%. The major respiratory quinone was MK-8. The major fatty acids were 16 : 1 ω7c, 16 : 0, 14 : 0, 15 : 0 iso, 16 : 1 ω5c, and 18 : 1 ω7c. Strain OSK2A^T could utilize H₂, ethanol, acetate, lactate, pyruvate, and formate as substrates with Fe(III)-citrate as electron acceptor. Amorphous Fe(III) hydroxide, Fe(III)-NTA, fumarate, malate, and elemental sulfur were utilized as electron acceptors with either acetate or ethanol as substrates. Results obtained from physiological, DNA-DNA hybridization, and chemotaxonomic tests support genotypic and phenotypic differentiation of strain OSK2A^T from its closest relative. The isolate is assigned as a novel subspecies with the name *Geobacter sulfurreducens* subsp. *ethanolicus*, subsp. nov. (type strain OSK2A^T=DSMZ 26126^T=JCM 18752^T).

Key Words—ethanol; Fe(III) reduction; *Geobacter*; lotus field

Introduction

Dissimilatory reduction of Fe(III) oxides is an important process for the oxidation of organic matter in terrestrial anoxic soils (Lovley, 1991, 1997; Thamdrup, 2000). This process is dominated by *Geobacter* spe-

cies (Anderson et al., 1998; Coates et al., 1996; Lovley et al., 2011; Snoeyenbos-West et al., 2000), contributing to the global cycling of metals and carbon (Lovley et al., 2011). The genus *Geobacter* was established by Lovley et al. (1993) with *Geobacter metallireducens* GS-15^T isolated from the Potomac River, the first species to be described under the genus, followed by *Geobacter sulfurreducens* PCA^T (Caccavo et al., 1994). Since then, other representatives belonging to the genus *Geobacter* have been described, with 16 valid species already reported at the time of writing. *G. sulfurreducens* PCA^T is one of the most studied *Geobacter* species as a model organism in various fields of research, such as biochemical and molecular stud-

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ies on the respiratory mechanisms of Fe(III) oxide (Kim et al., 2005; Lloyd et al., 2003; Lovley et al., 2011; Rollefson et al., 2009; Yun et al., 2011), microbial fuel cells (MFC) (Bond and Lovley, 2003), development of genetic manipulation techniques (Coppi et al., 2001; Mahadevan et al., 2011; Park and Kim, 2011; Ueki and Lovley, 2010), and the first genome analysis within the genus *Geobacter* (Methe et al., 2003).

The successful isolation of OSK2A^T as a novel Fe(III)-reducing bacterium described as a novel subspecies within the *Geobacter sulfurreducens*, with 99.6% similarity based on the 16S rRNA gene sequence and the capability of growth with ethanol as a substrate, will supplement the findings and add to the growing list of type strains within the genus *Geobacter*. Characterization of this strain will also provide insight into features that may lead to improved current production in MFC. Despite the high similarity of OSK2A^T to *G. sulfurreducens* PCA^T, there are some distinguishable phenotypic characteristics, such as the ability to utilize ethanol, temperature growth range, and other morphological and physiological differences between them. Here, we report the taxonomic characterization of strain OSK2A^T assigned as a novel subspecies belonging to *Geobacter sulfurreducens* subsp. *ethanolicus* subsp. nov. (type strain=DSMZ 26126^T=JCM 18752^T). In addition, new metabolic information obtained from this study and others (Lovley, 2006; Lovley et al., 2011; Speers and Reguera, 2011) validates an emendation to the original description of *Geobacter sulfurreducens* PCA^T which is proposed here.

Materials and Methods

Isolation and growth conditions. Mud samples for enrichment were collected from a lotus field in Japan, and isolation was performed in roll tubes with agar as the solidifying agent. Purity of the isolate was checked by microscopy and the absence of growth in an anaerobic heterotrophic medium (NIH thioglycolate broth, Difco) amended with pyruvate (20 mM).

Geobacter sulfurreducens PCA^T (DSM 12127^T) purchased from DSMZ was used as the reference strain for all tests under our laboratory conditions.

Morphological and physiological tests. For morphological analysis, strain OSK2A^T was grown with acetate and amorphous Fe(III) hydroxide and pieces of glass, washed with PBS, and fixed for scanning electron microscopy using phosphate-buffered saline

(pH 7) containing 2% glutaraldehyde. Fixed samples were dehydrated through a stepwise increase in ethanol series (50, 60, 70, 80, 90, 95, and 99.5%) followed by 100% overnight. Ethanol was removed from fixed samples through a paper filter at room temperature, and samples were immediately coated with OsO₄ in an osmium coater (Neoc-ST, Meiwaforis). The OsO₄-coated plates were observed by SEM at 5 kV (S-4800, Hitachi). Motility was observed for cultures grown on ethanol (20 mM) and amorphous Fe(III) hydroxide (ca. 100 mM) and examined by phase contrast microscopy in accordance with the method described by Childers et al. (2002).

Physiological tests on growth ranges for temperature, pH, and NaCl concentration were performed with acetate (10 mM) and fumarate (20 mM), in a basal medium slightly modified compared with that of Widdel and Pfennig (1977), used previously to culture "*Geobacter luticola*" OSK6^T (Viulu et al., 2012). Tests for all growth ranges and optima for strain OSK2A^T and the reference strain PCA^T were performed in duplicate and the growth was monitored using a spectrophotometer by measuring OD at 560 nm.

Fe(III) utilization and growth tests. Fe(III) utilization and growth of the strain were observed at 30°C with acetate as an electron donor and Fe(III)-NTA as an electron acceptor. Cell growth was determined by measuring protein concentrations and direct cell counting with SYBR Gold fluorescent dye with a Bicinchoninic Acid (BCA) Protein Assay kit (Pierce Biotechnology) according to the manufacturer's instructions, with slight modifications compared with a previously described method (Viulu et al., 2012).

The same composition of bicarbonate-buffered basal medium described above was used for all phenotypic characterizations in which acetate or Fe(III)-citrate was used as the electron donor or acceptor, respectively. Utilization of electron donors was examined at 30°C in the presence of Fe(III)-citrate (50 mM) with the following substrates (concentrations in mM given in parentheses, unless otherwise stated): formate (10), propionate (10), butyrate (10), pyruvate (10), lactate (10), fumarate (10), succinate (10), ethanol (10), butanol (10), glucose (10), phenol (1), benzoate (1), toluene (1), methanol (5), propanol (10), isopropanol (10), H₂ (ca. 62 kPa), and CH₄ (ca. 62 kPa). Utilization of electron acceptors was examined with nitrate (20), malate (20), fumarate (40), Fe(III)-NTA (10), amorphous Fe(III) hydroxide (50), sulfate (20), el-

elemental sulfur (3.0 g/L), sulfite (20), and thiosulfate (20) in the presence of acetate (10).

16S rRNA gene amplification and sequence analyses. The 16S rRNA gene of strain OSK2A^T was amplified by PCR using primers EU10F and U1500R. Sequencing was performed with an ABI PRISM 3100 genetic analyzer (Applied Biosystems), according to previously described procedures (Nakamura et al., 2011). For phylogenetic analyses, 16S rRNA gene sequences of the type strains of the genus *Geobacter* and other related taxa were aligned using the online alignment tool SINA (Pruesse et al., 2007) and manually edited in ARB (Ludwig et al., 2004). Aligned sequences were imported into MEGA 5.0 and PHYML 3.0.1 (Guindon and Gascuel, 2003; Tamura et al., 2011) to estimate phylogenetic trees using neighbor-joining (Saitou and Nei, 1987) and maximum likelihood (Felsenstein, 1981) methods, respectively, with the Jukes-Cantor model (Jukes and Cantor, 1969). Reliabilities of the bootstrap values were inferred by both methods in accordance with Hall (2008).

Analyses of respiratory quinone and cellular fatty acids. Cells for respiratory quinone analysis were extracted in chloroform/methanol, purified by TLC in hexane/benzene/chloroform mixture, and determined by HPLC according to a previously described procedure (Collins et al., 1982). Analyses of cellular fatty acids were carried out by the Identification Service of the DSMZ, Braunschweig, Germany. *G. sulfurreducens* PCA^T was used as the reference strain for both respiratory quinone and fatty acid analyses. Cells for both strains were cultured on acetate (20 mM) and Fe(III)-citrate (100 mM) until early stationary phases for chemotaxonomic analyses.

PCR-based fingerprinting analyses of repetitive extragenic palindromic (rep) elements. Genomic DNA for rep-PCR was obtained for strain OSK2A^T from cells grown with ethanol (10 mM) and Fe(III)-citrate (50 mM), while strain PCA^T cells were obtained from acetate (10 mM) and Fe(III)-citrate (50 mM). Rep-PCR amplifications were performed with REP2-I and REP1R-I primers according to PCR conditions described by Versalovic et al. (1991) and Sung et al. (2006). The rep-PCR products were electrophoresed in 1.5% agarose gel with 1 × TAE buffer at 18 V for 5 h. Resulting band patterns were obtained using a UV trans-illuminator equipped with a digital camera (GelDoc XR, Bio-Rad).

G+C content and DNA-DNA hybridization analyses.

For determination of the G+C content (mol%), cells of strain OSK2A^T and the reference strain PCA^T were grown with either ethanol (20 mM) or acetate (20 mM) and Fe(III)-citrate (100 mM) and were extracted according to the protocol described by Versalovic et al. (1991). The G+C content was determined by HPLC according to previously described procedures of Tamaoka and Komagata (1984). DNA-DNA hybridization analyses (Cashion et al., 1977; De Ley et al., 1970; Huss et al., 1983) were performed by the Identification Service of the DSMZ, Braunschweig, Germany.

Results and Discussion

Characteristics of the strain

Strain OSK2A^T was isolated from lotus field sediments by a roll tube method with agar as the solidifying agent, with colonies appearing red and spherical in shape. No growth was observed upon cultivation in the medium for heterotrophic anaerobes. Further microscopy observation of uniformity in cell morphology showed that strain OSK2A^T was eventually purified. The novel strain OSK2A^T is a rod-shaped bacterium, 0.76–1.65 μm long and 0.28–0.45 μm wide (Fig. 1). Strain OSK2A^T is motile when observed with ethanol and amorphous Fe(III) hydroxide under phase contrast microscopy, unlike the closely related strain PCA^T, which is not motile. The original description of strain PCA^T also lacked motility (Caccavo et al., 1994) and genomic information for strain PCA^T indeed indicates that its flagellum system is impaired (Ueki et al., 2012). Similar to the reference strain, OSK2A^T thrives in 0–1.0% NaCl and grows at an optimum temperature of 30–37°C with a growth range of 20–40°C, while the

Fig. 1. Morphology of strain OSK2A^T observed by scanning electron microscopy. Bar indicates 1 μm.

closest relative grows at a similar optimum temperature but with a significant difference in temperature range of 10–45°C. On the other hand, strain OSK2A^T grows at a pH range of 6.0–8.0, which is comparable to that of its closest relative. The lack of motility of its most closely related taxon, in contrast to OSK2A^T, and the different temperature growth range observed clearly differentiate OSK2A^T from PCA^T as two separate type strains. All the morphological and physiological results are summarized in Table 1.

In accordance with the hallmark of all other *Geobacter* species, strain OSK2A^T thrives by coupling the reduction of Fe(III) oxides (Fe(III)-NTA, Fe(III)-citrate, and amorphous Fe(III) hydroxide) to the oxidation of acetate, coinciding with an increase in cell number

and protein concentration (Fig. 2). Both the novel strain OSK2A^T and the reference strain PCA^T utilized the following electron donors with Fe(III)-citrate: acetate, lactate, pyruvate, and formate, while only the novel strain could utilize ethanol. The original description of strain PCA^T (Caccavo et al., 1994) indicated that it did not utilize carboxylic acids; however, this study and others (Lovley, 2006; Lovley et al., 2011; Speers and Reguera, 2011) found otherwise. Acetate or ethanol as the substrate with the following electron acceptors was utilized by the novel strain OSK2A^T: amorphous Fe(III) hydroxide, Fe(III)-NTA, malate, fumarate, and elemental sulfur (Table 1).

Ethanol as the distinguishing substrate showed growth when measured at OD₅₆₀ (Fig. S1), unlike the

Table 1. Characteristics of strain OSK2A^T differentiating it from closely related members of the genus *Geobacter*.

Characteristics	1	2	3	4
Cell width (μm)	0.28–0.45	0.5	0.6	0.5
Cell length (μm)	0.76–1.65	2–3	1.0–2.0	2–4
Motility	+	–*	–	+
Temperature range (°C)	20–40	10–45*	30 [‡]	30–35 [‡]
pH range	6.0–8.0	5.6–8.0	ND	6.7–7.0 [‡]
Electron acceptor usage				
Fe(III)-citrate	+	+*	–	+
Nitrate	–	–*	ND	+
Fumarate	+	+*	ND	–
Malate	+	+*	ND	ND
Elemental sulfur	+	+*	ND	–
Electron donor usage				
H ₂	+	+*	+	–
Formate	+	+*	+	–
Propionate	–	–*	+	+
Butyrate	–	–*	+	+
Lactate	+	+*	–	–
Succinate	–	–*	–	ND
Benzoate	–	–*	+	+
Ethanol	+	–*	+	+
Propanol	–	–*	ND	+
Butanol	–	–*	ND	+
Phenol	–	–*	–	+
Toluene	–	–*	+	+
DNA G+C content (mol%) [†]	61.2 ^{LC}	61.9 ^{LC*}	57.3 ^{LC}	56.6 TM
16S rRNA gene similarity against strain OSK2A ^T (%)	100	99.6	95.6	95.6

Taxa: 1, strain OSK2A^T (data from this study); 2, *G. sulfurreducens* PCA^T and DSM 12127^T (Caccavo et al., 1994, and this study); 3, *G. grbiciae* TACP-5^T (Coates et al., 2001); 4, *G. metallireducens* GS-15^T (Lovley et al., 1993). All strains utilize acetate and pyruvate as an electron donor. +, positive; –, negative; ND, no data available in the original reference. *, data from this study using DSM 12127^T; ‡, optimum; †, determined by HPLC (LC) or melting temperature (TM).

Fig. 2. Growth of strain OSK2A^T with acetate and Fe(III)-NTA as electron donor and acceptor, respectively.

(a) Open diamond denotes cell number and filled square represents protein concentration. (b) Acetate was utilized with concomitant reduction of Fe(III)-NTA over time; open triangle, acetate concentration; filled circle, Fe(II) concentration. Data are presented as means of triplicate incubations with standard deviations as error bars.

reference strain with no growth after 10 days of cultivation. Strain OSK2A^T has phenotypic characteristics almost identical to those of the phylogenetically closest relative (*G. sulfurreducens* PCA^T), except for ethanol, which the novel strain can utilize but which strain PCA^T cannot. The majority of *Geobacter* species can utilize ethanol as a substrate for growth with concomitant reduction of Fe(III); however, PCA^T does not have this ability and this seems to be indicative of the whole genome sequence of PCA^T, which does not possess the genes for ethanol utilization (genes of neither NAD⁺ nor NADP⁺-dependent aldehyde dehydrogenase were found, although those of alcohol dehydrogenase exist), distancing OSK2A^T from PCA^T. Other compari-

sons of phenotypic characteristics between the novel strain and its most closely related strain are given in Table 1.

Our preliminary comparisons of current production between strain OSK2A^T and PCA^T in microbial fuel cells (MFC) in the presence of acetate as the substrate showed the former producing a significantly higher maximum current density than the latter, namely, 6.5 times higher (7.1 and 1.1 A/m², respectively). Previous studies conducted with an isolate named KN400 and the wild-type strain of *G. sulfurreducens* also showed similar results to this study (Yi et al., 2009). In the previous study, strain KN400 produced higher current than the wild-type PCA^T strain. Furthermore, strain KN400 has 100% similarity to *G. sulfurreducens* PCA^T, based on the 16S rRNA gene, but a comparison of the complete genomes of KN400 and PCA^T strains showed divergence of genes involved in current production (Butler et al., 2012). Similarly, the difference in current production between OSK2A^T and PCA^T strains may support strain OSK2A^T as a separate strain from PCA^T, albeit belonging to the same species. On the other hand, although current production has not been officially recognized as a standard criterion for the taxonomic classification of *Geobacter* species, there is an increasing number of studies on their promise for current production in MFCs, and it is becoming an integral component of their physiological description (Lovley et al., 2011, and references therein).

Respiratory quinone and fatty acid compositions

The novel strain has MK-8 as the major respiratory quinone, as commonly reported for the genus *Geobacter* (Hedrick et al., 2009; Kunapuli et al., 2010; Lovley et al., 2011; Viulu et al., 2012). Unlike the respiratory quinones, fatty acids are specific for each strain. Strain OSK2A^T contains almost the same major fatty acids ($\geq 1 \text{ mol}\%$), having just one less than its closest relative: 16 : 1 $\omega 7c$, 16 : 0, 14 : 0, 15 : 0 iso, 16 : 1 $\omega 5c$, and 18 : 1 $\omega 7c$ (Table 2), in agreement with the findings of Hedrick et al. (2009). Other fatty acids absent in the novel strain but present in the closely related strain PCA^T, albeit in tiny proportions ($< 1 \text{ mol}\%$), are 17 : 1 iso $\omega 9c$, 17 : 0 iso, 15 : 1 iso F, and 13 : 0 iso. On the other hand, 18 : 1 $\omega 7c$ 11-methyl ($< 1 \text{ mol}\%$) is found in the novel strain but is absent in the reference strain PCA^T. Thus, fatty acid composition could not clearly differentiate the novel strain from its most closely related strain PCA^T; rather, the findings of

Table 2. Fatty acid compositions of strain OSK2A^T and its closest relative, strain PCA^T.

Total lipid fatty acids (mol%)	OSK2A ^T	PCA ^T
16 : 1 ω 7c	47.63	49.31
16 : 0	33.55	29.53
14 : 0	7.11	4.89
15 : 0 iso	3.89	5.73
16 : 1 ω 5c	2.48	2.52
18 : 1 ω 7c	2.24	3.95
16 : 0 3OH	Tr	1.11
16 : 1 iso I	Tr	Tr
18 : 0	Tr	Tr
13 : 1 at 12–13	Tr	Tr
15 : 0 anteiso	Tr	Tr
18 : 1 ω 5c	Tr	Tr
17 : 1 anteiso ω 9c	Tr	Tr
15 : 0 iso 3OH	Tr	Tr
18 : 1 ω 7c 11-methyl	Tr	–
17 : 1 iso ω 9c	–	Tr
17 : 0 iso	–	Tr
15 : 1 iso F	–	Tr
13 : 0 iso	–	Tr

Both strains were grown in 20 mM acetate and 100 mM Fe(III)-citrate until early stationary phase. Fatty acid analyses were carried out by the Identification Service and Dr. Brian Tindall, DSMZ, Braunschweig, Germany. Values of ≥ 1 mol% of fatty acids are shown. tr, less than 1 mol%; –, not detected.

both the respiratory quinone and the fatty acid compositions of OSK2A^T and its most closely related taxon strengthened the similarities shared between them as belonging to the same species within the genus *Geobacter*.

Genetic studies

Analysis of the near complete sequences of the 16S rRNA gene (1,358 bp) and eventual construction of the phylogenetic tree as described above revealed that strain OSK2A^T is positioned within the genus *Geobacter* (Fig. 3) and is almost identical to *G. sulfurreducens* PCA^T, with 99.6% similarity, as well as 95.6% or lower similarity to other *Geobacter* species (Table 1).

Although the novel strain is more than 99% similar to the most closely related strain based on the 16S rRNA gene, rep-PCR band patterns of the genomic DNA for both the novel strain and the reference strain PCA^T revealed distinguishable fingerprints between the two, indicating that they are indeed separate strains (Fig. 4).

The G+C content of genomic DNA for strain OSK2A^T

is 61.2 mol%, which is slightly lower but almost identical to that of the most closely related strain PCA^T (61.9 mol%), supporting their close phylogenetic relationship (Table 1). Previous values obtained for *G. sulfurreducens* by Coates et al. (2001) showed a lower G+C content than values shown here, probably due to the different protocol employed in this study (Tamaoka and Komagata, 1984) as opposed to the protocol of Mesbah et al. (1989) reported by Coates et al. (2001). Furthermore, although the DNA-DNA hybridization value of 60.7% (average of duplicate values: 57.2 and 64.2%) strongly supports strain OSK2A^T as a novel species (Stackebrandt and Goebel, 1994; Wayne et al., 1987), similar to strains TACP-2^T and GS-15^T, which share greater than 99% similarity based on their 16S rRNA gene sequences and yet exhibit less than 70% DNA-DNA homology (Coates et al., 2001), phenotypic characteristics do not differ starkly between them, so the two strains belong to the same species.

Strain OSK2A^T and the most closely related strain PCA^T showed very similar phenotypic and chemotaxonomic traits, agreeing with the closeness of their relationship based on the 16S rRNA gene sequence. However, ethanol utilization and the motility of strain OSK2A^T, which are both absent for strain PCA^T, clearly distinguish the novel strain from its most closely related taxon. In addition, the limited temperature range observed for the novel strain compared with that of the most closely related strain PCA^T also delineates the two. On the other hand, although 16S rRNA gene sequence and G+C content showed high similarity between strain OSK2A^T and PCA^T, a DNA-DNA hybridization value of 60.7% and distinct genomic fingerprints revealed by rep-PCR indicate distinctive features between them. All traits stated above corroborate classifying OSK2A^T as a novel subspecies belonging to *Geobacter sulfurreducens*. On the basis of all the similarities and differentiating characteristics observed between strain OSK2A^T and its closest relative (strain PCA^T), it is proposed that strain OSK2A^T be classified as a novel subspecies of *Geobacter sulfurreducens* with the name *Geobacter sulfurreducens* subsp. *ethanolicus*, subsp. nov.

Emended description of *Geobacter sulfurreducens* (Caccavo et al., 1994)

The description of *Geobacter sulfurreducens* is based on the data from Caccavo et al. (1994), with the following modifications and additional features: utiliz-

Fig. 3. Phylogenetic tree reconstructed from 16S rRNA gene sequences with Jukes-Cantor corrections, showing the placement of strain OSK2A^T among closely related members of the family *Geobacteraceae*.

The tree was constructed using MEGA5. Percentages at nodes show bootstrap values calculated by neighbor-joining (NJ) and maximum likelihood (ML) methods from 1,000 replicates with the Jukes-Cantor correction model. NJ/ML values at or above 70% reliability are shown, whereas values less than 70% are indicated by hyphens. Bars, 0.02 base substitutions per site.

Fig. 4. Rep-PCR fingerprints of strains PCA^T (A) and OSK2A^T (B).

The first lane shows the 1-kb size marker (SibEnzyme).

es formate, lactate, and pyruvate as substrates for growth with Fe(III) as an electron acceptor. Grows at 10–45°C and pH 5.6–8.0.

Description of *Geobacter sulfurreducens* subsp. *ethanolicus*, subsp. nov.

Geobacter sulfurreducens subsp. *ethanolicus* (etymologies: e.tha.no'li.cus. N.L. n. *ethanol* ethanol; L. suff. *-icus* suffix used with various meanings; N.L. masc. adj. *ethanolicus* belonging to ethanol, in reference to the ability of the species to utilize ethanol as a substrate for growth).

The cells are Gram-negative, motile, rod-shaped, strictly anaerobic, 0.76–1.65 µm long, and 0.28–0.45 µm wide. Growth occurs at 20–40°C with an optimum of 30–37°C, pH 6.0–8.1 (optimum pH 7.0) and can tolerate up to 1% NaCl. Electron donors utilized in the presence of Fe(III)-citrate include H₂, ethanol, acetate, lactate, pyruvate, and formate. Other electron donors tested but not utilized are propionate, butyrate, succinate, malate, fumarate, benzoate, butanol, methanol, propanol, iso-propanol, phenol, toluene, glucose, and yeast extract. Amorphous Fe(III) hydroxide, Fe(III)-citrate, Fe(III)-NTA, fumarate, malate, and elemental sulfur are utilized as electron acceptors with either ace-

tate or ethanol as substrates, while nitrate, sulfate, sulfite, and thiosulfate are not utilized. The major respiratory quinone is MK-8. The major fatty acids are 16 : 1 ω 7c, 16 : 0, 14 : 0, 15 : 0 iso, 16 : 1 ω 5c, and 18 : 1 ω 7c. The G+C content of the genomic DNA is 61.2 mol%.

The type strain, OSK2A^T (=DSMZ 26126^T=JCM 18752^T), was isolated from lotus field sediments in Aichi Prefecture, Japan.

Geobacter sulfurreducens subsp. *sulfurreducens* (Caccavo et al., 1994) is hereby created based on the description of *Geobacter sulfurreducens* reported by Caccavo et al. (1994).

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Supplementary Materials

Fig. S1. Growth of strain OSK2A^T, observed at 30°C with ethanol and fumarate as electron donor and acceptor, respectively.

Data are presented as means of OD₅₆₀ in triplicate incubations and error bars represent standard deviation values.

Supplementary figures are available in our J-STAGE site (<http://www.jstage.jst.go.jp/browse/jgam>).

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