Supporting Information for manuscript

Microbial electrosynthesis of butyrate from carbon dioxide

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Summary:

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Microbial community analysis methods

DNA was extracted from cell pellets using the FastDNA® SPIN kit for soils (MP, Biomedicals) following the manufacturer's instructions. Partial 16S rRNA gene fragments were obtained by PCR amplification using the bacterial universal primers 357F and 907R. Reaction mixtures and PCR amplification conditions have been described previously (Lane, 1991). A 44 base pair GC clamp sequence was added to the 5' end of 357F primer for separation of PCR products by denaturing gradient gel electrophoresis (DGGE) (Muyzer and Smalla, 1998). All chemicals and Taq polymerase used in PCR amplifications were provided by Qiagen (Qiagen Ltd. Sussex, UK). PCR amplifications were performed in a 9700GeneAmp thermal cycler (Applied Biosystems, Foster City, CA). When necessary, different PCR products of the same sample were pooled and concentrated to an appropriate concentration of 100 ng/µL. Twenty-five µL of concentrated 16S rRNA PCR products were loaded on 6% (vol/vol) acrylamide-bis-acrylamide gels with a 40 to 65 % urea-formamide denaturing gradient (Bäckman et al., 2003). DGGE was performed in a Ingeny phorU system (INGENY, The Netherlands) as described previously (Prat et al., 2009). DGGE gel was run for 17 h at 160V and stained for 30 min with Sybr® Gold (Molecular Probes Europe, Invitrogen Corporation, UK), for visualization under UV excitation. The more intense bands of every position in the gel were excised using a sterile scalpel. The DNA fragments were recovered by elution in Tris/HCl buffer (10 mM at pH 7.4) at 65ºC during 2 hours and re-amplified as described above. Sequencing in both directions of 16S rRNA gene fragments obtained from re-amplification of excised DGGE bands, was performed by the Macrogen service (Macrogen, Korea). Sequences were examined for the presence of chimeras using the Uchime algorithm (Edgar et al., 2011), manually refined using the Bioedit v7.0 package and aligned using the ClustalW software. Aligned sequences were analyzed with the

BLASTn® program at the National Center for Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/) and bacterial species identified as closer similarities to known sequences using the nucleotide collection database. Partial 16S rRNA gene sequences were submitted to GenBank public database with accession numbers from KM489062 to KM489069.

References

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Table S1. *Mineral medium composition.*

Minerals		Trace elements		Vitamins	
Component	**g L^{-1}**	**Component**	**mg·L^{-1}**	**Component**	**µg·L^{-1}**
KH_2PO_4	1	Nitrilotriacetic acid	20.0	Biotin	20.0
NaCl	1	$MnSO_4 \cdot H_2O$	10.0	Folic acid	20.0
NH_4Cl	0.25	$Fe(SO_4)_2(NH_4)_2 \cdot 6H_2O$	8.0	Pyridoxine hydrochloride	100.0
MgOH	0.05	$CoCl_2.6H_2O$	2.0	Thiamine hydrochloride	50.0
KCl	0.1	$ZnSO_4 \cdot 7H_2O$	0.002	Riboflavin	50.0
$CaCl_2$	0.03	$CuCl_2 \cdot 2H_2O$	0.2	Nicotinic acid	50.0
$BrCH_2CH_2SO_3Na$	6.3	$NiCl_2 \cdot 2H_2O$	0.2	DL- calcium pantothenate	50.0
		$Na_2MoO_4 \cdot 2H_2O$	0.2	Vitamin B12	1.0
		Na_2SeO_4	0.2	p- aminobenzoic acid	50.0
		Na_2WO_4	0.2	Lipoic acid (Thioctic acid)	50.0

Table S2. *Most probable sequence identification of DGGE bands. Similarity values and closest match to sequences of identified bacteria in GenBank reference RNA database are indicated. Accession numbers appear in parentheses.*

DGGE band	Closest Bacterial species	Identities (%)
1	*Clostridium carboxidivorans* P7 (NR_104768.1) *Clostridium scatologenes* K29 (AB610570) *Clostridium drakei* FP (NR_114863.1)	100
2	*Clostridium ljungdahlii* DSM13528 (NR_074161.1) *Clostridium ragsdalei* (DQ020022) *Clostridium autoethanogenum* DSM10061 (CP006763.1)	100
3	Uncultured *Firmicutes* clone (GU559846.1)	94