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Title:

Matching different inorganic compounds as hybrid electron donors to improve CO_2 fixing by non-photosynthetic microbial flora without hydrogen

Number of:

Pages: 6

Figures: 0

Tables: 4

Supporting Information

Non-photosynthetic CO_2 fixing micro-organism. Two NPMFs capable of fixing CO_2 without hydrogen were isolated from sea water and their sediments collected from the Yellow Sea, East China Sea, South China Sea and Antarctic waters. The mixture of these two NPMFs was then domesticated in the laboratory for more than 6 months under aerobic and anaerobic conditions.

DNA extraction and PCR-DGGE. The microbial flora was cultured with 0.2% of each electron donor under different gas conditions for 96 h, after which the total DNA was extracted. The bacterial DNA was extracted using a soil DNA kit (D5625-01, United States, Omega) according to the manufacturer's instructions. Primers 341f with a GC-clamp (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTAGGGGAGGCAGCAG-3') and 534r (5'-ATTACCGCGGCTGCTGG-3') were used to amplify the V3 region of the bacterial 16S rDNA. The PCR mixtures had a final volume of 25 μL and contained 2.5 μl 10×PCR buffer, 1 U Taq DNA Polymerase, 1.0 μl of each dNTP (2.5 mmol L-1), 0.5 μl of each primer (10 μM) and 50ng of DNA as the template. The samples were amplified by subjecting the mixtures to the following conditions: 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 50 s and a final extension at 72 °C for 10 min. DGGE was conducted using the DCodeTM Universal Mutation Detection System (BioRad, USA). The PCR products were electrophoresed directly in a 10% (v/v) polyacrylamide gel in 1×TAE buffer containing a linear gradient that ranged from 30% to 60% denaturant (100% denaturant was equivalent to 7 mol·L-1 urea and 40% (v/v) deionized formamide). Electrophoresis was performed at 60 °C under a constant voltage of 80 V. After 15 h of electrophoresis, the gel was stained with ethidium bromide (EB) for 15 min and then photographed. The contrast of the bands in different lanes was determined by Quantity One 4.6.2 (BioRad, USA), and the Dice Coefficient (Cs) was defined as the following:

$$Cs = (2L/L_0) \times 100\%$$

*where L was the amount of duplicate bands in both lanes and L_0 was the total amount of bands in both lanes.

Sequencing of DGGE bands. Prominent bands were excised from the DGGE gel for 16S rDNA fragment sequencing. The fragments were then re-amplified by PCR and purified using a B type Mini-DNA Rapid Purification Kit (BioDev, China), after which they were cloned using the pMD19-T plasmid vector system (TaKaRa, Japan). The DNA sequences were then determined by a commercial service (Shanghai Invitrogen Biotechnology Co., LTD., China). The vector sequence was cut off and the

remaining nucleotides were compared to those available in GenBank using the BLAST program to identify the most similar 16S rDNA fragments.

Estimation of CO_2 fixing efficiency. The total organic carbon (TOC) value reflecting the microbial CO_2 fixing efficiency was analyzed using a Shimadzu TOC-VCPH total organic carbon analyzer (Shimadzu Seisakusho Co. Ltd., Kyoto, Japan). To reduce the impact of inorganic carbon on the analyses, the pH of the sample was adjusted to about 4.0 prior to TOC analysis.

Table S1 CCD under aerobic condition

Name	Units	-1 level	+1 level	-alpha	+alpha
A: $NaNO_2$	%, w/v	0.25	0.75	0.0795518	0.920448
B: $Na_2S_2O_3$	%, w/v	0.50	1.00	0.329552	1.17045
C: Na_2S	%, w/v	0.75	1.25	0.579552	1.42045

Table S2 CCD under anaerobic condition

Name	Units	-1 level	+1 level	-alpha	+alpha
A: $NaNO_2$	%, w/v	0.55	1.05	0.379552	1.22045
B: $Na_2S_2O_3$	%, w/v	0.60	1.10	0.429552	1.27045
C: Na_2S	%, w/v	0.75	1.25	0.579552	1.42045

Table S3 Experimental and predicted results under aerobic condition by Experimental design for the three variables

Std order	Factors (w/v)			TOC (mg/L)		
	$NaNO_2$	$Na_2S_2O_3$	Na_2S	Experimental (a, b)		Predicted
1	0.25	0.50	0.75	5.13	5.5	6.20
2	0.25	0.50	1.25	87.98	97.98	91.97
3	0.75	0.50	0.75	5.04	4.93	5.02
4	0.75	0.50	1.25	77.28	81.78	81.72
5	0.25	1.00	0.75	8.298	9.72	9
6	0.25	1.00	1.25	36.06	37.54	38.19
7	0.75	1.00	0.75	41.62	43.48	44.89
8	0.75	1.00	1.25	95.26	91.24	91.97
9	0.50	0.75	0.58	5.256	4.974	4.41
10	0.50	0.75	1.42	112.4	109.08	110.46
11	0.08	0.75	1.00	4.26	4.776	4.16
12	0.92	0.75	1.00	49.18	48.5	47.61
13	0.50	0.33	1.00	56.6	50.22	51.84
14	0.50	1.17	1.00	62.66	70.78	65.45
15	0.50	0.75	1.00	54.96	46.62	45.29
16	0.50	0.75	1.00	38.776	40.02	45.29
17	0.50	0.75	1.00	38.4	40.6	45.29
18	0.50	0.75	1.00	53.52	40.56	45.29
19	0.50	0.75	1.00	41.12	40.45	45.29
20	0.50	0.75	1.00	58.22	53.42	45.29

*the value of initial TOC was 0.576mg/L. Cultured for 96h

Table S4 Experimental and predicted results under anaerobic condition by Experimental design for the three variables

Std order	Factors (w/v)			TOC (mg/L)		
	$NaNO_2$	$Na_2S_2O_3$	Na_2S	Experimental (a, b)		Predicted
1	0.80	0.85	0.58	3.88	3.16	3.13
2	0.55	0.60	0.75	6.19	6.81	7.10
3	0.55	1.10	0.75	57.96	56.70	59.10
4	1.05	0.60	0.75	4.95	4.22	4.71
5	1.05	1.10	0.75	106.50	116.62	112.16
6	0.38	0.85	1.00	45.62	38.02	39.12
7	0.80	0.43	1.00	12.36	18.52	14.48
8	1.22	0.85	1.00	114.86	104.62	109.61
9	0.55	0.60	1.25	90.20	95.82	95.24
10	0.55	1.10	1.25	5.91	6.28	6.68
11	1.05	0.60	1.25	88.58	96.60	93.15
12	1.05	1.10	1.25	108.92	102.34	106.25
13	0.80	0.85	1.42	118.88	123.96	119.12
14	0.80	1.27	1.00	82.24	87.54	82.96
15	0.80	0.85	1.00	86.64	86.56	83.51
16	0.80	0.85	1.00	77.56	85.12	83.51
17	0.80	0.85	1.00	85.70	84.94	83.51
18	0.80	0.85	1.00	80.38	82.48	83.51
19	0.80	0.85	1.00	78.92	82.58	83.51
20	0.80	0.85	1.00	84.52	85.80	83.51

*the value of initial TOC was 0.643mg/L. Cultured for 96h