Inventory of Supporting Information

Glutamate production from aerial nitrogen using nitrogen-fixing bacterium *Klebsiella oxytoca*

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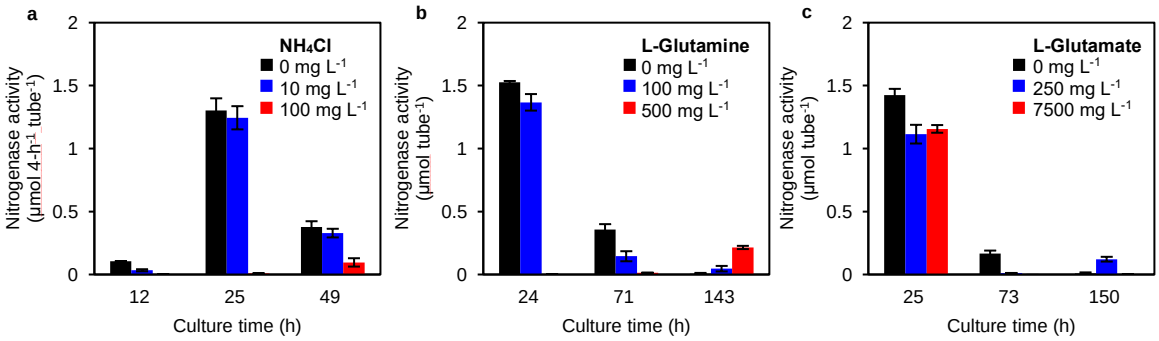

Extended Data 1. Effect of nitrogen compounds on nitrogenase activity of *K. oxytoca* NG13.

K. oxytoca NG13 cells were diazotrophically grown in KDC medium supplied with NH_4Cl (**a**), L-glutamine (**b**), and L-glutamate (**c**) with indicated concentrations. Culture was performed with 5 mL medium in a non-hermetic ϕ18 test tube at 25°C statically. Nitrogenase activity was measured at each time point using independent triple biological replicates. Data are shown as the mean of three biological replicates with standard deviation.

Extended Data 2. Effect of carbon source on nitrogenase activity of *K. oxytoca* NG13.

Carbon source	Nitrogenase activity (μmol 4-h^{-1} $tube^{-1}$)
Glucose	0.40 ± 0.01
Fructose	0.45 ± 0.03
Sucrose	0.83 ± 0.15
Citrate	0.11 ± 0.01
Malate	0.32 ± 0.02
Succinate	N.D.
2-Oxoglutarate	N.D.
Lactate	N.D.
Acetate	N.D.
Formate	N.D.
Ethanol	N.D.

Culture was performed with 5 mL Rennie salt basal medium[1] supplied with indicated carbon sources at the concentration of 15 g L^{-1} in a non-hermetic ϕ18 test tube at 25°C statically. Maximum nitrogenase activity observed during 14 d culture are shown as the mean of three biological replicates with standard deviation. N.D. means Not Detected.

Extended Data 3. Effect of organic acids combined with glucose on nitrogenase activity of *K. oxytoca* NG13 and CgCS strain and glutamate production of CgCS strain.

Carbon source (g L^{-1})	Nitrogenase activity (μmol 4-h^{-1} $tube^{-1}$)		Glutamate production (mg L^{-1})
	NG13	CgCS strain	CgCS strain
Glucose (7.5)	0.63 ± 0.01	0.25 ± 0.01	28.5 ± 1.9
Glucose (15)	0.70 ± 0.05	0.28 ± 0.01	26.0 ± 1.1
Glucose (7.5) and citrate (7.5)	1.66 ± 0.10	0.54 ± 0.02	109.0 ± 3.8
Glucose (7.5) and malate (7.5)	1.14 ± 0.04	0.46 ± 0.03	56.1 ± 2.1
Glucose (7.5) and succinate (7.5)	1.50 ± 0.09	0.56 ± 0.02	63.6 ± 2.3
Glucose (7.5) and 2-oxoglutarate (7.5)	1.05 ± 0.09	0.39 ± 0.01	79.3 ± 2.2

Culture was performed with 5 mL Rennie salt basal medium supplied with indicated carbon sources in a non-hermetic ϕ18 test tube at 25°C statically. Maximum nitrogenase activity and glutamate production observed during 6 d culture are shown as the mean of three biological replicates with standard deviation.

Extended Data 4. Effect of different concentrations of citrate on nitrogenase activity of *K. oxytoca* NG13 and CgCS strain and glutamate production of CgCS strain.

Citrate (g L^{-1})	Nitrogenase activity (μmol 4-h^{-1} $tube^{-1}$)		Glutamate production (mg L^{-1})
	NG13	CgCS strain	CgCS strain
0	0.46 ± 0.01	0.21 ± 0.01	46.4 ± 11.4
1.0	0.45 ± 0.04	0.22 ± 0.01	60.3 ± 12.3
2.5	0.67 ± 0.01	0.36 ± 0.01	71.2 ± 4.3
5.0	0.86 ± 0.06	0.46 ± 0.01	90.5 ± 5.8
7.5	1.12 ± 0.05	0.52 ± 0.01	86.9 ± 1.3
15	1.33 ± 0.06	0.47 ± 0.01	98.1 ± 0.1

Culture was performed with 5 mL Rennie salt basal medium supplied with 7.5 g L^{-1} of glucose and indicated concentrations of citrate in a non-hermetic ϕ18 test tube at 25°C statically. Maximum nitrogenase activity and glutamate production observed during 8 d culture are shown as the mean of three biological replicates with standard deviation.

Extended Data 5. Effect of heterologous expression of various citrate synthase and 2-metylcitrate synthase genes in *K. oxytoca* NG13.

Strain	Nitrogenase activity (μmol 4-h^{-1} $tube^{-1}$)	Glutamate production (mg L^{-1})
NG13	1.10 ± 0.02	N.D.
CgCS	0.53 ± 0.04	79.0 ± 1.3
CgMCS	0.69 ± 0.03	68.0 ± 0.3
EcMCS	0.56 ± 0.01	85.4 ± 2.1
NG13	1.35 ± 0.02	N.D.
CgCS	0.52 ± 0.02	84.5 ± 6.0
KoCS	0.90 ± 0.01	6.8 ± 0.2

Culture was performed with 5 mL KDC medium in a non-hermetic ϕ18 test tube at 25°C statically. Maximum nitrogenase activity and glutamate production observed during 8 d culture are shown as the mean of three biological replicates with standard deviation. N.D. means Not Detected.

Extended Data 6. Effect of culture volumes on nitrogenase activity and glutamate production of CgCS strain.

Volume (mL)	Nitrogenase activity (μmol 4-h^{-1} $tube^{-1}$)	Glutamate production (mg L^{-1})
2.5	0.42 ± 0.01	157.2 ± 3.8
5.0	0.52 ± 0.02	84.5 ± 6.0
10	0.91 ± 0.03	60.6 ± 5.4

Culture was performed with indicated volumes of KDC medium in a non-hermetic ϕ18 test tube at 25°C statically. Maximum nitrogenase activity and glutamate production observed during 8 d culture are shown as the mean of three biological replicates with standard deviation.

Supplementary Table 1. Plasmids used in this study.

Name	Description
pCCS	Expression of CgCS gene under the *trc* promoter, pSC101 *ori*, Tet^R
pCMCS	Expression of CgMCS gene under the *trc* promoter, pSC101 *ori*, Tet^R
pEMCS	Expression of EcMCS gene under the *trc* promoter, pSC101 *ori*, Tet^R
pKCS	Expression of KoCS gene under the *trc* promoter, pSC101 *ori*, Tet^R
pKCT	Expression of CitS gene under the *trc* promoter, pCDF *ori*, Spc^R

87 **Supplementary Table 2. Oligonucleotide primers used in this study.**

	Sequence (5' to 3')	Amplification of
1	CGATTAAATAAGGAGGAATAAACCATGTTTGAAAGGGATATC	CgCS gene
2	CGGGTACCGAGCTCGAATTCTTAGCGCTCCTCGCGAGG	CgCS gene
3	GAATTCGAGCTCGGTACC	pMW118
4	GGTTATTGTCTCATGAGCGG	pMW118
5	CCGCTCATGAGACAATAACCAATTCTCATGTTTGACAG	Tet^R cassette of 707-FLPe
6	GGATCTTCACCTAGATCCTTATTCAGGTCGAGGTGGCCCG	Tet^R cassette of 707-FLPe
7	AAGGATCTAGGTGAAGATCC	pMW118
8	GGGTGTTGGCGGGTGTCGGGCGCTCACTGCCCGCTTTC	pMW118
9	CCCGACACCCGCCAACACCC	*lacI* and P_{trc} region of pTrcHis2-TOPO/lacZ
10	GGTTTATTCCTCCTTATTTAATCG	*lacI* and P_{trc} region of pTrcHis2-TOPO/lacZ
11	CGATTAAATAAGGAGGAATAAACCATGTCCAGCGCCACAACC	CgMCS gene
12	CGGGTACCGAGCTCGAATTCTTAACGCTTTTCAATGGG	CgMCS gene
13	CGATTAAATAAGGAGGAATAAACCATGAGCGACACAACGATC	EcMCS gene
14	CGGGTACCGAGCTCGAATTCTTACTGGCGCTTATCCAG	EcMCS gene

15	CGATTAAATAAGGAGGAATAAACCATGTCTGATGCTAAAGCA	KoCS gene
16	CGGGTACCGAGCTCGAATTCTTAGCGGGCGATATCGTT	KoCS gene
17	CGATTAAATAAGGAGGAATAAACCATGACTAATATGAGCCAGGC	CitS gene
18	CGCTAGTAGACGAGTCCATGTTACATCATCATGCCGAAC	CitS gene
19	CATGGACTCGTCTACTAG	*lacI*-pCDF *ori*-Spc^R region of pCDFDuet
20	AACATTATCCAGAACGGGAGTCCTAATGCAGGAGTCGC	*lacI*-pCDF *ori*-Spc^R region of pCDFDuet
21	CTCCCGTTCTGGATAATG	P_{trc} region of pTrcHis2-TOPO/lacZ

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Supplementary Fig. 1. Sequence of the open reading frame encoding a putative CS in *K. oxytoca* NG13 (KoCS).

ATGTCTGATGCTAAAGCAAAAATCACCCTGGGTGGTGATACTGCTATCGAACTGGATGTGCTAAAGG
GCACGCTCGGTCAGGATGTTATTGATATTCGTAGTCTTGGTTCAAAAGGCGTATTTACTTTTGACCC
TGGTTTCACCTCAACCGCATCCTGCGAATCTAAAATCACCTTTATCGACGGTGATGAAGGTATCCTG
CTGCACCGCGGTTTTCCGATCGATCAGTTAGCAACCGACTCCAACTATCTGGAAGTATGCTACATCC
TGCTGAACGGTGAGAAGCCGACTCAGGCCCAGTACGACGAATTCAAAACAATCGTTACTCGCCACAC
TATGATTCACGAACAGATTACCCGTCTGTTCCACGCGTTCCGCCGCGATTCACATCCGATGGCCGTT
ATGTGCGGTATCACCGGCGCGCTGGCCGCGTTCTATCACGATTCGCTGGATGTGAATAACCCACGCC
ATCGCGATATCGCCGCATTCCGCCTGCTCTCCAAGATGCCGACGATGGCGGCAATGTGTTACAAATA
TTCTATCGGTCAGCCTTTCGTTTATCCGCGCAACGACCTCTCCTACGCGGGTAACTTCCTGAATATG
ATGTTCTCCACGCCGTGTGAAAAATATGAAGTGAACCCGATTCTGGAACGCGCGATGGACCGTATCC
TGATCCTGCACGCCGACCACGAACAAAACGCTTCGACCTCCACCGTGCGTACCGCCGGCTCTTCCGG
CGCGAACCCGTTTGCCTGCATCGCAGCGGGCATTGCCTCCCTGTGGGGACCGGCGCACGGCGGCGCC
AACGAAGCGGCGCTGAAGATGCTCGAAGAGATCAGCTCCGTTGAACACATTCCGGAATTCGTTCGTC
GCGCGAAAGACAAAAACGACTCTTTCCGCCTGATGGGCTTTGGCCACCGGGTGTACAAAAACTACGA
CCCGCGCGCCACCGTTATGCGTGAAACCTGCCATGAAGTGCTGAAAGAGCTGGGCACCAAAGACGAT
CTGCTGGAAGTGGCTATGGAGCTTGAGCACATCGCGCTTAACGACCCGTACTTCATTGAGAAGAAAC
TTTACCCTAACGTCGATTTCTACTCCGGTATCATCCTCAAAGCGATGGGCATCCCATCCTCCATGTT
TACCGTTATCTTCGCGATGGCGCGTACCGTGGGCTGGATTGCGCACTGGAACGAAATGCACAGCGAC
GGCATGAAAATAGCCCGTCCGCGTCAGCTGTATACCGGCTACGAAAAGCGCGATTTCAAAAACGATA
TCGCCCGCTAA

Supplementary Fig. 2. Sequence of the open reading frame encoding a putative CitS in *K. oxytoca* NG13.

ATGACTAATATGAGCCAGGCTCCATCTGCAGAGAAAAAAGGCGTTAGCGATATTCTGGGGTTTAAAA
TCTTCGGCATGCCGCTACCGCTTTACGCCTTTGCGTTAATCACTTTACTACTTTCACACTTTTATAA
TGCCCTGCCGACCGACATTGTCGGCGGCTTCGCCATCATGTTTATTATTGGCGCCGTTTTTGGAGAA
ATTGGCAAACGCCTGCCGATCTTCAATAAATATATCGGCGGCGCGCCGGTGATGATCTTCCTCGTGG
CCGCCTATTTCGTTTATGCAGGCATTTTCACCCAGAAAGAAATTGATGCGATCACTAATGTGATGGA
TAAAAGTAACTTCCTGAACTTATTCATCGCAGTATTGATTACTGGTGCAATCCTCTCGGTTAACCGT
AAGCTGCTGCTGAAATCTCTGCTGGGTTATATTCCGACCATTTTAATGGGGATCCTCGGCGCATCCA
TCTTCGGGATTCTCATCGGCTTGTGCTTCGGTATCTCCATCGACCGCATTATGATGCTGTACGTCCT
GCCGATTATGGGTGGCGGCAACGGCGCGGGCGCGGTGCCGCTATCTGAAATTTATCACTCCGTCACC
GGCCGTTCGCGTGAAGAGTACTACTCCACCGCGATTGCTATCCTGACCATCGCCAACATCTTTGCCA
TCGTGTTTGCCGCGCTGCTCGATATTATCGGTAAAAAGCACACCTGGCTGAGCGGCGAAGGCGAGCT
GGTGCGCAAGGCCTCTTTCAAAGTAGAAGATGATGAAAAAGCGGGCCAGATTACCCATCGCGAAACG
GCCGTCGGCCTGGTGCTCGCCACAACCTGCTTCCTGCTGGCCTACGTTATCGCCAAGAAAATTCTGC
CAAGCATTGGCGGCGTATCTATCCACTATTTCGCCTGGATGGTGCTGATCGTCGCGGCGCTGAACGC
TTCCGGCCTCTGCTCGCCTGAGATTAAAGCTGGCGCTAAACGCCTATCTGACTTCTTCTCTAAGCAA
CTGCTGTGGGTACTGATGGTCGGCGTCGGCGTGTGCTACACCGACCTGCAGGAAATTATCGACGCGA
TTACCTTTGCGAACGTCATCATCGCGGCGGTGATCGTCGTGGGCGCGGTCATCGGCGCGGCCATCGG
CGGCTGGATGATTGGCTTCTTCCCGATTGAATCCGCGATCACCGCCGGTCTGTGCATGGCTAACCGC
GGCGGCTCGGGCGACCTGGAAGTACTCTCTGCCTGTAACCGTATGAATCTTATCTCTTATGCGCAAA
TCTCCTCCCGTCTGGGCGGCGGTATTGTGCTGGTCATTGCCAGCATCGTGTTCGGCATGATGATGTA
A

Reference

1. Rennie, R. J. A single medium for the isolation of acetylene-reducing (dinitrogen-fixing) bacteria from soils. *Can. J. Microbiol.* **27**, 8–14 (1981).