

Supplementary Information

Global investigation of an engineered nitrogen-fixing *Escherichia coli* strain reveals regulatory coupling between host and heterologous nitrogen-fixation genes

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Supplementary Figures

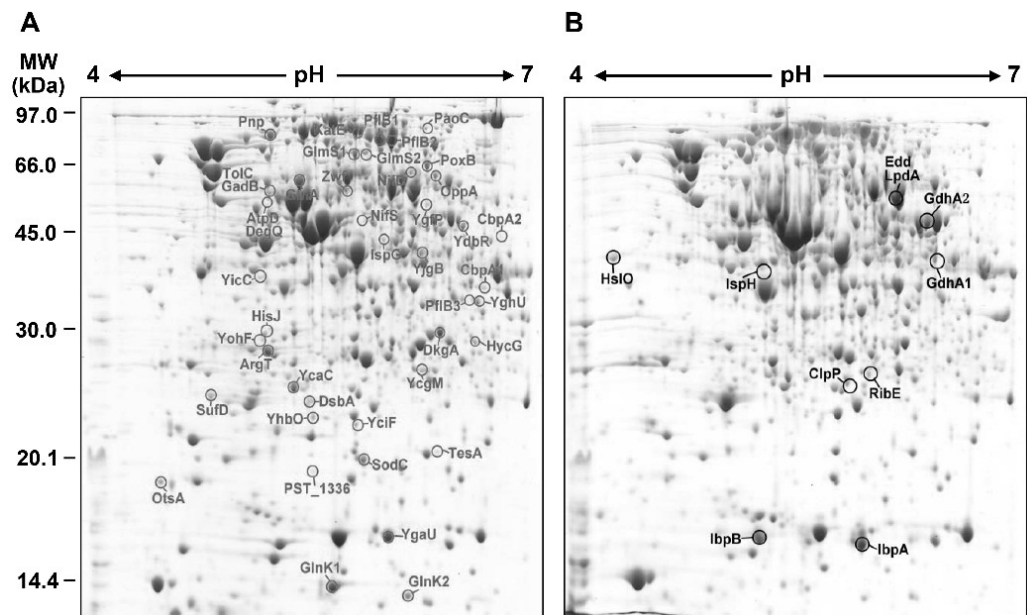


Figure S1. Differential protein synthesis patterns of *E. coli* EN-01 under nitrogen-fixation conditions.

Coomassie Blue stained the two-dimensional protein gels of total protein from nitrogen-fixation conditions and nitrogen excess conditions in parallel. Circles indicate protein spots differentially expressed between the two conditions. (A) Total protein under nitrogen-fixation condition. Gray circles, proteins induced under this condition. (B) Total protein under nitrogen-excess conditions. Black circles, proteins repressed under nitrogen-fixation conditions. The circled spot changes were observed in three repeat experiments. Proteins were identified by MALDITOF-MS analysis.

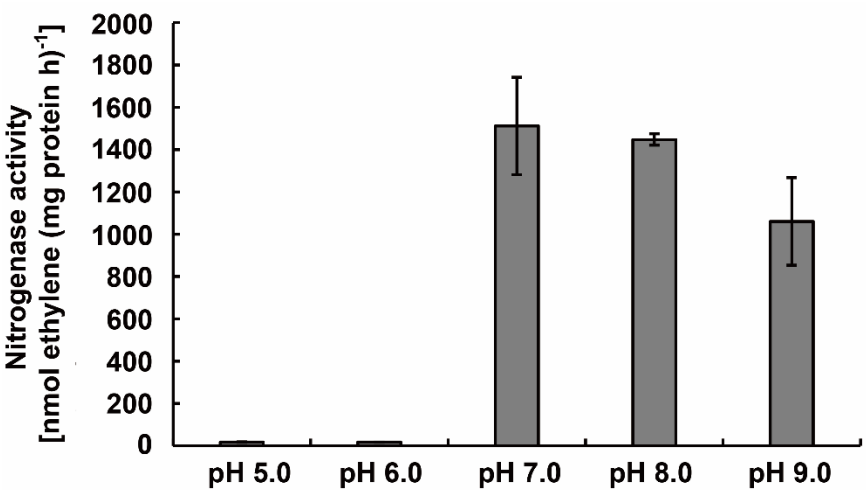


Figure S2. *P. stutzeri* A1501 nitrogenase activity under microaerobic conditions when cells were cultured in minimal medium without nitrogen with varying pH.

All experiments were performed three times.

Supplementary Tables

Table S1. Genes with similar expression patterns in *E. coli* EN-01 and *P. stutzeri* A1501 under nitrogen-fixation and nitrogen-excess conditions.

Gene	EN-01		A1501		Product
	Fold change	ID	Fold change	ID	
Upregulated					
<i>glnK</i>	86.88	b0450	6.85	PST0502	nitrogen regulatory protein P-II
<i>glgA</i>	11.68	b3429	2.23	PST2137	glycogen synthase
<i>iciA</i>	3.66	b2916	2.03	PST3130	chromosome initiation inhibitor
<i>osmC</i>	5.81	b1482	3.86	PST0265	osmotically inducible protein OsmC
<i>katE</i>	15.47	b1732	2.27	PST0039	catalase
<i>amtB</i>	55.28	b0451	2.55	PST0504	ammonium transporter
<i>amtB</i>	55.28	b0451	3.86	PST0503	ammonium transporter
<i>yeaG</i>	20.34	b1783	2.07	PST0722	serine protein kinase PrkA
<i>glnL/ntrB</i>	10.36	b3869	2.86	PST0350	nitrogen regulation protein NtrB
<i>glnG/ntrC</i>	10.61	b3868	3.19	PST0349	nitrogen regulation protein NtrC
<i>ynfM</i>	26.45	b1596	2.4	PST0565	putative transport protein YnfM
<i>rutA</i>	22.56	b1012	10.74	PST3597	hypothetical protein
<i>rutB/ycdL</i>	16.73	b1011	7.91	PST3598	isochorismatase family protein ycdL
Downregulated					
<i>fpr</i>	-2.41	b3924	-2.04	PST1518	ferredoxin--NADP reductase
<i>acnB</i>	-2.19	b0118	-2.36	PST2046	aconitate hydratase 2
<i>fdx</i>	-2	b2525	-2.07	PST3037	ferredoxin
<i>accC</i>	-2.48	b3256	-2.52	PST0186	acetyl-CoA carboxylase, biotin carboxylase
<i>accB</i>	-2.05	b3255	-2.68	PST3273	biotin carboxyl carrier protein (BCCP)
<i>infA</i>	-2.91	b0884	-5.05	PST2297	translation initiation factor IF-1
<i>queA</i>	-2.9	b0405	-2.14	PST3052	S-adenosylmethionine:trna ribosyltransferase-isomerase
<i>topA</i>	-2.24	b1274	-2.19	PST1729	DNA topoisomerase I
<i>creA</i>	-8.28	b4397	-2.41	PST0960	CreA family protein
<i>secG</i>	-2.95	b3175	-3.05	PST3315	preprotein translocase, SecG subunit
<i>phoP</i>	-4.81	b1130	-2.02	PST0389	two-component response regulator PhoP

Table S2. Up-regulated and down-regulated proteins in *E. coli* EN-01 under nitrogen-fixation conditions compared with nitrogen-excess conditions.

Protein function ^a	Protein name ^a	ORF no. ^a	Fold change ^b	Transcriptional ratios ^c	Experimental pI/MW(kDa)	EN01-1 ^d	EN01-2 ^d	EN01- 3 ^d	EN01- NH ₄ ⁺ - 1 ^e	EN01- NH ₄ ⁺ - 2 ^e	EN01- NH ₄ ⁺ - 3 ^e
Upregulated											
Energy production and conversion											
xanthine dehydrogenase family protein, molybdopterin-binding subunit	PaoC	b0284	4.51	2.64	6.28 /89.43	10677.52	11983.71	8080.42	2473.21	2272.84	2064.76
formate acetyltransferase	PflB	b0903	9.07	3.61	5.89 /82.08	79820.07	407578.31	309101.28	33823.87	17139.11	36831.71
F0F1 ATP synthase subunit beta	AtpD	b3732	Present ^f	4.35	5.01 /57.70	7653.83	5997.1	5206.97	0	0	0
hydrogenase 3 and formate hydrogenase complex, HycG subunit	HycG	b2719	Present ^f	24.80	6.64 /29.10	19537.53	20459.2	13388.17	0	0	0
Amino acid transport and metabolism											
glutamine synthetase	GlnA	b3870	2.03	7.61	5.26 /61.97	289832.63	37147.68	118378.93	57434.42	80955.52	80633.23
cystine transporter subunit	FliY	b1920	2.31	-1.64	5.06 /27.32	20701.87	35823.57	57987.79	17916.95	18431.98	13205.36
oligopeptide ABC transporter, periplasmic oligopeptide-binding protein	OppA	b1243	2.36	2.47	6.36 /66.12	40116.44	46987.46	33417.05	16323.41	20914.87	13926.92
pyruvate dehydrogenase	PoxB	b0871	2.43	4.87	6.30 /70.51	65159.8	79142.16	52377.86	24985.06	27228.57	28608.11

histidine ABC transporter, periplasmic histidine-binding protein	HisJ	b2309	4.15	9.19	4.98 /29.54	36150.55	26584.92	22719.2	6428.93	9904.91	4274.55
asparagine synthetase B	AsnB	b0674	4.79	1.46	5.55 /67.49	49417.91	78407.34	62968.32	14520.67	15260.93	15743.42
multifunctional acyl-CoA thioesterase I and protease I and lysophospholipase L1	TesA	b0494	Present ^f	3.44	6.38 /20.45	6190.78	9500.38	7482.43	0	0	0
peptidase B	PepB	b2523	Present ^f	0.90	5.61 /49.92	12920.84	26648.82	11392.6	0	0	0
glutamate decarboxylase isozyme	GadB	b1493	Present ^f	25.14	5.01 /59.64	31924.04	27675.11	17496.52	0	0	0
putative transport protein	YdcS	b1440	Present ^f	1.82	6.64 /43.66	29133.55	27857.91	36215.28	0	0	0
predicted oxidoreductase, Zn-dependent and NAD(P)-binding	YbdR	b0608	Present ^f	3.08	6.57 /48.83	30937.75	45376.91	27138.29	0	0	0
nitrogen regulatory protein P-II 2	GlnK	b0450	Present ^f	86.88	6.14 /14.03	58043.93	68251.67	42932.55	0	0	0
selenocysteine synthase	SelA	b3591	2.16	1.12	6.44 /58.27	8310.03	9456.8	6966.4	5634.95	1923.29	3904.54
lysine-arginine-ornithine-binding periplasmic protein	ArgT	b2310	Present ^f	35.73	5.00 /27.90	212837.19	119965.22	105171.81	0	0	0
Nucleotide transport and metabolism											
deoxyribose-phosphate aldolase	DeoC	b4381	3.76	1.19	5.70 /29.02	76300.9	79586.59	74272.4	25008.19	17381.48	18838.22
guanine deaminase	YgfP	b2883	Present ^f	2.07	6.30 /55.73	14275.27	8858.43	15291.98	0	0	0
Carbohydrate transport and metabolism											
alpha,alpha-trehalose-phosphate synthase	OtsA	b1896	2.19	14.34	4.20 /18.84	26775.55	35598.46	28366.25	20257.74	11660.89	9577.1

phosphopyruvate hydratase	Eno	b2779	2.78	1.79	5.46 /50.13	238485.81	335433.31	243209.45	138489.92	108317.13	47516.21
glyceraldehyde-3-phosphate dehydrogenase	GapA	b1779	2.71	-1.01	6.81 /38.17	301419.25	252047.86	269862.31	64522.14	43432.99	195470.06
glycerol-3-phosphate transporter periplasmic binding protein	UgpB	b3453	Present [£]	0.85	6.40 /49.58	13698.9	19931.49	16862.25	0	0	0
glucose-6-phosphate 1-dehydrogenase	Zwf	b1852	Present [£]	2.00	5.63 /61.05	16686.32	8395.39	54276.13	0	0	0
Coenzyme transport and metabolism											
nicotinamide-nucleotide adenyllyltransferase	NadR	b4390	Present [£]	1.78	5.61 /49.92	12920.84	26648.82	11392.6	0	0	0
glycogen branching enzyme	GlgB	b3432	2.09	3.52	6.41 /86.12	7467.03	7851.56	5053.94	2240.98	2915.56	4582.13
Lipid transport and metabolism											
4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	IspG	b2515	Present [£]	2.19	5.93 /45.28	8618.21	10410.06	8816.28	0	0	0
predicted oxidoreductase with NAD(P)-binding Rossmann-fold domain	YohF	b2137	Present [£]	3.11	4.94 /28.94	24960.61	9695.64	9657.03	0	0	0
Translation, ribosomal structure and biogenesis											
polynucleotide phosphorylase/polyadenylase	Pnp	b3164	3.80	3.03	5.03 /84.20	36250.27	92173.78	95961.63	11099.99	41358.55	6600.91
selenocysteine-specific translation elongation factor	SelB	b3590	2.14	1.51	6.60 /77.51	6848.07	9243.59	5522.94	3440.22	3434.1	3220.38
Transcription											

DNA-binding transcriptional regulator CsiR	CsiR	b2664	Present ^f	4.20	6.73 /25.68	4297.85	4668.76	3109.85	0	0	0
Cell wall/membrane/envelope biogenesis											
L-glutamine:D-fructose-6-phosphate aminotransferase	GlmS	b3729	2.38	3.79	5.70 /76.99	83182.41	105947.39	81678.21	28565.22	38858.22	46462.52
outer-membrane lipoprotein carrier protein	LolA	b0891	3.12	-2.01	5.71 /21.04	23890.07	25584.98	22370.58	11224.33	7815.73	4003.94
outer membrane protein TolC	TolC	b3035	Present ^f	2.51	5.01 /59.64	31924.04	27675.11	17496.52	0	0	0
Posttranslational modification, protein turnover, chaperones											
cysteine desulfurase activator complex subunit	SufD	b1681	Present ^f	2.92	6.90 /55.84	6544.38	5925.19	4065.63	0	0	0
glutathione S-transferase YghU	YghU	b2989	3.22	5.68	6.68 /33.76	38692.62	34271.93	26826.43	9706.74	11543.7	9724.58
curved DNA-binding protein CbpA	CbpA	b1000	Present ^f	2.80	6.96 /35.52	32523.53	23415.64	23012.08	0	0	0
periplasmic protein disulfide isomerase I	DsbA	b3860	4.32	3.24	5.39 /24.01	55759.93	29482.02	52869.19	10653.49	10870.27	10440.11
serine peptidase DegQ	DegQ	b3234	Present ^f	2.47	5.01 /57.70	7653.83	5997.1	5206.97	0	0	0
conserved protein	YbeL	b0643	Present ^f	-1.10	5.04 /18.04	22022.03	12809.27	9323.94	0	0	0
Inorganic ion transport and metabolism											
DNA protection during starvation protein	Dps	b0812	3.87	1.53	6.08 /19.52	79388.73	75383.64	74407.08	21475.36	15061.94	22691.99
hydroperoxidase II	KatE	b1732	2.94	15.47	5.70 /88.15	95539.57	72345.97	57848.5	12035.92	21784.89	42976.7
superoxide dismutase	SodC	b1646	Present ^f	2.94	5.76 /19.95	114789.43	108092.42	88218.53	0	0	0
thiosulfate transporter subunit	CysP	b2425	Present ^f	1.15	6.82 /35.63	20128.07	13530.59	24539.7	0	0	0

Secondary metabolites biosynthesis, transport and catabolism											
predicted hydrolase	YcaC	b0897	3.00	3.36	5.22 /25.00	144939.47	97448.28	141008.89	49178.2	34413.05	44121.52
fumarylacetoacetate hydrolase family protein	YcgM	b1180	Present ^f	2.63	6.26 /26.24	21708.94	19513.85	20872.69	0	0	0
Signal transduction mechanisms											
putative sensor-like histidine kinase YfhK	YfhK	b2556	Present ^f	1.65	6.17 /67.51	18207.94	16189.23	9317.45	0	0	0
two-component response regulator	ArcA	b4401	2.09	-5.26	5.23 /29.69	39667.48	62332.28	82985.18	31040.23	34255.95	23194.07
DNA-binding transcriptional regulator CpxR	CpxR	b3912	2.69	-1.06	5.52 /29.19	22428.25	18636.93	14576.23	5213.51	8048.4	7435.03
General function prediction only											
quercetinase activity in vitro	YhhW	b3439	2.29	-0.94	5.18 /32.82	21831.43	10486.6	19326.6	11612.08	6623.29	4357.37
2,5-didehydrogluconate reductase A	DkgA	b3012	3.44	2.07	6.40 /29.46	92496.3	106684.42	86604.19	25515.1	35565.52	21959.24
periplasmic protein	OsmY	b4376	20.31	-1.14	5.36 /21.41	151591.67	118169.62	171191.2	6721.91	10278.41	4706.62
predicted oxidoreductase with NAD(P)-binding Rossmann-fold domain	YhhX	b3440	Present ^f	1.65	6.52 /48.13	15423.54	10153.25	5881.94	0	0	0
oxidoreductase, zinc-binding dehydrogenase family	YjgB	b4269	Present ^f	2.09	6.24 /42.12	47531.85	39563.63	41745.54	0	0	0
predicted intracellular protease	YhbO	b3153	Present ^f	7.50	5.38 /22.84	64092.88	29909.31	67314.84	0	0	0
Function unknown											
hypothetical protein	YigA	b3810	2.21	-1.01	5.47 /22.72	25648.86	15764.65	16649.71	8065.59	9922.33	8329.08

conserved protein	YicC	b3644	2.11	2.88	4.97 /38.15	22058.33	10631.31	9523.56	5006.41	6441.15	8573.92
predicted protein	YgaU	b2665	5.06	5.62	5.96 /16.34	245207.84	253176.19	234093.84	52365.71	45143.2	47247.07
putative structural proteins	YciF	b1258	Present ^f	2.62	5.71 /22.35	19012.46	20388.78	18002.55	0	0	0
Nitrogen fixation island											
nitrogenase metallocusters biosynthesis protein NifS	NifS ^g	PST135 1	Present ^f	51.3	5.78 /53.42	6963.21	14656.29	3414.32	0	0	0
MoFe protein, alpha subunit	NifD ^g	PST132 7	Present ^f	32.4	6.17 /67.51	18207.94	16189.23	9317.45	0	0	0
Predicted dehydrogenases and related proteins	PST133 6 ^g	PST133 6	Present ^f	39.6	5.38 /19.38	24088.09	8742.44	19414.71	0	0	0
Downregulated											
RNA processing and modification											
oligoribonuclease	Orn	b4162	-3.03	-1.18	4.73 /24.43	9098.19	14068.15	9330.53	36131.91	29164.15	33109.08
Energy production and conversion											
dihydrolipoamide dehydrogenase	LpdA	b0116	-2.99	-2.75	6.18 /57.58	56598.43	127645.84	25557.87	254452.25	57837.09	315874.13
phosphoenolpyruvate carboxykinase (ATP)	Pck	b3403	absent	-1.17	5.59 /68.04	0	0	0	36430.63	38259.81	48119.72
predicted oxidoreductase	YbdH	b0599	-3.58	1.42	5.90 /43.78	8774.57	10979.1	8114.74	30006.88	43497.24	26280.76
oxygen-insensitive NAD(P)H nitroreductase	NfnB	b0578	-2.16	1.54	5.96 /26.37	28804.92	41919.6	26287.59	53080.47	143290.31	12978.92
Amino acid transport and metabolism											
acetolactate synthase I, large subunit	IlvB	b3671	absent	0.73	5.30 /68.75	0	0	0	38016.14	73289.27	35485.25

threonine deaminase	IlvA	b3772	absent	-1.55	5.69 /63.73	0	0	0	24481.38	28379.67	38401.97
O-succinylhomoserine (thiol)- lyase	MetB	b3939	absent	1.14	6.45 /44.68	0	0	0	24239.77	34008.5	24223.99
glutamate dehydrogenase, NADP-specific	GdhA	b1761	-7.40	-2.83	6.44 /52.06	29211.08	30002.09	30655.72	277676.16	217072.55	170477.48
5,10-methylenetetrahydrofolate reductase	MetF	b3941	-8.69	1.96	6.26 /30.84	7299.61	7103.46	4704.97	11787.5	93968.05	60224.76
dihydroxyacid dehydratase	IlvD	b3771	-8.00	1.02	5.81 /74.30	13936.62	15733.89	6622.83	101661.64	96741.55	92073.8
branched-chain amino acid aminotransferase	IlvE	b3770	-5.22	-1.53	5.56 /35.82	18327.35	21701.1	14432.3	63254.97	117118.41	103894.3
shikimate kinase I	AroK	b3390	-2.41	1.86	5.37 /20.38	16142.32	8690.47	18526.2	33093.54	41515.46	30049.91
cysteine synthase A	CysK	b2414	-2.35	-1.71	5.93 /36.78	14016.04	18586.75	13035.83	34556.94	37422.72	35246.63
phospho-2-dehydro-3- deoxyheptonate aldolase	AroG	b0754	-2.74	-1.44	6.61 /41.26	36909.09	31800.61	35035.91	94650.8	117022.41	72191.32
Nucleotide transport and metabolism											
deoxycytidine triphosphate deaminase	Dcd	b2065	-2.40	-1.33	5.89 /26.07	9384.1	10606.33	8500.86	23506.97	21230.95	23630.9
nucleoside diphosphate kinase	Ndk	b2518	-4.11	-1.27	5.82 /16.17	22277.23	26338.69	22684.02	102015.91	105794.41	85167.12
adenine phosphoribosyltransferase	Apt	b0469	-2.30	1.89	5.29 /24.80	30153.17	29483.78	38498.63	75689.65	75418.02	74900.48
Carbohydrate transport and metabolism											
6-phosphogluconate dehydrogenase	Gnd	b2029	-2.44	1.05	5.03 /52.58	88572.82	47881.77	70907.34	233766.09	69702.33	203984.17

galactose-1-epimerase (mutarotase)	GalM	b0756	-4.06	1.19	4.52 /46.18	4161.61	7899.85	4001.38	26761.48	19006.74	19442.58
dihydroxyacetone kinase subunit M	DhaM	b1198	-2.37	1.74	4.28 /61.44	2577.76	5994.25	3071.64	12839.21	7310.6	7472.04
Coenzyme transport and metabolism											
riboflavin synthase subunit alpha	RibE	b0415	-2.54	-5.56	5.94 /25.86	12778.35	14581.08	11278.82	38269.34	31827.47	28207.88
GTP cyclohydrolase II	RibA	b1277	-2.56	-1.86	5.90 /24.91	11777.34	11443.32	8796.5	29363.88	27978.73	24642.35
S-adenosylmethionine synthetase	MetK	b2942	-2.82	1.13	4.93 /50.27	26681.05	29343.18	20474.42	90838.11	33628.05	91264.6
glutamate-1-semialdehyde aminotransferase	HemL	b0154	-3.38	1.24	4.41 /49.33	6183.41	8573.31	8262.15	36710.5	23063.72	18065.61
pyridoxamine kinase	PdxY	b1636	absent	1.49	6.53 /30.40	0	0	0	8598.31	9861.19	8770.25
Lipid transport and metabolism											
4-hydroxy-3-methylbut-2-enyl diphosphate reductase	IspH	b0029	-2.33	-2.10	5.13 /39.24	3669.75	4141.6	3417.17	10769.77	7088.45	8333.4
Translation, ribosomal structure and biogenesis											
tyrosyl-tRNA synthetase	TyrS	b1637	-2.22	1.24	5.84 /50.21	37375.21	58452.7	31579.82	81569.73	82705.83	118206.37
ribosome recycling factor	Frr	b0172	-3.08	1.27	5.91 /24.41	7840.33	8898.43	8490.84	31657.99	26231.36	19713.46
50S ribosomal protein L7/L12	RplL	b3986	-3.18	1.39	4.28 /14.56	47803.42	66588.53	63704.26	229539.64	171419.98	165383.13
methionyl tRNA synthetase	MetG	b2114	-3.61	2.44	5.79 /80.87	10972.85	33312.61	4419	52287.39	59628.54	64036.46
leucyl-tRNA synthetase	LeuS	b0642	-2.28	2.71	5.11 /96.46	7332.34	9844.02	10359.16	21477.2	20512.73	20778.93
Transcription											
transcriptional repressor MprA	MprA	b2684	-5.29	-1.05	6.15 /21.06	8752.04	1545.35	6165.73	29917.84	29967.06	27190.46

transcriptional regulator kdgR	IclR	b4018	-6.48	1.20	5.44 /32.09	24074.2	16642	27680.72	150553.41	151445.09	141386.81
transcription antitermination protein NusG	NusG	b3982	absent	1.80	6.38 /21.60	0	0	0	6977.88	8770.56	5386.64
DNA-binding transcriptional regulator OxyR	OxyR	b3961	-2.22	1.84	6.47 /37.29	19712.31	12220.15	11013.55	28567.06	36083.06	30754.15
Cell wall/membrane/envelope biogenesis											
alanine racemase	Alr2	b4053	absent	-1.08	6.85 /44.53	0	0	0	4928.02	5321.1	4894.04
3-deoxy-manno-octulosonate cytidyltransferase	KdsB	b0918	-2.00	1.17	5.09 /32.68	23208.32	10632.43	12552.83	42314.24	28138.5	22163.18
glucose-1-phosphate thymidyltransferase	RfbA	b2039	-3.04	1.53	5.53 /31.73	16907.38	23722.25	17684.05	73826.11	51039.49	52174.43
GDP-mannose 4,6-dehydratase	Gmd	b2053	absent	1.62	6.14 /46.98	0	0	0	25036.07	5950.76	8019.78
outer membrane protein A	OmpA	b0957	-2.50	1.86	5.90 /18.97	11913.64	9947.09	7138.24	26625.68	23994.02	21946.15
D-alanyl-alanine synthetase A	DdlB	b0092	-3.02	3.52	4.80 /49.47	11585.98	11187.74	8210.42	38246.95	33031.5	22161.79
Posttranslational modification, protein turnover, chaperones											
ATP-dependent Clp protease proteolytic subunit	ClpP	b0437	absent	-2.38	5.77 /25.10	0	0	0	20241.49	10250.34	15811.18
heat shock protein IbpA	IbpA	b3687	-15.46	-4.33	5.88 /16.01	14930.61	16020.35	8425.25	288347.63	49751.41	270715.31
alkyl hydroperoxide reductase subunit C	AhpC	b0605	-5.36	-1.44	4.82 /23.40	63761.68	87968.7	142460.13	272085.38	834374.38	471427.28
Hsp33-like chaperonin	HslO	b3401	-3.18	-16.38	4.02 /41.23	13268.13	19124.97	13699.24	36743.54	49281.96	60575.85
alkyl hydroperoxide reductase, F52a subunit, FAD/NAD(P)-binding	AhpF	b0606	-2.08	-0.95	5.60 /60.81	24979.74	51586.84	28670.05	73367.01	53821.85	91792.66

peroxiredoxin	Bcp	b2480	-2.02	3.09	4.87 /18.25	29997.48	24790.01	14579.71	61259.92	55028.6	23709.66
heat shock chaperone IbpB	IbpB	b3686	-2.02	-5.71	5.11 /15.88	63345.59	157825.98	155647.95	282802.44	259774.81	217381.23
General function prediction only											
ClpXP protease specificity-enhancing factor	SspB	b3228	-3.75	-1.56	4.16 /26.45	7673.35	10968.57	12966.14	24000.53	53391.7	41024.33
modulator of drug activity B	MdaB	b3028	-3.17	-1.83	6.26 /23.94	5124.2	4836.35	4320.23	17199.98	14016.38	14083.69
global DNA-binding transcriptional dual regulator H-NS	Hns	b1237	absent	-1.34	5.57 /17.03	0	0	0	23813.91	28365.97	24136.01

a Protein names, accession numbers and descriptions are from EcoCyc database. The data are grouped according to their biological function.

b Translation level ratios (cells under nitrogen-fixation conditions/cells under nitrogen-excess conditions).

c Transcriptional ratios obtained from DNA microarray experiments (see Materials and Methods) are indicated.

d Data set of EN01-1, EN01-2 and EN01-3 was the gray intensity of protein from three repetitions under nitrogen-fixation conditions.

e Data set of EN01-NH₄⁺-1, EN01-NH₄⁺-2 and EN01-NH₄⁺-3 was the the gray intensity of protein from three repetitions under nitrogen-excess conditions.

f Absent: all proteins were detectable only under nitrogen-fixation conditions and were absent under nitrogen-excess conditions.

g Protein-coding genes located on the nitrogen fixation island.

Table S3. Transcriptionally altered genes in recombinant *E. coli* strain EN-01 under nitrogen-fixation conditions compared with the nitrogen-excess conditions.

Gene name	Locus_tag	Change ratio	Functional description
nitrogen regulatory system genes and nitrogen assimilation related genes			
<i>rpoN</i>	b3202	3.64	RNA polymerase, sigma 54 (sigma N) factor
<i>ntrB</i>	b3868	10.61	nitrogen regulation protein NR(I), <i>glnG</i>
<i>ntrC</i>	b3869	10.36	nitrogen regulation protein NR(II), <i>glnL</i>
<i>glnB</i>	b2553	2.12	regulatory protein P-II for glutamine synthetase
<i>glnK</i>	b0450	86.88	nitrogen assimilation regulatory protein for GlnL, GlnE, and AmtB
<i>amtB</i>	b0451	55.28	ammonium transporter
<i>glnA</i>	c4819	7.61	glutamine synthetase
<i>glnD</i>	b0167	5.27	PII uridylyl-transferase / uridylyltransferase
<i>glnH</i>	b0811	2.74	glutamine ABC transporter periplasmic protein
<i>glnP</i>	b0810	9.04	glutamine transporter subunit
<i>glnQ</i>	b0809	9.16	glutamine ABC transporter ATP-binding protein
<i>gltB</i>	b3212	14.16	glutamate synthase, large subunit
<i>gltD</i>	b3213	7.49	glutamate synthase, 4Fe-4S protein, small subunit
<i>gdhA</i>	b1761	-2.83	glutamate dehydrogenase
<i>nac</i>	b1988	124.98	nitrogen assimilation transcriptional regulator
<i>gltI</i>	b0655	3.56	glutamate and aspartate transporter subunit
<i>gltJ</i>	b0654	3.84	glutamate and aspartate transporter subunit
<i>gltK</i>	b0653	5.27	glutamate and aspartate transporter subunit
<i>gltL</i>	b0652	4.18	glutamate and aspartate transporter subunit
<i>gltP</i>	z5676	11.78	glutamate/aspartate:proton symporter
<i>gadC</i>	b1492	12.19	predicted glutamate/gamma-aminobutyric acid antiporter
<i>hisM</i>	b2307	10.45	histidine/lysine/arginine/ornithine transporter subunit M
<i>hisP</i>	b2306	4.52	histidine/lysine/arginine/ornithine transporter subunit P
<i>hisQ</i>	b2308	10.30	histidine/lysine/arginine/ornithine transporter subunit Q
<i>lysP</i>	b2156	3.31	lysine transporter
<i>argT</i>	b2310	35.73	lysine/arginine/ornithine transporter subunit
<i>hisJ</i>	b2309	9.19	histidine transport system histidine-binding periplasmic protein
<i>yqjU</i>	b3089	2.34	sodium:serine/threonine symporter
<i>potC</i>	b1124	2.03	polyamine transporter subunit
<i>potD</i>	b1123	2.18	polyamine transporter subunit
<i>gsiB</i>	b0830	4.77	predicted peptide transporter subunit: periplasmic-binding component of ABC superfamily
<i>oppA</i>	b1243	2.47	oligopeptide transporter subunit
<i>oppC</i>	b1245	2.17	oligopeptide transporter subunit
<i>oppD</i>	b1246	3.09	oligopeptide transporter subunit
<i>gsiC</i>	b0831	2.02	predicted peptide transporter subunit: membrane component of ABC superfamily

<i>ddpA</i>	b1487	7.49	putative hemin-binding lipoproteinputative hemin-binding lipoprotein
<i>dppA</i>	b3544	6.89	dipeptide transporter
<i>dppB</i>	b3543	2.07	dipeptide/heme transporter
<i>dppD</i>	b3541	2.26	dipeptide/heme transporter
<i>gsiA</i>	b0829	2.34	glutathione transporter ATP-binding protein, ABC superfamily
<i>sapA</i>	b1294	2.41	antimicrobial peptide transport ABC transporter periplasmic binding protein
<i>sapB</i>	b1293	2.54	antimicrobial peptide transport ABC transporter permease
<i>sapC</i>	b1292	2.45	antimicrobial peptide transport ABC transporter permease
<i>sapD</i>	b1291	2.8	antimicrobial peptide transport ABC system ATP-binding protein
<i>astD</i>	b1746	17.79	succinylglutamic semialdehyde dehydrogenase
<i>astE</i>	b1744	14.33	succinylglutamate desuccinylase
<i>astB</i>	b1745	16.68	succinylarginine dihydrolase
<i>astC</i>	b1748	41.67	succinylornithine transaminase, PLP-dependent
<i>astA</i>	c2147	36.61	arginine succinyltransferase
<i>gabD</i>	b2661	2.3	succinate-semialdehyde dehydrogenase I, NADP-dependent
<i>gabP</i>	b2663	2.53	gamma-aminobutyrate transporter
<i>gabT</i>	b2662	5.61	4-aminobutyrate aminotransferase, PLP-dependent
<i>flkB</i>	ECs5185	3.46	peptidyl-prolyl cis-trans isomerase
<i>codA</i>	c0456	1.16	cytosine deaminase
<i>codB</i>	b0336	1.63	cytosine transporter
<i>nupC</i>	b2393	1.43	nucleoside (except guanosine) transporter
<i>ydcS</i>	b1440	1.82	predicted spermidine/putrescine transporter subunit
<i>ydcT</i>	b1441	1.80	putative ATP-binding component of a transport system
<i>ydcU</i>	b1442	1.83	predicted spermidine/putrescine transporter subunit
<i>ydcV</i>	c1867	1.58	predicted spermidine/putrescine transporter subunit
<i>ydcW</i>	b1444	-1.11	gamma-aminobutyraldehyde dehydrogenase
<i>ydcG</i>	b1006	13.1	pyrimidine permease
<i>ydcF</i>	b1005	4.94	hypothetical protein
Central carbon pathways related genes			
<i>pgi</i>	b4025	3.40	glucose-6-phosphate isomerase
<i>fbp</i>	b4232	1.63	fructose-1,6-bisphosphatase
<i>tpiA</i>	b3919	-1.15	triosephosphate isomerase
<i>fba</i>	b2097	2.33	fructose-bisphosphate aldolase
<i>gapC</i>	b1417	2.20	glyceraldehyde 3-phosphate dehydrogenase
<i>pgk</i>	b2926	1.14	phosphoglycerate kinase
<i>gpmA</i>	b0755	-1.21	phosphoglycerate kinase

<i>eno</i>	b2779	1.79	phosphopyruvate hydratase
<i>pykF</i>	b1676	8.28	pyruvate kinase
<i>ldhA</i>	b1380	-4.18	D-lactate dehydrogenase
<i>adhE</i>	b1241	3.12	bifunctional acetaldehyde-CoA/alcohol dehydrogenase
<i>pta</i>	b2297	4.27	phosphate acetyltransferase
<i>ackA</i>	b2296	2.54	acetate kinase
<i>aceE</i>	b0114	-3.01	pyruvate dehydrogenase subunit E1
<i>aceF</i>	z0125	-2.11	dihydrolipoamide acetyltransferase
<i>lpdA</i>	b0116	-2.75	lipoamide dehydrogenase
<i>pflB</i>	z1248	5.44	formate acetyltransferase 1
<i>fdhF</i>	b4079	6.59	selenopolypeptide subunit of formate dehydrogenase H
<i>ppc</i>	b3956	10.64	phosphoenolpyruvate carboxylase
<i>maeB</i>	b2463	-5.36	predicted phosphotransacetylase
<i>gltA</i>	z0873	-2.73	type II citrate synthase
<i>acnB</i>	z0128	-3.29	bifunctional aconitate hydratase 2, 2-methylisocitrate dehydratase
<i>icdA</i>	b1136	1.31	isocitrate dehydrogenase
<i>frdA</i>	b4154	-3.56	fumarate reductase flavoprotein subunit
<i>aceA</i>	b4015	-2.15	isocitrate lyase
<i>aceB</i>	b4014	-5.69	malate synthase
<i>mdh</i>	b3236	-1.16	malate dehydrogenase
<i>zwf</i>	b1852	2.02	glucose-6-phosphate 1-dehydrogenase
<i>gnd</i>	c2556	1.15	6-phosphogluconate dehydrogenase
<i>rpiA</i>	b2914	1.27	ribose-5-phosphate isomerase A
<i>rpiB</i>	b4090	1.23	ribose-5-phosphate isomerase B
<i>tktA</i>	b2935	2.72	transketolase
<i>tktB</i>	b2465	17.14	transketolase
<i>talA</i>	b2464	7.64	transaldolase
<i>edd</i>	b1851	-2.34	phosphogluconate dehydratase
<i>eda</i>	b1850	-2.85	keto-hydroxyglutarate-aldolase
<i>appA</i>	b0980	2.74	phosphoanhydride phosphorylase
<i>appB</i>	b0979	3.37	cytochrome bd-II oxidase subunit II
<i>appC</i>	b0978	7.63	cytochrome bd-II oxidase subunit I
<i>hyaA</i>	b0972	7.98	hydrogenase-1 small subunit
<i>hyaB</i>	b0973	5.71	hydrogenase 1 large subunit
<i>hyaC</i>	b0974	5.32	hydrogenase 1 b-type cytochrome subunit
<i>hyaD</i>	b0975	4.81	hydrogenase 1 maturation protease
<i>hyaE</i>	b0976	2.52	hydrogenase-1 operon protein HyaE
<i>hyaF</i>	b0977	2.27	hydrogenase-1 operon protein HyaF
<i>fhlA</i>	b2731	2.15	DNA-binding transcriptional activator
<i>hycA</i>	b2725	13.41	formate hydrogenlyase regulatory protein HycA
<i>hycB</i>	b2724	19.03	formate hydrogenlyase subunit 2
<i>hycC</i>	b2723	13.51	formate hydrogenlyase subunit 3

<i>hycD</i>	b2722	15.02	membrane-spanning protein of hydrogenase 3
<i>hycE</i>	b2721	14.89	formate hydrogenlyase subunit 5 precursor
<i>hycF</i>	b2720	18.84	formate hydrogenlyase complex iron-sulfur subunit
<i>hycG</i>	b2719	24.8	formate hydrogenlyase complex iron-sulfur subunit
<i>hycH</i>	b2718	9.82	formate hydrogenlyase maturation protein
<i>ndh</i>	b1109	2.41	respiratory NADH dehydrogenase 2/cupric reductase
<i>napH</i>	b2204	2.04	ferredoxin-type protein essential for electron transfer from ubiquinol to periplasmic nitrate reductase (NapAB)
<i>narG</i>	b1224	2.26	nitrate reductase 1, alpha subunit
<i>narH</i>	b1225	2.29	nitrate reductase 1, beta (Fe-S) subunit
<i>narI</i>	b1227	2.02	nitrate reductase 1, gamma (cytochrome b(NR)) subunit
<i>narJ</i>	b1226	3.05	molybdenum-cofactor-assembly chaperone subunit (delta subunit) of nitrate reductase 1
<i>narW</i>	b1466	2.33	nitrate reductase 2 (NRZ), delta subunit (assembly subunit)
<i>narY</i>	b1467	2.63	nitrate reductase 2 (NRZ), beta subunit
<i>narZ</i>	b1468	3.77	nitrate reductase 2 (NRZ), alpha subunit
<i>narU</i>	b1469	3.63	nitrate/nitrite transporter
<i>nirD</i>	b3366	3.03	nitrite reductase, NAD(P)H-binding, small subunit
<i>norR</i>	b2709	4.92	Anaerobic nitric oxide reductase DNA-binding transcriptional activator
<i>tatB</i>	b3838	2.44	TatABCE protein translocation system subunit
<i>tatA</i>	b3836	2.49	TatABCE protein translocation system subunit
<i>tatE</i>	b0627	4.43	TatABCE protein translocation system subunit
<i>pflB</i>	b0903	5.44	formate acetyltransferase 1
<i>adhP</i>	c1911	3.56	alcohol dehydrogenase
<i>yjgB</i>	b4269	2.09	predicted alcohol dehydrogenase
Oxygen protection related genes			
<i>sodC</i>	b1646	2.94	superoxide dismutase, Cu, Zn
<i>katE</i>	b1732	15.47	hydroperoxidase HP(II)(III) (catalase)
<i>ccmD</i>	b2198	4.13	cytochrome c biogenesis protein
<i>nrfA</i>	b4070	2.24	nitrite reductase, formate-dependent, cytochrome
<i>osmC</i>	b1482	5.81	Lipoyl-dependent Cys-based peroxidase, hydroperoxide resistance; salt-shock inducible membrane protein; peroxiredoxin
<i>yhbO</i>	b3153	7.5	stress-resistance protein
<i>dps</i>	b0812	1.53	DNA starvation/stationary phase protection protein Dps

Table S4. Peak area of organic acids in the medium from *E. coli* EN-01 and *P. stutzeri* A1501 under nitrogen-fixation conditions.

organic acids	<i>E. coli</i> DH10B		<i>E. coli</i> EN-01		<i>P. stutzeri</i> A1501	
	0 h	45 h	0 h	45 h	0 h	10 h
Pyruvic acid	0	1222.4	0	560.9	0	252.4
Succinic acid	0	231.1	0	212.2	0	0
Lactic acid	0	675.8	0	366.1	3228.6	3047
Formic acid	0	372.8	0	404.7	0	0
Acetic acid	0	389.9	0	422.1	0	0

Table S5. Bacterial strains and plasmids used in this study.

Bacterial Strains	Genotype or description	Reference or source
<i>E.coli</i>		
DH10B	Wild-type strain of <i>E. coli</i>	Laboratory stock
EN-01	<i>E. coli</i> DH10B strain bearing the NFI of <i>Pseudomonas stutzeri</i> A1501	Han <i>et al.</i>
<i>Pseudomonas stutzeri</i>		
A1501	Wild-type strain of <i>Pseudomonas stutzeri</i>	Laboratory stock

Table S6. List of primers used in this study.

Gene name	Primers	Sequence (5' - 3')
PST1302	PST1302F	CGCATACGCCAGCAACTCG
	PST1302R	CCGCTGTTCTTCAATGCCTC
PST1303	PST1303F	TCAAGAGCACGCCAAATCA
	PST1303R	TGACCGTGCTCGCAACAGA
<i>nifQ</i>	PST1304F	GAACAGGACGGCGGCTAC
	PST1304R	TGCTCTTCTCCAAAACAATC
PST1305	PST1305F	TCAACGGGCCTGGATGG
	PST1305R	CGACTACGCCGAGCAACA
<i>nifB</i>	PST1306F	CCTGGATCTACTGGAACAAC
	PST1306R	ATGCGGCTGACCTCTTT
<i>rnfB</i>	PST1316F	ATTCTGGTTCTTGCACTGAT
	PST1316R	CGCCTCGATTTCCTTGAT
<i>rnfC</i>	PST1317F	CCCTCGCACATTCCCTT

	PST1317R	TGCTTGACGTAATCGTTCTT
<i>rnfD</i>	PST1318F	CGCTGGTCTTCGTCATT
	PST1318R	ACCTGGCGGGTCCACTTCG
<i>rnfG</i>	PST1319F	TCCAGTTCATCGCCCTCG
	PST1319R	CCACCGTCCTTCTTCACC
<i>rnfE</i>	PST1320F	CGGCAGCGGCACGTTGTT
	PST1320R	CCAGCACGAAGCCCAGA
<i>rnfH</i>	PST1321F	CTGAGCAAGCAAAAGGTC
	PST1321R	CGTCGTCATCGTCGTCT
<i>nifY2</i>	PST1322F	CATCGCCTCCAACAACG
	PST1322R	GGAGACCACATAGAGCACC
PST1325	PST1325F	ACCAGAGCGAGGTCAATCCC
	PST1325R	ATGCCGAGGAACAGGTAGCG
<i>nifH</i>	PST1326F	GATGATGGCGATGTATGC
	PST1326R	TCGGTGTTGCGGCTGTT
<i>nifT</i>	PST1329F	CAAGAAGGACCAGGAAGAA
	PST1329R	AGGGTGATCGGCAGTTT
<i>nifY</i>	PST1330F	CTCTACACCCTGAGCATCG
	PST1330R	GGCCGCATGACCTTGAC
PST1331	PST1331F	AGCGAGCACCTGGTATCC
	PST1331R	GCAGTGCTGGCTGGAGTTGA
PST1332	PST1332F	CTGTTCCGCTTCATCCGC
	PST1332R	GCTTTCCTCAGGCAGACGCT
<i>nifE</i>	PST1333F	TACCAAGAACCTCGGCAACC
	PST1333R	CGATGTTGTATTCACCGATCAG
<i>nifN</i>	PST1334F	GCGTTCGCCAAGGTGTTCT
	PST1334R	GCTGCTTGTCGCAAATGGT
<i>nifX</i>	PST1335F	ACCAGCATTTTCGGTTCGTC
	PST1335R	TTGTCCTCGTTGCCGTCCT
PST1336	PST1336F	GGCACCTACGACACCTGG
	PST1336R	TCGCCGACGATGGGGAT
PST1341	PST1341F	GCGATTTCTTCGGCAGCAT
	PST1341R	CGAACTCGGCATAGACCTG
PST1342	PST1342F	CAGCATCGCCAACAAGACC

	PST1342R	CACCCTCGGCAATCACCC
PST1344	PST1344F	TTCCAGTCATCGCAACAGAG
	PST1344R	CGGCGGATAGAGCAGGT
<i>modC</i>	PST1345F	CTGGAAGTCAACGGCGAACG
	PST1345R	TCACCACATGGTCCCAGTCC
<i>modB</i>	PST1346F	GTGGGCTCGGTGCTTTACT
	PST1346R	ACGCTGAAGAAGGTGTCCC
<i>modA</i>	PST1347F	AGCCGCTTCACCTATGCCA
	PST1347R	GCGGTCTAAGGTTTCCAGTG
PST1348	PST1348F	ATGCAGGCCGAATACGAGC
	PST1348R	ATTGATTGCGTGCGCTGGT
<i>nifU</i>	PST1350F	GTGACGTCGGCTCGCTGAG
	PST1350R	CTCGTCGACGGTCAGCCC
<i>nifS</i>	PST1351F	TGAACAAGGTCGGCATCG
	PST1351R	TGTCCATCGCACGCATAA
<i>nifV</i>	PST1352F	CGATACGGTGGGAGTAATGG
	PST1352R	CGTTGACCGTGGTGTGATAT
<i>cysE</i>	PST1353F	GCACGGTGGTCGGTATCC
	PST1353R	GGTCGGGAATCAGGTAATGG
PST1354	PST1354F	TGTTCCGGCGAGGGCAAGG
	PST1354R	GGGGAAAGGTGAAAATGGC
<i>nifW</i>	PST1355F	GCGCTGGAAGAGCTGGTAT
	PST1355R	AGGGCACGCCGAAGAAGTT
<i>nifZ</i>	PST1356F	GTCTGCCGCTCTACGAATATG
	PST1356R	CAGCCCTGATCGAGGAAAT
<i>nifM</i>	PST1357F	CTGGGCTTTCACCTGCTGT
	PST1357R	TGGTGCCGCTCGTATTGAC
PST1358	PST1358F	CCTACATGCAGCATCTGGACG
	PST1358R	CTTCTCCAGCTCGACCGTTC
<i>nifF</i>	PST1359F	GTTTCTCGATGCGATGGG
	PST1359R	TGAGGTTGCTCTGGTTGTCC
16s	16SF	CCTACGGGAGGCAGCAG
	16SR	ATTACCGCGGCTGCTGG