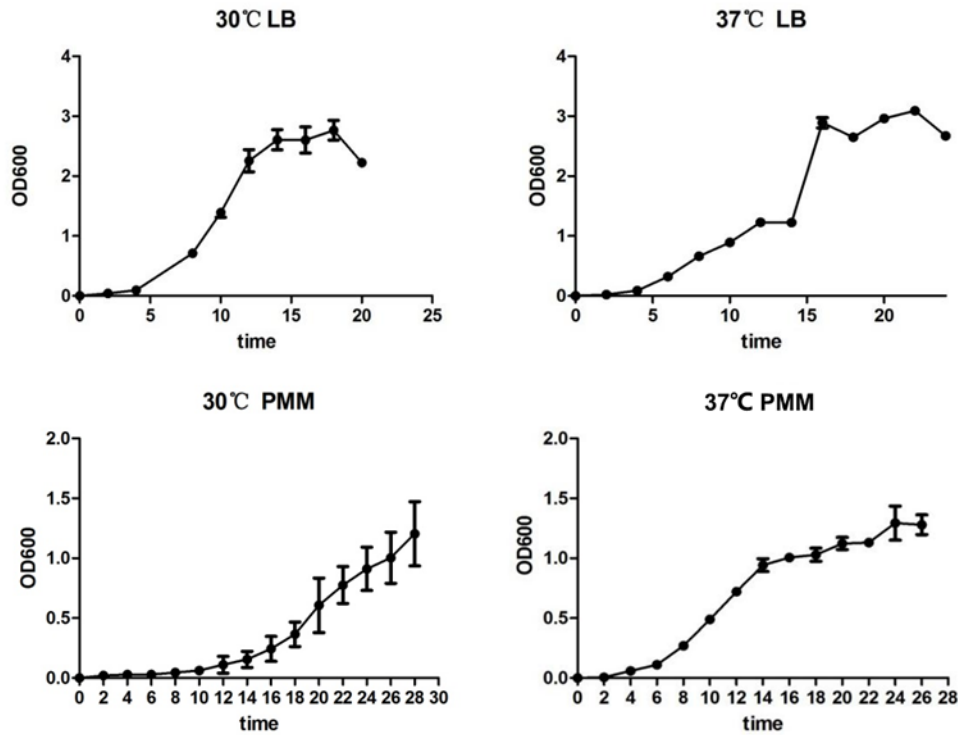


1 Supplementary Information



2

3 Figure S1. The growth curve of *P. stutzeri* DSM4166 under four culture
 4 conditions. The strain DSM4166 was cultured in LB liquid medium and PMM
 5 liquid medium at 30°C and 37°C respectively. For each culture condition, three
 6 time point were selected for RNA-seq analysis according the growth curve. 30°C
 7 LB: 10h, 14h, 18h; 37°C LB, 30°C PMM and 37°C PMM: 12h, 16h, 24h.

8

9 Table S1. Oligonucleotides for cloning of screened endogenous promoter

10 regions from *P. stutzeri* DSM4166.

Primer name	Sequence (5'-3')	PCR product
pBBR1-1	CCTCATAAAGGCCAAGAAGGGCGGAAAGTCCAAATTGT AAGCTAGCCATATGGCTTACCG	pBBR1 vector
pBBR1--2	ATCTGCATAAAACTGTTGTAAT	
firefly-1	ATGGAAGACGCCAAAAACAT	<i>firefly</i>
firefly-2	TTACAATTTGGACTTTCCGCC	
03385-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TAAAATTATTTTCGCCTCAACGC	P03385
03385-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGACTTCACCTCGATCG	
03670-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TAGCTCAAGTAAGCGACGACC	P03670
03670-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TCAGCGTTCCCCAGCTT	
04125-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TTGGTCGTTCTGTTTTGTTGT	P04125
04125-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGGGGCGCTCCTAATTG	
05750-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGGATCGGCTCCAGTTCAGG	P05750
05750-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TCGAAACCTCCAGTTTGC	
06545-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGGCTGGCGTCCTGAATAG	P06545

06545-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TTTGATGTCTCCTTGAACC	
07275-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGCTGCCTCCATAGTGGTTC	
07275-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGGCTTGGGGGCGACG	P07275
07800-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TCAGAGAATCCTCGTTTCAA	
07800-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGACAATAACGGGTACTG	P07800
07835-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TATGGTTCATCTCGTTGAAGG	
07835-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TTGAAAGTCTCCTGCAGA	P07835
09495-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TCGCATCAGCAACGGTTCAC	
09495-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGGTGTTTGCCTCCAAG	P09495
09555-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TTACCTGATGACACCCCGGTC	
09555-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGTCTGGTCACCTTCGATA	P09555
10035-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TTTCATGGTGAGCTCCTGTTG	
10035-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGATGACAACCTCTTGAC	P10035
10410-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGCAGTTCGTTTCATGTGCC	P10410

10410-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGGTGATCTCTCCACATTAGT	
10530-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGATCGAACAAAGGGGCCTT	
		P10530
10530-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TAAATTCAGCTCTACGAC	
11665-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGCGCATAACGGTTCTAGG	
		P11665
11665-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGCTGTTATCCCTCTGCA	
11670-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGGACATTTAGCGTTAACTCTC	
		P11670
11670-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGCTGGACTCCGTCTT	
11845-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TTTGGCTGGATGATCGGAAC	
		P11845
11845-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGTTGATTCCTGCTGTTTT	
12445-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TCATTCAGGAAGCATCACGGC	
		P12445
12445-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TTTCTTGTTCTCCTCGTTGG	
12505-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TCGTTTCGGTTCTTCCTGG	
		P12505
12505-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGTATGGCTCCGCTTG	
16030-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TCCATCACAGTACCAAGCC	
		P16030

16030-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGTCTTTGTATCCTTGAAAC	
17330-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TATTTGACCCTTGCGTCAG	
		P17330
17330-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGTCGTTCAAATTA ACTAC	
18460-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGGCAGTTACGGCGTCGG	
		P18460
18460-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGGGATGTCCTCCATTTG	
18725-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TACGAGAAACGATGATACTTGG	
		P18725
18725-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TTAAGAGATGTCACCTTTC	
19520-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGCGCTTCCCTATCCACGAC	
		P19520
19520-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TTTACGGAGTCACTCCAAA	
19680-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TTGAGGTTTCCTTCACAACAG	
		P19680
19680-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TGGCTTGCTCCATGGCG	
21820-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGTTGGGGGCTTTACGCGCT	
		P21820
21820-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TCCGACCGCCAGAGTACCGT	
22180-1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TGCCAAGGCGGCGGGATCG	
		P22180

22180-2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TTGGTGCAGCTTGTGGTGC	
P _{genta} -1	GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA TTGAAGGCACGAACCCAGTTG	
P _{genta} -2	ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA TCGTTGCTGCTCCATAAC	P _{genta}

12 Table S2. Oligonucleotides used for construction of DSM4166-attB strain.

Primer name	sequence (5'-3')	PCR product
pK18mob-1	GCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCC ACCTCACCCATCTGGTCCATCATGATGAATTCGTAATCAT GGTCATAG	pK18mob vector
pK18mob-2	GGCACTGGCCGTCGTTTTAC	
19520-1	GTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCC ATGGCAACGAAACTCTGG	PSTAA_19520
19520-2	GTGGAGTACGCGCCCGGGGAGCCCAAGGGCACGCCCTG GCACCCGCACCGCGGCTTCGAGACCGTCCCTTGGAGACG GTGTAAGAC	
19520-check-F	AAACGATGTCGCCGATCTTC	Crossover-L colony PCR check
lac-seq	AGGCACCCCAGGCTTTACAC	
pK18-seq	CGGGCCTCTTCGCTATTAC	Crossover-R colony PCR check
19520-check-R	TAGAGGGGGCGAGACATAAG	

13

14

15 Table S3. Oligonucleotides used for construction of DSM4166-P12445-nifA,
 16 DSM4166-P11670-nifA and DSM4166-P12445-nifHDK-P11670-nifA strains.

Primer name	sequence (5'-3')	PCR product
p15A-1	AGATCCGAAAACCCCAAGTT	p15A vector
p15A-2	AGATCCTTTCTCCTCTTTAGATC	
nifA-1	ATGAACGCCACATTTGCCGA	<i>nifA</i>
nifA-2	TGGAGATCCTTAAGATCCGTAAGTTGGGGTTTTCGGATCT TCAGATCTTGCGCATATGAA	
P12445-F	AGAAAAGAATTCAAAAGATCTAAAGAGGAGAAAGGATCTC ATTCAGGAAGCATCACGGC	P12445 for P12445-nifA
P12445-R with p15A HA	GGGTCGGGGCGACGGCGCGTTTCGGCAAATGTGGCGTTC ATTTCTTGTTCTCCTCGTTGG	
P11670-F with p15A HA	AAAAGAATTCAAAAGATCTAAAGAGGAGAAAGGATCTTTA GCGTTAACTCTCTTCTTGC	P11670 for P11670-nifA
P11670-R with nifA HA	GGGTCGGGGCGACGGCGCGTTTCGGCAAATGTGGCGTTC ATGCTGGACTCCGTCTTCGTG	
P12445-F	AGAAAAGAATTCAAAAGATCTAAAGAGGAGAAAGGATCTC ATTCAGGAAGCATCACGGC	P12445 for P12445-nifHDK- P11670-nifA
P12445-R with nifH HA	TTCCACCCTTCCCGTAAATAGCGCATTGACGCATTGCCAT TTCTTGTTCTCCTCGTTGGA	
nifH-F	ATGGCAATGCGTCAATGCGC	nifHDK for P12445-nifHDK- P11670-nifA
nifK-R	TCAACGAACCAGGTCGTAGT	
P11670-F with nifK HA	CATGCAGAGCACCGACTACAACTACGACCTGGTTCGTTGA TTAGCGTTAACTCTCTTCTTGC	

P11670-R with nifA HA	GGGTCGGGGCGACGGCGCGTTTCGGCAAATGTGGCGTTC ATGCTGGACTCCGTCTTCGTG	P11670 for P12445-nifHDK- P11670-nifA
19520-check-F	AAACGATGTCGCCGATCTTC	
nifA-5out	TGTTGCCGTGCTTGAGGATG	<i>attL</i> site colony PCR check
lac-seq	AGGCACCCCAGGCTTTACAC	
nifA-3out	GTACGCGAACTGGAAAAGT	<i>attR</i> site colony PCR check

18 Table S4. Oligonucleotides for quantitative real-time PCR.

Primer name	Sequence (5'-3')
GAPDH-F	CAATGGGTATGGGCGTATCG
GAPDH-R	AGGTGGCTGAGGTCGTTGAT
nifA-qRT-F	CAAGTGGAACAGCACCGT
nifA-qRT-R	GGCGAGTTGTAGTGGATGG
nifB-qRT-F	GACCATCACCATCAACTGC
nifB-qRT-R	TTGACCTTCACCAGGATGC
nifD-qRT-F	CGCTTGAGCAATCGAAGAA
nifD-qRT-R	AGACCACACCCTTCGAGC
nifE-qRT-F	TCAAGTCCTGGTCGGTGAT
nifE-qRT-R	CCTCCTCGGTGGATTCTT
nifF-qRT-F	CCGATTGCGAGAACGAAAG
nifF-qRT-R	CCCATCGCATCGAGAACTC
nifH-qRT-F	GGATGACCTGGACTTCGTCT
nifH-qRT-R	GCATACATCGCCATCATCTC
nifK-qRT-F	AACCCTGGCAGTTGGAAA
nifK-qRT-R	TGACCTTCATCAGGAACTCG
nifM-qRT-F	ATCACCGATACAGAGGTCAGC
nifM-qRT-R	GGAAATCCTCGTTGACGGT
nifN-qRT-F	TTCGCCAAGGTGTTCTTCG

nifN-qRT-R	GATGCTGCTTGTGCGCAAATG
nifQ-qRT-F	AGCATCTATGGCGCGACCT
nifQ-qRT-R	TCTTCCAGCGCATGTCCTG
nifS-qRT-F	TCAAGGTGGACAAGAAGGG
nifS-qRT-R	CGCATCGCTATGGAACAT
nifT-qRT-F	GATCAGCCGTAACAAGAATG
nifU-qRT-F	TGTCGCTGAAGGTCGATCC
nifU-qRT-R	TGATCTTCAGCGCCTCGTC
nifV-qRT-F	GATACGGTGGGAGTAATGGAG
nifV-qRT-R	GCACTTCCAGCTCCAGATC
nifW-qRT-F	CTGAACTTCTTCGGCGTG
nifW-qRT-R	TGACACAGGTAGTCGTGATAGC
nifX-qRT-F	CGTTCGTGTTCTACGGCATCG
nifX-qRT-R	TTGTCCTCGTTGCCGTCCT
nifY-qRT-F	AGCTGAACGGCGTGGATAC
nifY-qRT-R	GTTGCGCAGGCGGTTTAG
nifZ-qRT-F	TGGATACCCAACCTGTTCG
nifZ-qRT-R	AATAGACGTGGTACTGGGTGC
rnfA-qRT-F	GGTCATGGTGATCTTCGCC
rnfA-qRT-R	AAAGCCATCGCCAGCAAC
rnfB-qRT-F	ATCCATTGCGTGTTGAGC

rnfB-qRT-R	AGTGGTCCAGCGTCAGTGT
rnfC-qRT-F	CCTACAGCATCGTCATCGCC
rnfC-qRT-R	TGGCGGATCAGCTGCTTG
rnfD-qRT-F	CTGTATCTGTTCGGCTGGCC
rnfD-qRT-R	CGTCAGTGCCAAACAGCCAG
rnfE-qRT-F	CATTGGCTGGAAATCACCG
rnfE-qRT-R	TTGAACGCCAGCACGAAG
rnfG-qRT-F	CTACGACAACGATCCGCTG
rnfG-qRT-R	GACGGTGCTGATCTGGAAG
rnfH-qRT-F	ACGGCAAGTTCGTCAAAC
rnfH-qRT-R	CGTCATCGTCGTCTTCATC

20 Table S5. Nitrogenase activity of DSM4166, DSM4166-TPnif-1, 2, 3.

Strains	C ₂ H ₄ yield (nmol)	Protein (mg)	Nitrogenase activity (nmol C ₂ H ₄ /mg protein/h)	Nitrogenase activity mean	SD
DSM4166	34.293	0.537	15.969	14.690	1.203
	31.124	0.536	14.517		
	30.162	0.555	13.583		
DSM4166-TPnif-1	39.848	0.543	18.332	19.632	1.482
	47.469	0.559	21.246		
	39.937	0.517	19.317		
DSM4166-TPnif-2	146.261	0.481	75.997	58.328	16.943
	79.563	0.471	42.218		
	112.502	0.495	56.770		
DSM4166-TPnif-3	260.971	0.438	148.956	135.230	17.159
	196.988	0.425	115.992		
	231.779	0.412	140.740		

22 Table S6. Nitrogenase activity of DSM4166, DSM4166-P11670-nifA,
 23 DSM4166-P12445-nifA, DSM4166-P12445-nifHDK-P11670-nifA.

Strains	C ₂ H ₄ yield (nmol)	Protein (mg)	Nitrogenase activity (nmol C ₂ H ₄ /mg protein/h)	Nitrogenase activity mean	SD
DSM4166	17.274	0.320	13.504	14.784	2.240
	19.604	0.364	13.477		
	18.482	0.266	17.370		
DSM4166-P11670-nifA	265.102	0.325	204.170	221.612	15.671
	322.443	0.344	234.505		
	299.074	0.331	226.162		
DSM4166-P12445-nifA	351.252	0.232	378.492	387.006	59.166
	473.975	0.263	449.968		
	370.642	0.279	332.559		
DSM4166-P12445-nifHDK-P11670 -nifA	880.690	0.266	828.014	754.206	74.153
	705.220	0.259	679.712		
	794.522	0.263	754.892		

25 Table S7. Quantitative real-time PCR (qRT-PCR) analysis of *nif* genes in
 26 DSM4166 and DSM4166-P12445-nifA strains.

Genes	Strains	Relative quantification (RQ)			RQ mean	SD
<i>nifH</i>	DSM4166	1.521	0.704	0.774	1.000	0.453
	DSM4166-P12445-nifA	83.774	121.889	70.747	92.137	26.577
<i>nifD</i>	DSM4166	1.512	0.688	0.800	1.000	0.447
	DSM4166-P12445-nifA	91.379	112.729	70.443	91.517	21.143
<i>nifK</i>	DSM4166	1.520	0.719	0.761	1.000	0.451
	DSM4166-P12445-nifA	131.673	152.841	76.567	120.360	39.375
<i>nifT</i>	DSM4166	1.411	0.864	0.725	1.000	0.362
	DSM4166-P12445-nifA	116.534	172.091	94.982	127.869	39.785
<i>nifY</i>	DSM4166	1.490	0.712	0.798	1.000	0.427
	DSM4166-P12445-nifA	118.705	143.046	111.488	124.413	16.535
<i>nifE</i>	DSM4166	1.627	0.642	0.731	1.000	0.545
	DSM4166-P12445-nifA	62.007	41.976	47.827	50.603	10.300
<i>nifN</i>	DSM4166	1.608	0.634	0.757	1.000	0.530
	DSM4166-P12445-nifA	41.962	42.482	30.402	38.282	6.829
<i>nifX</i>	DSM4166	1.623	0.641	0.736	1.000	0.542
	DSM4166-P12445-nifA	93.974	75.231	87.539	85.581	9.524
<i>nifB</i>	DSM4166	1.442	0.712	0.846	1.000	0.388
	DSM4166-P12445-nifA	69.933	105.061	47.762	74.252	28.893

<i>nifQ</i>	DSM4166	1.284	0.791	0.925	1.000	0.255
	DSM4166-P12445-nifA	87.466	93.915	83.240	88.207	5.376
<i>nifU</i>	DSM4166	1.610	0.637	0.753	1.000	0.532
	DSM4166-P12445-nifA	44.794	50.469	31.657	42.307	9.650
<i>nifS</i>	DSM4166	1.590	0.632	0.778	1.000	0.516
	DSM4166-P12445-nifA	28.446	15.967	17.529	20.647	6.799
<i>nifV</i>	DSM4166	1.589	0.661	0.750	1.000	0.512
	DSM4166-P12445-nifA	58.775	39.128	50.991	49.631	9.894
<i>nifW</i>	DSM4166	1.570	0.667	0.764	1.000	0.496
	DSM4166-P12445-nifA	59.162	69.138	68.035	65.445	5.469
<i>nifZ</i>	DSM4166	1.480	0.675	0.845	1.000	0.424
	DSM4166-P12445-nifA	27.134	30.292	23.372	26.933	3.464
<i>nifM</i>	DSM4166	1.506	0.677	0.817	1.000	0.444
	DSM4166-P12445-nifA	59.802	50.525	57.063	55.797	4.766
<i>nifF</i>	DSM4166	1.506	0.650	0.845	1.000	0.449
	DSM4166-P12445-nifA	38.330	49.814	26.934	38.359	11.440
<i>rnfA</i>	DSM4166	1.500	0.705	0.796	1.000	0.435
	DSM4166-P12445-nifA	48.482	29.354	34.645	37.494	9.877
<i>rnfB</i>	DSM4166	1.588	0.628	0.784	1.000	0.516
	DSM4166-P12445-nifA	30.266	24.332	22.576	25.725	4.030
<i>rnfC</i>	DSM4166	1.540	0.608	0.852	1.000	0.483

	DSM4166-P12445-nifA	24.818	34.969	20.661	26.816	7.360
<i>rnfD</i>	DSM4166	1.562	0.629	0.809	1.000	0.495
	DSM4166-P12445-nifA	50.756	44.312	46.634	47.234	3.264
<i>rnfG</i>	DSM4166	1.356	0.683	0.961	1.000	0.338
	DSM4166-P12445-nifA	16.900	17.784	17.378	17.354	0.442
<i>rnfE</i>	DSM4166	1.352	0.760	0.887	1.000	0.312
	DSM4166-P12445-nifA	48.717	66.485	51.510	55.571	9.555
<i>rnfH</i>	DSM4166	1.356	0.751	0.893	1.000	0.316
	DSM4166-P12445-nifA	53.763	63.742	50.193	55.899	7.023

28 Table S8. Quantitative real-time PCR (qRT-PCR) analysis of *nifH* gene in
 29 DSM4166, DSM4166-P11670-nifA and DSM4166-P12445-nifHDK-P11670-nifA
 30 strains.

Strains	Relative quantification(RQ)			RQ mean	SD
DSM4166	0.706	1.028	1.266	1.000	0.281
DSM4166-P11670-nifA	70.747	46.309	45.232	54.096	14.430
DSM4166-P12445-nifHDK-P11670-nifA	544.604	564.803	413.648	507.685	82.062

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