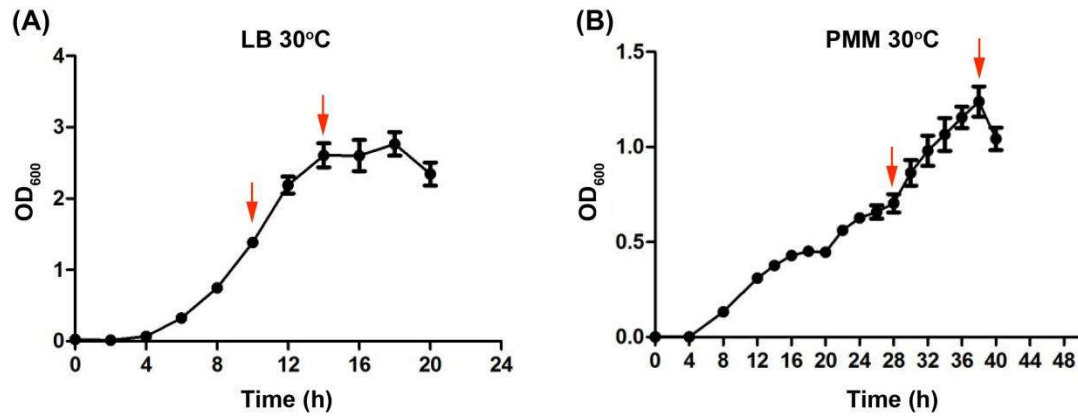


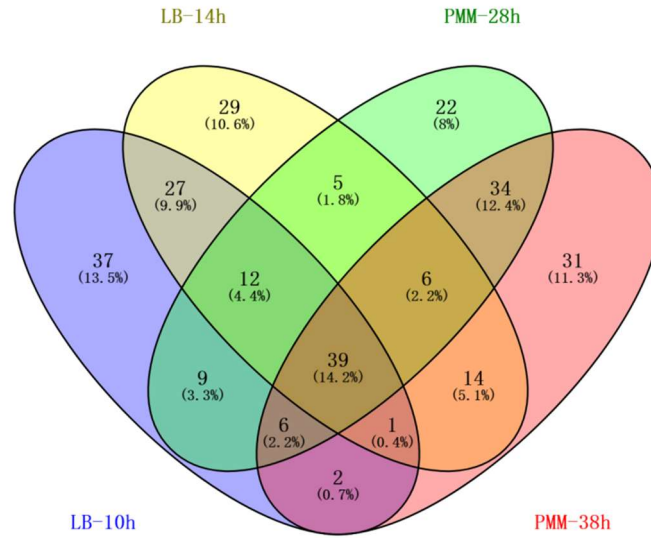
## 1 Supplemental Information



2

3 Fig. S1. The growth curve of *P. stutzeri* DSM4166 in the LB medium and the  
4 PMM medium. For each culture condition, two time point (indicated by the red  
5 arrow) were selected for RNA-seq analysis according the growth curve.

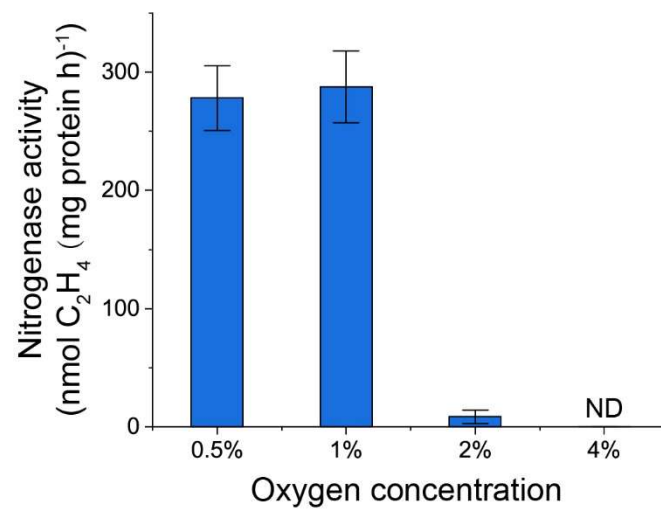
6



7

8 Fig. S2. Venn diagram of genes which were selected in 3% cut off according to  
 9 gene expression level in RNA-seq. Expression levels of genes from each  
 10 sample were ranked from the highest to the lowest based on their values of  
 11 read counts. There were 133 genes in the 3% cutoff in the four samples (Table  
 12 S2). Venn diagram was drawn according to Table S2.

13



14

15 Fig. S3. The nitrogenase activity of the wild-type DSM4166 strain cultivated in  
16 the nitrogen-free medium K at different oxygen concentrations.

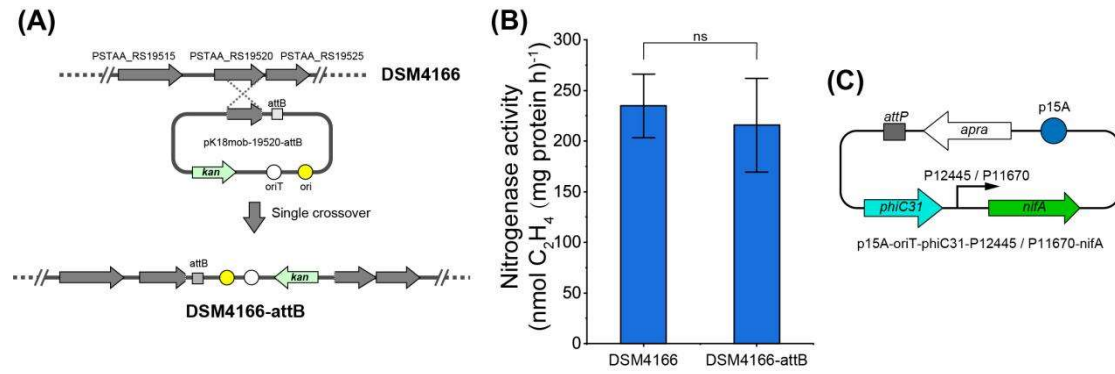


Fig. S4. **(A)** Schematic of construction of DSM4166-attB strain. **(B)** The nitrogenase activity of DSM4166 and DSM4166-attB. **(C)** Schematic of the plasmid containing expression cassette: P12445-nifA or P11670-nifA. Error bars indicate the standard deviations of three replicates (n=3). The P-value cutoff for all the plots is 0.05. \* P<0.05, \*\* P<0.01, and \*\*\* P<0.001. ns, not significant. Two-sided Student's t-test was used to compute statistical significance.

29 Table S3. The sequence of screened endogenous promoters from *P. stutzeri*  
 30 DSM4166.

| Promoter ID        | Sequence (5'-3')                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| P <sub>genta</sub> | TGAAGGCACGAACCCAGTTGACATAAGCCTGTTTCGGTTCGTAAACTG<br>TAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAACCTTGAC<br>CGAACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGT<br>TATGACTGTTTTTTGTACAGTCTATGCCTCGGGCATCCAAGCAGCAA<br>GCGCGTTACGCCGTGGGTTCGATGTTTGATGTTATGGAGCAGCAACG                                                                                                                                                                                                                                                           |
| P22180             | GCCAAGGCGGCGGGATCGTACCTGCGATCCTGCCGCTGCAGCTGAT<br>CATTTTCTAACCAGCATGATTA AAAAGTGATCGACACGCCGTAAAGAT<br>CTCCGTACACTTGCGCCCAGCACCAACAAGCTGCACCA                                                                                                                                                                                                                                                                                                                                                                          |
| P17270             | CCGGTACGCAGCGCGTTTCGCGCGTTGCGTCAGAGGGTGGCTGTCA<br>GCAGGCACGGTCATGCCTCTTTATTAAACACGGGGTCAATCCCCGGC<br>CTGTTCACTCAAGGAGCCCTACC                                                                                                                                                                                                                                                                                                                                                                                           |
| P19520             | GCGCTTCCCTATCCACGACACGGCCGTGCAGCGCACGGCCTGTACG<br>TGTGCTTGAACCGTTATCTCGATACTGTGCGCGCACCATACGCCGC<br>AGGCCTGCCTTGCTATCACTCGGATTAATACCTAAACAGGAGCATAG<br>CTACGCAAACCTGCTGAACCAGAGCCTGCAGAGGCGACAAACGGCAA<br>CAAAACGCGCCAAACCCTTGAGAATCAAGGCGCGTAAGCCTTCTTCG<br>TCGTGTTTCTGACGACATTTTTTTAGTCTAAGCCCCTAATATCATTAAG<br>CCTTGGTGCTTGCACTTTGAATTTCGGATCGATATCATCAGCCTCGGC<br>CAACCTATTGGGGAAGACCCGCCCGCCGAATGCCGCTCGTTCCCT<br>TCCGGAAGAGAGCGAGCACCGTAAACGGCAGGGTGTAAGGACCG<br>GTTCGAAGGACTTCGAACCAACACAACATTTGGAGTGA CTCCGTAA |
| P06545             | GGCTGGCGTCCTGAATAGGTTAATGGGCCTGCGAAGAGGCGCATCT<br>TACCGAGCCTGGCGCGGGTGTTCAAAGCCTGGCTACTGGCGCGCCG<br>CAACGGCCGTACAGCCAGCAGCAGCCACTCGGCGAAGAAATCCAA<br>AATAAAGCTCGCTTTGCTCCAGAAAAACGGTATTGTTACTGGGCTGC<br>TGCTGCATGTGTCACCGTGAATCGCTACATGTTTAGATTTTCGATCCAA<br>ATATCGATACGACTCCTATCTCCTTGACACAGTGATGTTTCGGGCCTT<br>TTAGCCCGCACTTTATTCTTCAAACTTGTTCAAGGAGACATCAA                                                                                                                                                          |
| P03385             | AAAATTATTTTCGCCTCAACGCCGCGTCATCCGCGGCCCCAGACGCTGT<br>TGTCAGCCGAACGGCAGCGCCGAAAACCCCTCGCGCACCTTGACCT<br>TGCCGAATCCGAACCTATCCTTGAGTTCAGGCCATCCAAATAAACGAA                                                                                                                                                                                                                                                                                                                                                                |

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|--------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|        | GCCGGGAAACGGCTGACGGAATTCAGGGTTTCGACCATTAGGTGTT<br>CGGGGGAACCTCCACTGGCCGCCACTTCGGCCGTGCTCCAGAGTGCC<br>TGATGACGCGCCTATTTCGTGCATCGATCGAGGTGAAGTC                                                                                                                                                                                                                                                                                             |
| P10035 | GGTGAGCTCCTGTTGCGCTGCGCGCTGTGCTGGTCGACCCGCCTCA<br>ACTACAGCATACACCTATACATGGTTATTCTGAATGTACAGCTGATGGC<br>GCCCCGTTTCGTTGCAGAAAGAGCCGCCACCGTCGGTACCGCAGGGA<br>GCCTGAAGCGCGTATTGACGGCCCGCCAAGGATGCGACACCGCGTT<br>GCTGAGCGTTATTCTGCCGGCAGCCGCCTTCTTGTACAAGAAAAGTC<br>GCAAGATCTTGTACACCACATATTGACTTGTACAAGCCGAAGAATATG<br>ATGGCATCAAGTTGTACAGAAATTCATCTTGTACAAGAGGTTGTCATC                                                                       |
| P19680 | TGAGGTTTCCTTCACAACAGCGAATACGATGGACCGCGCGATTGAGA<br>GCCTCGCAAGTACCTTTTACAAGGAAATGCCAGTGCCGCTGATCTGT<br>AGTTTTACTACTAAAGAGTATTTTACGGGGGCTGATCCCGCGCCGTT<br>TTGTAGTAAACTACAGACGCCCGGCCCTACCCCGGCACTCGTCCA<br>AGAACATTACACGCACCGGCCACAAAGCCGGTAGCGAACAGGCGA<br>CCGATTCTGGAAGCCTTTCCGCCATGGAGCAAGCC                                                                                                                                            |
| P03670 | GCGACGACCTTGCGTCTACAGCCCGAGCGACTCGCGACAGGCTGAT<br>GGCTGGTGGCTCTTGCCACCGGCCTTTTTCCGTTATGGAAAGCCAGC<br>CGCGCTGGCTCTTCCTCGGAAGCCACCTACAGGTGGCGCGAACCAT<br>GGGGTTCGCAAGATTTTCTGGCTGCTCCCGTCGGGAGAAGCCAACA<br>AGCAGGTGACCAAGCTGGGGAACGCTG                                                                                                                                                                                                      |
| P20930 | ACCAAAAAAAGCGCCAACGTCGATCATCCGGGGACAGTCTCCGTTG<br>GCGCGCATGTCTCGCGAGGCTTTCCGGGCTTCCTACCCCGGAAAG<br>AATGTGACGCTGATCTCATATTGATATCAGCGAATCCGCGATCACAA<br>GCGTACTTAGGGAGTACCTTCTTGGAGGTATTTGCCTGCTTGGCACA<br>GCAAACCTTGACAGAATTATAAGTATGTACAATAAATCTATCTTGACG<br>GCCGCAACCCTGAACGCGATGGCCGGTCTATTTCTGGCATGCGAGC<br>GCCTTTGCCGGTTTTCGGCTGTTGCCAGCTCGGTGCGCTGCTAGACT<br>CCCTATTCCGCGCAGACTCACGAGGGATGCCGGATCAACCGAAAAC<br>AACGAGAAAAGGAGTGCATCC |
| P04125 | GGTCGTTCTGTTTTGTTGTTGTTTGCGAAAGGGTCATGCACAGAACCG<br>CTGTTCTACCGCAAAAGTAGAAAGAAAATCTCTCCGCGTCACGGCCAGA<br>CTGTTAATCGACACGCGACGGTGGGGTGATCCCCTGCCGATCGCCGGC<br>GTGGAGAGACAACCGTGCGGTTTCTCCATGACTGCGTCAGGCGGGTAG<br>CGAACAACGCCACCCTGACTCCGATAACAACAAATTAGGAGCGCCCC                                                                                                                                                                          |

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|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| P12415 | AAGTTCTCCTTTTGGTCGGTTCGCCGAAGCGCAACTATTATCAGAATT<br>GCGAGCGGGTCAGTAAGGCGATTGAGATAACTGGCAGCTGTATTGCG<br>CCTGAAGCCTAAAAAAGAGGCTATGGGCGGGAACCGAAGCGGCGTC<br>ATTTGCTCTTATTGCTTGATGGCGCTTTGCAATCCTGGCTGAAGAAAA<br>ACAAGGAGTTTCCGCT                                                                                                                                                                                                                     |
| P07800 | CAGAGAATCCTCGTTTCAAAGGTGAAGACTAGCGCGGTGCCGTTTGT<br>CGGCCTAGTCTGAAAAGTAACCGGAGCCGCCCGGATTCCCATGCGT<br>GGCGTTTCTGGCTGAATGTCCATACTGGAGGTTTTGATCGATGAGTA<br>CATTACGGACAGCAGTACCCGTTATTGTC                                                                                                                                                                                                                                                             |
| P09555 | TGACACCCCGGTGCGGAAAGGTTATGCGCTAGCCTGGAATGGCCGG<br>ATTGCTTTACCCGTAACACCTGCGGCGCCGAAGTCGAAGCTCGGCG<br>CCGTAGTTTTAGTAGGAATCCCAGCTCACAAAGCCGGGATTTCACTC<br>TGAAAATTTGATGACCAGCAGGAGCATCCGGGTTGTGCCCGGGAAC<br>TATCTGCGCGGTCCGTAGTGGCTTTACCTGAGTCGCTGCTTCAGAAC<br>TTGGGAAGTCATGCTGCGGTTTCCACGCTGGTGGTGTCTCCTTATCG<br>AAGGTGACCAGAC                                                                                                                      |
| P10530 | TCGAACAAAGGGGCCTTCGGGCCCTTTGTATTTGGCTAGCCGCCTT<br>GTTTTTTGCCGAGGGTGCCCTCATCTTTGCTGCAAGGGTATAGCCCA<br>TCCGTTTACCGCCGTATGGCCGTCGTAGAGCTGAATTT                                                                                                                                                                                                                                                                                                       |
| P09495 | CGCATCAGCAACGGTTCACCCCAGCGAGCCTCTCTGTCAACCCGCT<br>CCCGAATGTTTCACCGTGCCCGTGGGAGCGGCCTTGGCCGCTGAAG<br>CGCTTTTCCAACGTGCAACAGCACACAGCGATCTCCCTTCGCCACTA<br>CTTGGGAAACAGCTTCAGCCATCCCCCTAAAGTTTTCCCGTTGCGC<br>CCGAAAAGCTGGTCAAGCAAGTCCGCAGTATGGATGCGCTGGCGTA<br>GTGGTGGTGCGGTACCGGGGGCGCGAAAAAGTTCGAATCTGCCCTA<br>AAGCCAACCCAAACGGCGCCGATAAGCTAATCGAATGCGAACTCAAT<br>GGGTGCCTGGGCACAAGCCGGCCCGGAGTCGCAAGCTCAGGCAAC<br>CAAAGTAACTAGCGCCCTTGGAGGCAAACACC |
| P12505 | CGTTTCGGTTCTTCCTGGTGGGCGGCTGGCCCCGGCTGTTGACGCC<br>GATGCCAGCGTGGCCGTCATCGGCTGCTACAGACGGTCGCAGAGG<br>GCCGCAAGGGATGCGCCACTTTACTGCATTAACCCTCGTCTAGACTA<br>ACCTTGAACCTTCGTCTGTGCCGTCACTACTACCGACGAGCCTTT<br>CCCCAGCAAGCCAAGCGGAGCCATAC                                                                                                                                                                                                                 |
| P16030 | TCACAGTACCAAGCCTGTGCGCAGCGAGCTCCGTATCGATATGCGA<br>CACAGGCAGAAGCGCCTGCTTTACCGCCTTCTCGCTGACTGAAAAAC<br>AACACAGTCAGCCGTGGCTTTGACGATCCGCCGTAGCGGAGAAGCA                                                                                                                                                                                                                                                                                               |

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|        | <p>TGTCGAACCACCCTTCAAAAGCGCTGAAATGGCGCCGCCTGGACAG<br/> GCAAATTCTATGGCTCCGAGGGGTTGGCATTCCCCGGCGAGTTATC<br/> GTAGAGTGTGCCCACTGTGTTTGCATGGGTCGCTGTTGAATCGTGAC<br/> CTGGTGCAGTAGATCTTCAGATCCGCTACTCCGCTCGACTCTCTTAC<br/> TCCTTGCAACCAGTTCCAGCTTCCTGCTTTGCGGGGGCAATTCTACT<br/> TTTGAGTTTCAAGGATACAAAGAC</p>                                                                                |
| P05750 | <p>GGATCGGCTCCAGTTCAGGGCAAGTCGCCTCGTCTTGGGGCAGGCG<br/> GGCGAGGAAATAGCGCGAGGGTACTCGCTCCTGCGACCGGATTGAA<br/> CCCGCCCTAGAGCGTGGCGGGCGGCGTCGCATCGAGCTGGTATGC<br/> CGCTTGCTTTGGCTGCCTGGCTCTGAACGGAACGGCCCTGGCGAGC<br/> GGGGCCGAGGCAGTGAATAAAGAACATTGCCGATCATCAACGGAC<br/> GACTAGACTCAGGTCAGGCTGCGGCCGCTTCGCTGGTCAAGGCATC<br/> GAAGCGCCAGGCCCGAACCCGAGAATAAGAAAGCAAACCTGGAGGTT<br/> TCG</p> |
| P10410 | <p>GTGCCTTGGGCCTTAAGTCGAGAACGGTTCAGCTATAGTAGGCC<br/> CTTCGCCGAGCCCGGTTGGCGGGCATTGTTACTTATTTTGCAGGGTC<br/> GGGCCACAGTCGTGCGGCCCGGCTCCGGTTGAGTTTCCAACACTAA<br/> TGTGGAGAGATCACC</p>                                                                                                                                                                                                     |
| P02720 | <p>GGGAAATACGCTCCAAATAAAGCGGCTCTGGCGTGAATTATGGCCAA<br/> AGATAGCTTTTTATATGGTGAATAAACTGCTTCAGCCTTTTTATACTC<br/> CGAATCAATAGTGAATCCGGAAAAGCCTGTTGAGCGGGCGGGAGG<br/> CTGCA</p>                                                                                                                                                                                                            |
| P11845 | <p>TCGGAACGCCGAAAGGCGGCCAAACGGACTTCGAGGTCAGCGCAG<br/> CCGGTAGGCGCGCCTCGAGCCGTTAACAAGGAGGCATGTGGGAGA<br/> CCAGATCCAGTCCCGCAGGCCTTTTTTTATATGAATGAACCCACATTG<br/> TCTGGAATGTGGAGATTGGGCGGATTCTCGCCCGAAAACAGCAGGA<br/> ATCAAC</p>                                                                                                                                                         |
| P07835 | <p>GGTTCATCTCGTTGAAGGAACTTGATACGCCAGGTTCGACTCGCATA<br/> GGTATGCTTGGAAGATGGCGAATGCTGAGTCAGGGCGGTGAAGTTG<br/> CCAAGTGCCTTTTCGTGCTGCCACCTAAGCCATCCCCGGGAACG<br/> AAACAGCAGTCTGCAGGAGACTTTCA</p>                                                                                                                                                                                          |
| P18460 | <p>GGCAGTTACGGCGTCGGTGAAGGTTTGCGGGGCAGCCATTGTAGCG<br/> GCGCTGCCGTGGCTGCGCTGCGCCATGCTGCAAGAGGCCATCCGG<br/> CGAACGGATTGAACTTCGCCGGACCGTCCAACCTCCTACCTAGTAAGG<br/> GGCGGTTGCGGTGGCCCGGAGAGGGCCCCGCGTACCGCATTCCG<br/> CTGGCACACCAAATGGAGGACATCCC</p>                                                                                                                                      |

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| P18595 | <p> GTCGGGCTCCTCGAGTTTGCGCGGAGTATAAAGGCTCGCACCGACC<br/> GCGGCAGCGTCAAGCTGCCGACAGCCTACGGATCGCTCGACCGGCT<br/> TTTTTTACCTCCTGGCGGAAAACCTGCGAGCATCTGTGAGCCAGGTC<br/> ATGTCCAATGAAAAACTGACCTTGGCAGGGGAGCACAATGCTTATAA<br/> TCCCCTCCAAGAATATTGACGCCACCTAACTGACGATACTTGCTGAC<br/> GAGGCGTCGTTGTAAATCAAAGCGCTCGGCTATGCTGCGTCTGGAT<br/> CCGGTTCGACAGAAGAAACCCGCACAAGATTGCGGGCTCGCCCGGT<br/> ACCTTCCGCCTTGCCCTGCCGACTTGGTACCTATATAAGACGCTGC<br/> CGAATTCAAGCGTTGTGACCGCCGCAAAGCAGGCGACCGCAGGAGA<br/> GTCTCG </p> |
| P11670 | <p> TTAGCGTTAACTCTCTTCTTGCTGGGCGTGACACAGAATCCCGAATGC<br/> GCCGGGTAGGCGCCCTGACTCTCCATCTAGTCGAGCCAGAAGGGGA<br/> TTCGCCGCGGTGATGATGGATGCACCAATAGAGTGTGAGCGTTTGA<br/> ACGCCGAGCCGCGGTGGCAGCGTCAGGGTTCAAGAGGCGGGTCT<br/> GGTACAGGCTCGTCCCTTATGACTTGTGAGTCATCGCGGCAGTATAC<br/> CATCGAGCCCCCTCTGGCAGAACGGCATTGCGCATACGACGAACG<br/> AACATGTAGTTTCAGGCGATTGAGTGGGTACCCTCAAACGACGAT<br/> CGATGTCTAACCACGAAGACGGAGTCCAGC </p>                                                                                     |
| P12445 | <p> CACGGCGGCCCGTCGAAGGATTTGTCGAGCAACTGATTCTAGCAGGTCTG<br/> ATTACCGATCAATTGGCAGTCGGCTGGCCCACCACGCTCGGTGCAGGTC<br/> AAAAAACGAGACACCACCCTCACTCAAACAAGAGTTTGACTTCGGTATTC<br/> GACTAGTGGCCATCAAACCGTTAGGATTCGGCTGCCCGCCTGAACAATAA<br/> TCCGGCGGGCCGGAAGTGGTAAGACGTTTTGTAGCCTAACGAGACTATC<br/> GAACAAACCACTCCTGACCTTGACGCCCCGGCCCTAAAGACTAAGGTCT<br/> AAAGGCTGAAACAATGATGAAATGTTTTATTGCATGTCGCCGTAAGAGCG<br/> ACCTTCAGAGCATCATCAACGAGGAGAACAAGAA </p>                                                       |

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32 Table S4. Quality evaluation of samples for RNA-seq.

| Sample name | Concentration (ng $\mu\text{L}^{-1}$ ) | Total ( $\mu\text{g}$ ) | OD260/280 | OD260/230 | 23S/16S | RIN value |
|-------------|----------------------------------------|-------------------------|-----------|-----------|---------|-----------|
| LB-10h      | 1876                                   | 60.032                  | 2.066     | 1.712     | 1.8     | 9.7       |
| LB-14h      | 1992                                   | 63.744                  | 2.045     | 2.274     | 1.9     | 9.3       |
| PMM-28h     | 742                                    | 23.744                  | 2.05      | 1.364     | 1.6     | 10        |
| PMM-38h     | 964                                    | 30.848                  | 2.008     | 1.01      | 2.1     | 8.9       |

34 Table S5. Oligonucleotides for cloning of screened endogenous promoter  
 35 regions from *P. stutzeri* DSM4166.

| Primer name | Sequence (5'-3')                                                  | PCR product    |
|-------------|-------------------------------------------------------------------|----------------|
| pBBR1-1     | CCTCATAAAGGCCAAGAAGGGCGGAAAGTCCAAATTGT<br>AAGCTAGCCATATGGCTTACCG  | pBBR1 vector   |
| pBBR1--2    | ATCTGCATAAAACTGTTGTAAT                                            |                |
| firefly-1   | ATGGAAGACGCCAAAAACAT                                              | <i>firefly</i> |
| firefly-2   | TTACAATTTGGACTTTCCGCC                                             |                |
| 02720-1     | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TGGGAAATACGCTCCAAATAA  | P02720         |
| 02720-2     | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TTGCAGCCTCCCGCC        |                |
| 03385-1     | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TAAAATTATTTGCGCTCAACGC | P03385         |
| 03385-2     | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGACTTCACCTCGATCG      |                |
| 03670-1     | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TAGCTCAAGTAAGCGACGACC  | P03670         |
| 03670-2     | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TCAGCGTTCCCCAGCTT      |                |
| 04125-1     | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TTGGTCGTTCTGTTTTGTTGT  | P04125         |
| 04125-2     | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGGGGCGCTCCTAATTG      |                |
| 05750-1     | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TGGATCGGCTCCAGTTCAGG   | P05750         |

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|         |                                                                  |        |
|---------|------------------------------------------------------------------|--------|
| 05750-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TCGAAACCTCCAGTTTGC    |        |
| 06545-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TGGCTGGCGTCCTGAATAG   |        |
| 06545-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TTTGATGTCTCCTTGAACC   | P06545 |
| 07800-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TCAGAGAATCCTCGTTTCAA  |        |
| 07800-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGACAATAACGGGTACTG    | P07800 |
| 07835-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TATGGTTCATCTCGTTGAAGG |        |
| 07835-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TTGAAAGTCTCCTGCAGA    | P07835 |
| 09495-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TCGCATCAGCAACGGTTCAC  |        |
| 09495-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGGTGTTTGCCTCCAAG     | P09495 |
| 09555-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TTACCTGATGACACCCCGGTC |        |
| 09555-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGTCTGGTCACCTTCGATA   | P09555 |
| 10035-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TTTCATGGTGAGCTCCTGTTG |        |
| 10035-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGATGACAACCTCTTGAC    | P10035 |
| 10410-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TGCAGTTCGTTTCATGTGCC  | P10410 |

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|         |                                                                   |        |
|---------|-------------------------------------------------------------------|--------|
| 10410-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGGTGATCTCTCCACATTAGT  |        |
| 10530-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TGATCGAACAAAGGGGCCTT   |        |
|         |                                                                   | P10530 |
| 10530-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TAAATTCAGCTCTACGAC     |        |
| 11670-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TGGACATTTAGCGTTAACTCTC |        |
|         |                                                                   | P11670 |
| 11670-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGCTGGACTCCGTCTT       |        |
| 11845-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TTTGGCTGGATGATCGGAAC   |        |
|         |                                                                   | P11845 |
| 11845-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGTTGATTCCTGCTGTTTT    |        |
| 12415-1 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TAGCGGAAACTCCTTGTT     |        |
|         |                                                                   | P12415 |
| 12415-2 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TAAGTTCTCCTTTTGGTCCG   |        |
| 12445-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TCATTCAGGAAGCATCACGGC  |        |
|         |                                                                   | P12445 |
| 12445-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TTTCTTGTTCTCCTCGTTGG   |        |
| 12505-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TCGTTTCGGTTCTTCCTGG    |        |
|         |                                                                   | P12505 |
| 12505-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGTATGGCTCCGCTTG       |        |
| 16030-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TCCATCACAGTACCAAGCC    | P16030 |

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|---------|------------------------------------------------------------------|--------|
| 16030-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGTCTTTGTATCCTTGAAAC  |        |
| 17270-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TTCGGAATGACCGGTACGCAG |        |
|         |                                                                  | P17270 |
| 17270-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGGTAGGGCTCCTTGAG     |        |
| 18460-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TGGCAGTTACGGCGTCGG    |        |
|         |                                                                  | P18460 |
| 18460-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGGGATGTCCTCCATTTG    |        |
| 18595-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TGTCGGGCTCCTCGAGTTTG  |        |
|         |                                                                  | P18595 |
| 18595-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TCGAGACTCTCCTGCGG     |        |
| 19520-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TGCGCTTCCCTATCCACGAC  |        |
|         |                                                                  | P19520 |
| 19520-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TTTACGGAGTCACTCCAAA   |        |
| 19680-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TTGAGTTTTCTTCACAACAG  |        |
|         |                                                                  | P19680 |
| 19680-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGGCTTGCTCCATGGCG     |        |
| 20930-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TACCAAAAAAAGCGCCAACG  |        |
|         |                                                                  | P20930 |
| 20930-2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TGGATGCACTCCTTTTCTC   |        |
| 22180-1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TGCCAAGGCGGCGGGATCG   |        |
|         |                                                                  | P22180 |

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|                       |                                                                  |                    |
|-----------------------|------------------------------------------------------------------|--------------------|
| 22180-2               | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TTGGTGCAGCTTGTGGTGC   |                    |
| P <sub>genta</sub> -1 | GGCAGAATGCTTAATGAATTACAACAGTTTTTATGCAGA<br>TTGAAGGCACGAACCCAGTTG |                    |
| P <sub>genta</sub> -2 | ATGGCGCCGGGCCTTTCTTTATGTTTTTGGCGTCTTCCA<br>TCGTTGCTGCTCCATAAC    | P <sub>genta</sub> |

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37 Table S6. Oligonucleotides used for construction of DSM4166-attB strain.

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

38

39

40 Table S7. Oligonucleotides used for construction of DSM4166-P12445-nifA and

41 DSM4166-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<br>TCAGATCTTGCGCATATGAA  |                                      |
| P12445-F with<br>p15A HA | AGAAAAGAATTCAAAAGATCTAAAGAGGAGAAAGGATCTC<br>ATTCAGGAAGCATCACGGC   | P12445 for<br>P12445-nifA            |
| P12445-R with<br>nifA HA | GGGTCGGGGCGACGGCGCGTTTCGGCAAATGTGGCGTTC<br>ATTTCTTGTTCTCCTCGTTGG  |                                      |
| P11670-F with<br>p15A HA | AGAAAAGAATTCAAAAGATCTAAAGAGGAGAAAGGATCTT<br>TAGCGTTAACTCTCTTCTTGC | P11670 for<br>P11670-nifA            |
| P11670-R with<br>nifA HA | GGGTCGGGGCGACGGCGCGTTTCGGCAAATGTGGCGTTC<br>ATGCTGGACTCCGTCTTCGTG  |                                      |
| 19520-check-F            | AAACGATGTCGCCGATCTTC                                              | <i>attL</i> site colony<br>PCR check |
| nifA-5out                | TGTTGCCGTGCTTGAGGATG                                              |                                      |
| lac-seq                  | AGGCACCCCAGGCTTTACAC                                              | <i>attR</i> site colony<br>PCR check |
| nifA-3out                | GTACGCGAACTGGAAAAGT                                               |                                      |

42

43 Table S8. 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     |

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|            |                      |
|------------|----------------------|
| 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  |

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45 Table S9. Nitrogenase activity of DSM4166, DSM4166-P11670-nifA,

46 DSM4166-P12445-nifA.

| Strains                 | C <sub>2</sub> H <sub>4</sub> yield<br>(nmol) | Protein<br>(mg) | Nitrogenase activity<br>(nmol C <sub>2</sub> H <sub>4</sub> /mg<br>protein/h) | Nitrogenase<br>activity mean | SD     |
|-------------------------|-----------------------------------------------|-----------------|-------------------------------------------------------------------------------|------------------------------|--------|
|                         | 36.420                                        | 0.046           | 198.713                                                                       |                              |        |
| DSM4166                 | 48.073                                        | 0.047           | 256.980                                                                       | 234.657                      | 31.431 |
|                         | 48.328                                        | 0.049           | 248.278                                                                       |                              |        |
|                         | 81.800                                        | 0.052           | 391.352                                                                       |                              |        |
| DSM4166-<br>P11670-nifA | 97.459                                        | 0.050           | 487.197                                                                       | 431.281                      | 49.883 |
|                         | 100.974                                       | 0.060           | 415.293                                                                       |                              |        |
|                         | 183.665                                       | 0.052           | 882.071                                                                       |                              |        |
| DSM4166-<br>P12445-nifA | 199.990                                       | 0.053           | 945.076                                                                       | 954.687                      | 77.868 |
|                         | 242.804                                       | 0.059           | 1036.915                                                                      |                              |        |

47

48 Table S10. Quantitative real-time PCR (qRT-PCR) analysis of *nif* genes in  
 49 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 |

50 \*RQ was calculated using  $2^{-\Delta\Delta Ct}$  method. The expression level of *nif* genes in DSM4166  
51 wild-type strain were defined as 1. The RQ of *nif* genes in DSM4166-P12445-nifA strain  
52 represented the expression levels compared to the wild-type strain.  
53

54 Table S11. The concentration of extracellular ammonium produced by  
 55 DSM4166 and *nifA* overexpression strains.

| Strains                     | NH <sub>4</sub> <sup>+</sup> concentration (μM) |        |        | Average | STDEV |
|-----------------------------|-------------------------------------------------|--------|--------|---------|-------|
| DSM4166                     | 3.29                                            | 20.17  | 18.42  | 13.96   | 9.28  |
| DSM4166-P11670- <i>nifA</i> | 20.98                                           | 19.43  | 25.62  | 22.01   | 3.22  |
| DSM4166-P12445- <i>nifA</i> | 408.63                                          | 321.23 | 347.39 | 359.08  | 44.86 |

56