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A pathway for biological methane production using bacterial iron-only nitrogenase

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SUPPLEMENTARY INFORMATION

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Supplementary Materials included:

Supplementary Figure 1a, 1b, 1c

Supplementary Figure 2a, 2b, 2c

Supplementary Figure 3

Supplementary Figure 4

Supplementary Table 1

Supplementary Table 2

Supplementary Figures

1. ADU46342.1	I	P	G	V	M	T	I	R	G	C	A	Y	A	G	S	K	G	V	V	W	G	P	I	K	D	M	V	H	I	S	H	G	P	V	G	C
2. AAK78237.1	I	P	G	I	I	T	N	R	G	C	C	Y	A	G	C	K	G	V	V	M	G	P	I	K	D	M	I	H	I	T	H	G	P	I	G	C
3. AAM07249.1	I	P	G	I	I	T	N	R	G	C	A	F	A	G	T	K	G	V	V	F	G	P	I	K	D	M	V	H	L	V	H	G	P	I	G	C
4. AAM30418.1	I	P	G	I	I	T	N	R	G	C	A	F	A	G	T	K	G	V	V	F	G	P	I	K	D	M	V	H	I	V	H	G	P	I	G	C
5. AAM72762.1	V	P	G	I	I	T	Q	R	G	C	A	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	V	N	I	V	H	G	P	I	G	C
6. AAS94437.1	V	P	G	I	I	T	Q	R	G	C	C	Y	A	G	C	K	G	V	V	I	G	P	Y	G	D	M	V	H	I	V	H	G	P	V	G	C
7. AAW39637.1	V	P	G	L	I	T	M	R	G	C	A	Y	A	G	C	K	G	V	I	L	G	P	T	R	D	V	L	S	I	T	H	G	P	I	G	C
8. AAZ69153.1	I	P	G	I	I	T	N	R	G	C	A	F	A	G	T	K	G	V	V	F	G	P	I	K	D	M	V	H	L	V	H	G	P	I	G	C
9. ABB24388.1	V	P	G	I	I	T	Q	R	G	C	S	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	V	N	I	T	H	G	P	I	G	C
10. ABB28508.1	V	P	G	I	I	T	Q	R	G	C	A	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	V	N	I	V	H	G	P	I	G	C
11. ABK16710.1	I	P	G	I	I	T	Q	R	G	C	S	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	L	V	N	L	T	H	G	P	I	G	C
12. ABL64794.1	V	P	G	I	I	T	Q	R	G	C	C	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	V	N	I	V	H	G	P	I	G	C
13. ABM30104.1	V	P	G	I	I	T	Q	R	G	C	C	Y	A	G	C	K	G	V	V	I	G	P	Y	G	D	M	V	H	I	V	H	G	P	V	G	C
14. ABO51322.1	V	P	G	I	I	T	N	R	G	C	A	Y	A	G	C	K	G	V	V	L	G	P	L	K	D	I	V	H	I	T	H	G	P	I	G	C
15. ABP37358.1	V	P	G	I	I	T	Q	R	G	C	S	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	V	V	N	I	T	H	G	P	I	G	C
16. ABR34172.1	I	P	G	V	M	T	H	R	G	C	C	Y	A	G	C	K	G	V	V	L	G	P	L	K	D	I	V	L	L	T	H	G	P	I	G	C
17. ABR49648.1	I	P	G	I	I	S	N	R	G	C	C	F	A	G	C	K	G	V	V	L	G	P	L	K	D	V	V	H	I	V	H	G	P	I	G	C
18. ABS55663.1	I	P	G	I	I	S	Q	R	G	C	A	Y	A	G	C	K	G	V	V	V	A	P	I	K	D	M	I	T	I	T	H	G	P	V	G	C
19. ACD89765.1	V	P	G	I	I	T	Q	R	G	C	S	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	V	N	I	V	H	G	P	I	G	C
20. ACE04672.1	I	P	G	I	I	S	Q	R	G	C	S	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	V	N	L	V	H	G	P	I	G	C
21. ACF12018.1	V	P	G	I	I	T	Q	R	G	C	S	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	V	N	I	V	H	G	P	I	G	C
22. ACF13496.1	V	P	G	I	I	T	Q	R	G	C	C	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	L	N	I	V	H	G	P	I	G	C
23. ACF44168.1	V	P	G	I	I	T	Q	R	G	C	S	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	V	N	I	V	H	G	P	I	G	C
24. ACF46648.1	I	P	G	I	I	T	Q	R	G	C	S	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	V	N	L	V	H	G	P	I	G	C
25. ACL03217.1	V	P	G	L	M	G	Q	R	G	C	C	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	I	N	I	T	H	G	P	I	G	C
26. ACL09555.1	T	P	G	I	I	T	M	R	G	C	T	Y	A	G	C	K	G	V	I	L	G	P	T	R	D	I	V	N	I	T	H	G	P	I	G	C
27. ACL15931.1	I	P	G	I	I	S	Q	R	G	C	A	Y	A	G	C	K	G	V	V	V	G	P	I	K	D	M	V	H	I	V	H	G	P	V	G	C
28. ACN14082.1	V	P	G	I	I	T	Q	R	G	C	C	Y	A	G	C	K	G	V	I	M	G	P	T	R	D	I	V	N	L	V	H	G	P	I	G	C
29. ACS78511.1	I	P	G	I	I	T	M	R	G	C	T	Y	A	G	C	K	G	V	I	M	G	P	T	R	D	I	V	N	I	T	H	G	P	I	G	C
30. ACU88955.1	I	P	G	I	I	T	M	R	G	C	T	Y	A	G	C	K	G	V	I	L	G	P	T	R	D	I	V	N	I	T	H	G	P	I	G	C
31. ACV61912.1	I	P	G	I	I	T	N	R	G	C	A	Y	A	G	C	K	G	V	V	L	G	P	L	K	D	M	V	H	I	V	H	G	P	I	G	C
32. ADE55851.1	I	P	G	I	I	T	Q	R	G	C	S	Y	A	G	C	K	G	V	V	I	G	P	I	Y	D	I	C	H	I	T	H	G	P	I	G	C
33. ADG81560.1	I	P	G	I	I	T	N	R	G	C	A	Y	A	G	C	K	G	V	V	L	G	P	L	K	D	I	V	H	I	T	H	G	P	V	G	C
34. ADH87122.1	I	P	G	I	I	T	M	R	G	C	T	Y	A	G	C	K	G	V	I	M	G	P	T	R	D	I	V	N	L	V	H	G	P	I	G	C
35. ADK13575.1	V	P	G	V	I	T	S	R	G	C	C	Y	A	G	C	K	G	V	V	M	G	P	I	K	D	M	I	H	I	T	H	G	P	V	G	C
36. ADK82107.1	V	P	G	I	I	T	Q	R	G	C	C	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	I	N	I	T	H	G	P	I	G	C
37. ADK83813.1	I	P	G	I	I	T	M	R	G	C	T	Y	A	G	C	K	G	V	I	M	G	P	T	R	D	I	V	N	I	T	H	G	P	I	G	C
38. ADL52529.1	I	P	G	I	I	T	N	R	G	C	C	Y	A	G	C	K	G	V	V	L	G	P	V	K	D	M	V	H	I	V	H	G	P	I	G	C
39. ADL69354.1	I	P	G	I	M	T	N	R	G	C	S	Y	A	G	A	K	G	V	V	F	G	P	V	K	D	I	L	H	L	T	H	G	P	I	G	C
40. ADN01323.1	I	P	G	I	L	T	M	R	G	C	C	Y	A	G	C	K	G	V	V	L	G	P	T	R	D	I	L	Q	I	T	H	G	P	I	G	C

Supplementary Figure 1a | Multiple sequence alignment of a segment of representative NifD protein sequences. Position 75 of the *R. palustris* (ADU46342) NifD is highlighted with a red box. For simplicity, only 40 sequences are shown here.

1. ADU27824.1	I	P	G	S	I	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	L	I	H	G	P	V	G	C
2. AAM04635.1	I	A	G	S	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
3. AAZ71203.1	I	P	G	S	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
4. ABA23631.1	I	P	G	S	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	M	I	H	G	P	I	G	C
5. ACO76522.1	I	P	G	T	L	S	E	R	G	C	A	F	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	M	I	H	G	P	L	G	C
6. ADP69796.1	I	P	G	T	L	S	E	R	G	C	S	F	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	M	I	H	G	P	V	G	C
7. AGK14066.1	I	P	G	T	L	S	E	R	G	C	A	F	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	M	I	H	G	P	L	G	C
8. AGK18954.1	I	P	G	T	L	S	E	R	G	C	A	F	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	M	I	H	G	P	L	G	C
9. AGK98263.1	I	P	G	T	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	L	V	H	G	P	I	G	C
10. AIQ12504.1	I	P	G	T	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	M	I	H	G	P	V	G	C
11. AJA47505.1	I	P	G	T	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	L	V	H	G	P	I	G	C
12. AJA51493.1	I	P	G	T	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	L	V	H	G	P	I	G	C
13. AJE19761.1	I	P	G	T	L	S	E	R	G	C	A	F	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	M	I	H	G	P	L	G	C
14. AKB29650.1	-	-	-	-	M	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
15. AKB33577.1	-	-	-	-	M	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
16. AKB44498.1	I	P	G	S	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
17. AKB48005.1	I	P	G	S	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
18. AKB51721.1	I	P	G	S	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
19. AKB54345.1	I	P	G	S	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
20. AKB57578.1	I	P	G	S	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
21. AKB81589.1	I	P	G	S	I	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
22. AKG34370.1	I	P	G	T	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	L	K	D	T	I	Q	M	I	H	G	P	V	G	C
23. AKJ38129.1	I	P	G	S	L	S	E	R	G	C	S	Y	C	G	A	K	L	V	I	G	G	V	I	K	D	C	I	Q	M	I	H	G	P	V	G	C
24. BAH06671.1	I	P	G	T	L	S	E	R	G	C	S	F	C	G	S	K	L	V	I	G	G	V	L	K	D	T	I	Q	L	I	H	G	P	V	G	C
25. EDK33789.1	I	P	G	T	L	S	E	R	G	C	S	F	C	G	S	K	L	V	I	G	G	V	L	K	D	T	I	Q	L	I	H	G	P	V	G	C

Supplementary Figure 1b | Multiple sequence alignment of a segment of representative VnfD protein sequences. Position 57 of VnfD (ADU27824) (corresponds to the residue position 75 of *R. palustris* NifD) is highlighted with a red box.

1. ADU44702.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	M	S	H	G	P	V	G	C
2. ABC22195.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	Q	P	M	K	D	V	I	H	I	S	H	G	P	I	G	C
3. ABD90205.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	L	S	H	G	P	V	G	C
4. ABJ07809.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	L	S	H	G	P	V	G	C
5. ACF00151.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	M	S	H	G	P	V	G	C
6. ACF14288.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C
7. ACM03751.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	N	G	C
8. ACO80999.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C
9. ACS86388.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C
10. ADE84351.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	N	G	C
11. ADP72547.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C
12. ADQ79509.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	L	S	H	G	P	V	G	C
13. ADY57120.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	L	S	H	G	P	V	G	C
14. AEI37863.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C
15. AEO47910.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	Q	P	M	K	D	V	I	H	I	S	H	G	P	I	G	C
16. AFA47492.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	M	S	H	G	P	V	G	C
17. AGK13257.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C
18. AHF89180.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	L	S	H	G	P	V	G	C
19. AIF53399.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	M	S	H	G	P	V	G	C
20. AJQ26365.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	M	S	H	G	P	V	G	C
21. ALK10657.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C
22. BAH05371.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	A	I	H	M	S	H	G	P	V	G	C
23. BAR99382.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C
24. CCG07115.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	I	G	C
25. CUU43320.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C
26. EDK32424.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	A	I	H	M	S	H	G	P	V	G	C
27. AIQ17623.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C
28. AIQ57937.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	L	S	H	G	P	V	G	C
29. AIQ63607.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	M	S	H	G	P	V	G	C
30. CQR55107.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	L	S	H	G	P	V	G	C
31. AGF56849.1	I	P	G	S	I	T	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	M	S	H	G	P	I	G	C
32. AJA49856.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	M	S	H	G	P	I	G	C
33. AJA53844.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	M	S	H	G	P	I	G	C
34. AHJ12912.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	A	I	H	M	S	H	G	P	V	G	C
35. AAZ70500.1	I	P	G	T	I	S	E	R	G	C	A	F	C	G	A	K	H	V	I	G	A	P	M	K	D	V	I	H	L	C	H	G	P	V	G	C
36. AKB52430.1	I	P	G	T	I	S	E	R	G	C	A	F	C	G	A	K	H	V	I	G	A	P	M	K	D	V	I	H	L	C	H	G	P	V	G	C
37. AKB82412.1	I	P	G	T	I	S	E	R	G	C	A	F	C	G	A	K	H	V	I	G	S	P	M	K	D	V	I	H	L	C	H	G	P	V	G	C
38. AAM04629.1	I	P	G	T	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	A	P	M	K	D	V	I	H	V	S	H	G	P	V	G	C
39. AGK17546.1	I	P	G	S	I	S	E	R	G	C	A	Y	C	G	A	K	H	V	I	G	T	P	M	K	D	V	I	H	I	S	H	G	P	V	G	C

Supplementary Figure 1c | Multiple sequence alignment of a segment of representative AnfD protein sequences. Position 57 of AnfD (ADU44702) (corresponds to the residue position 75 of *R. palustris* NifD) is highlighted with a red box.

1. ADU46342.1	K	P	I	I	P	V	R	C	E	G	F	R	G	V	S	Q	S	L	G	H	H	I	A	N	D	V	I	R	D	W	V	F
2. AAK78237.1	I	Q	V	I	A	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	I	L	D	T	V	I	
3. AAM07249.1	I	K	V	I	P	A	R	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	S	N	S	L	M	E	H	L	I
4. AAM30418.1	I	K	V	I	P	A	R	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	S	N	A	L	M	E	N	L	I
5. AAM72762.1	C	N	V	F	G	F	S	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	V	F	K	H	M	V
6. AAS94437.1	I	P	V	M	A	F	N	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	K	I	F	D	M	A	L
7. AAW39637.1	I	K	V	F	G	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	Q	I	F	K	H	M	V
8. AAZ69153.1	I	K	V	I	P	A	R	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	S	N	A	L	M	E	H	L	I
9. ABB24388.1	C	N	V	F	G	F	S	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	V	F	K	H	M	V
10. ABB28508.1	C	N	V	F	G	F	S	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	V	A	N	N	G	V	F	K	H	M	V
11. ABK16710.1	I	N	V	F	A	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	I	F	K	H	V	V
12. ABL64794.1	C	N	V	F	G	F	S	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	V	F	K	H	M	V
13. ABM30104.1	I	P	V	M	A	F	N	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	K	I	F	D	M	A	L
14. ABO51322.1	V	P	V	M	A	F	N	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	I	L	M	D	R	V	I
15. ABP37358.1	C	N	V	F	G	F	S	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	I	F	K	H	M	V
16. ABR34172.1	I	D	V	I	S	F	P	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	K	L	I	K	S	V	I
17. ABR49648.1	I	Q	V	M	A	F	D	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	V	L	M	R	D	V	I
18. ABS55663.1	I	Q	V	L	A	Y	A	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	T	I	M	Q	N	V	I
19. ACD89765.1	C	N	V	F	G	F	S	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	V	F	K	H	M	V
20. ACE04672.1	C	N	I	F	G	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	Q	V	F	K	H	V	V
21. ACF12018.1	C	N	V	F	G	F	S	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	V	F	K	H	M	V
22. ACF13496.1	C	N	V	F	G	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	L	F	K	H	V	V
23. ACF44168.1	C	N	V	F	G	F	S	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	V	F	K	H	M	V
24. ACF46648.1	C	N	I	F	G	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	Q	V	F	K	H	V	V
25. ACL03217.1	I	N	I	F	G	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	I	F	K	H	V	V
26. ACL09555.1	I	N	I	F	A	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	Q	I	F	T	H	V	V
27. ACL15931.1	I	Q	V	L	S	F	N	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	N	L	M	E	H	V	I
28. ACN14082.1	I	N	V	F	G	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	I	F	T	H	V	V
29. ACS78511.1	C	N	V	F	G	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	Q	V	F	T	H	L	V
30. ACU88955.1	C	N	V	F	A	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	K	I	F	S	E	V	V
31. ACV61912.1	I	P	I	M	A	F	N	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	L	M	E	H	V	I
32. ADE55851.1	L	N	V	M	A	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	K	V	F	T	D	M	I
33. ADG81560.1	I	P	V	M	G	F	N	C	E	G	Y	R	G	V	S	Q	S	A	G	H	H	I	A	N	N	G	L	L	E	W	V	V
34. ADH87122.1	C	N	V	Y	A	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	Q	V	F	R	H	I	V
35. ADK13575.1	I	N	V	L	A	F	S	C	E	G	Y	K	G	V	T	Q	S	A	G	H	H	I	A	N	N	T	V	M	S	E	I	I
36. ADK82107.1	C	N	V	F	A	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	Q	I	F	K	H	V	V
37. ADK83813.1	C	N	V	F	A	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	Q	V	F	K	H	L	V
38. ADL52529.1	V	P	V	L	S	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	L	L	L	E	K	V	V
39. ADL69354.1	I	K	V	I	T	V	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	L	A	S	N	T	L	I	M	D	I	V
40. ADN01323.1	V	N	I	F	A	F	S	C	E	G	Y	K	G	V	S	Q	S	A	G	H	H	I	A	N	N	K	L	F	T	D	V	V

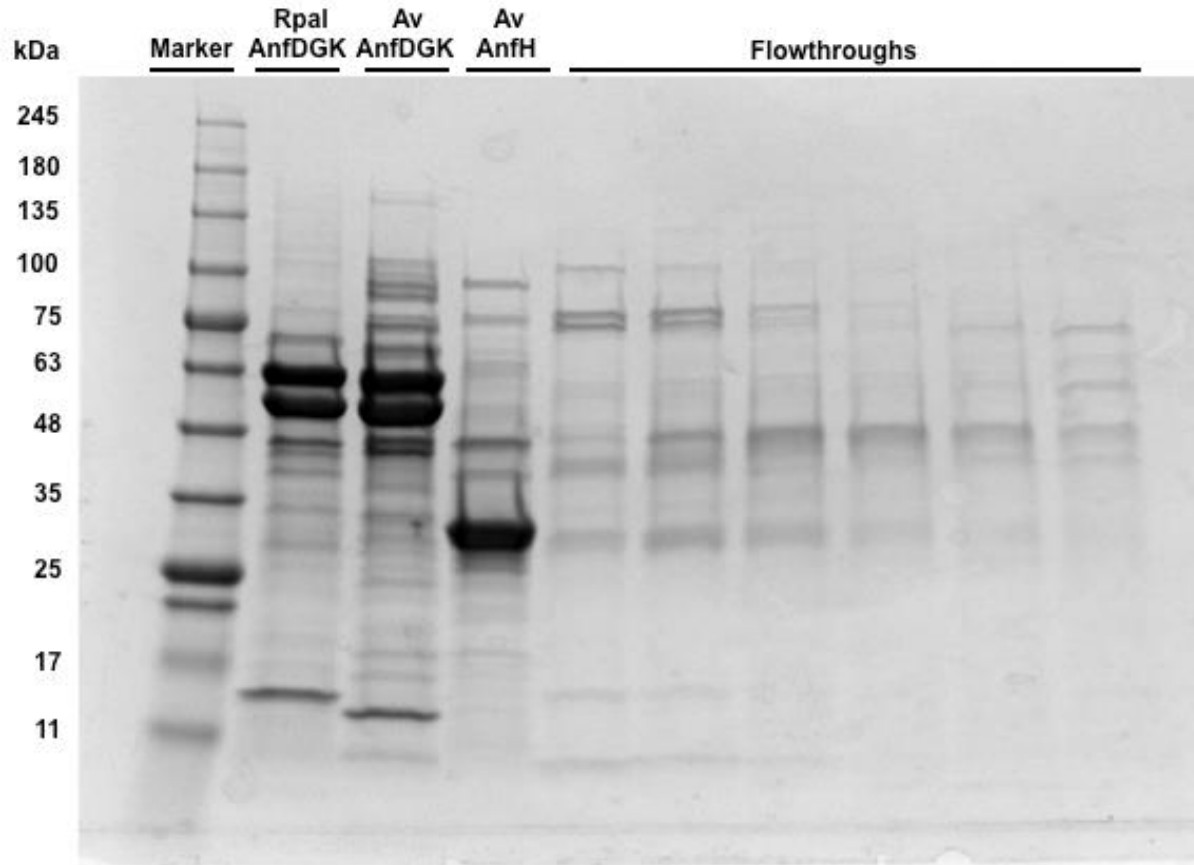
Supplementary Figure 2a | Multiple sequence alignment of a segment of representative NifD protein sequences. Position 201 in *R. palustris* NifD (ADU46342) is highlighted with a red box. For simplicity, only 40 sequences are shown here.

1. ADU27824.1	V	D	I	F	C	S	Q	C	A	G	F	A	G	V	S	Q	S	K	G	H	H	V	F	N	I	D	W	M	N	E	K	V
2. AAM04635.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	I
3. AAZ71203.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	I
4. ABA23631.1	V	D	I	F	C	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	V	L	N	I	A	W	I	N	E	K	V
5. ACO76522.1	V	D	V	F	T	V	E	C	P	G	F	S	G	V	S	Q	S	K	G	H	H	V	L	N	I	G	W	I	N	E	K	V
6. ADP69796.1	V	D	I	F	T	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	V	L	N	I	G	W	I	N	D	K	V
7. AGK14066.1	V	D	V	F	T	V	E	C	P	G	F	S	G	V	S	Q	S	K	G	H	H	V	L	N	I	G	W	I	N	E	K	V
8. AGK18954.1	V	D	V	F	T	V	E	C	P	G	F	S	G	V	S	Q	S	K	G	H	H	V	L	N	I	G	W	I	N	E	K	V
9. AGK98263.1	V	D	L	F	A	I	Q	C	P	G	F	A	G	V	S	Q	S	M	G	H	H	V	F	N	I	D	W	M	K	E	K	V
10. AIQ12504.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	V	L	N	M	G	W	I	N	E	K	V
11. AJA47505.1	V	D	L	F	A	V	Q	C	P	G	F	A	G	V	S	Q	S	M	G	H	H	V	F	N	I	D	W	M	K	E	K	V
12. AJA51493.1	V	D	L	F	A	V	Q	C	P	G	F	A	G	V	S	Q	S	M	G	H	H	V	F	N	I	D	W	M	K	E	K	V
13. AJE19761.1	V	D	V	F	T	V	E	C	P	G	F	S	G	V	S	Q	S	K	G	H	H	V	L	N	I	G	W	I	N	E	K	V
14. AKB29650.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	I
15. AKB33577.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	I
16. AKB44498.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	V
17. AKB48005.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	V
18. AKB51721.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	I
19. AKB54345.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	I
20. AKB57578.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	I
21. AKB81589.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	V
22. AKG34370.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	V	L	N	M	G	W	I	N	E	K	V
23. AKJ38129.1	V	D	I	F	V	V	E	C	P	G	F	A	G	V	S	Q	S	K	G	H	H	E	L	N	I	G	W	M	R	D	K	I
24. BAH06671.1	V	D	I	F	C	S	E	C	A	G	F	A	G	V	S	Q	S	K	G	H	H	V	F	N	I	S	W	M	N	E	K	V
25. EDK33789.1	V	D	I	F	C	S	E	C	A	G	F	A	G	V	S	Q	S	K	G	H	H	V	F	N	I	S	W	M	N	E	K	V

Supplementary Figure 2b | Multiple sequence alignment of a segment of representative VnfD protein sequences. Position 180 of VnfD (ADU27824) (corresponds to the residue position 201 of *R. palustris* NifD) is highlighted with a red box.

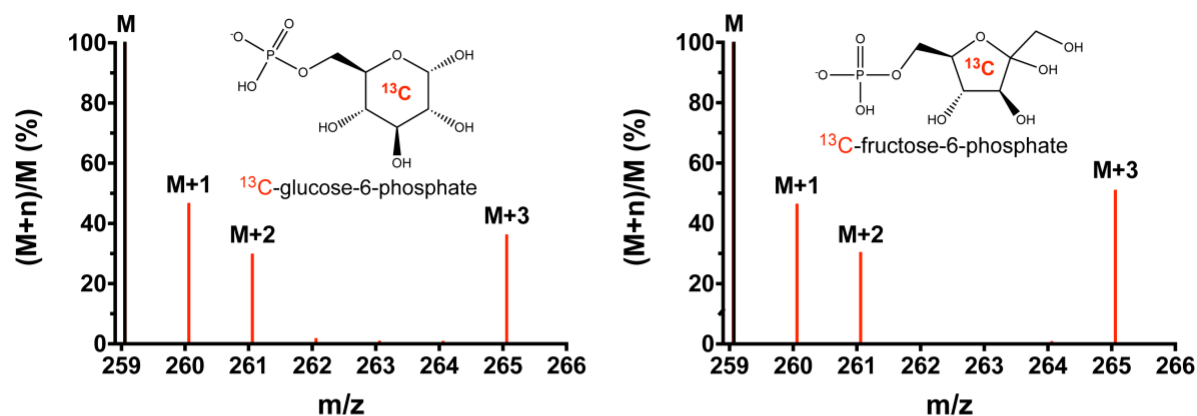
1. ADU44702.1	V	D	I	F	T	C	N	S	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	D	K	V
2. ABC22195.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	V	N	Q	K	V
3. ABD90205.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	L	N	Q	K	V
4. ABJ07809.1	I	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
5. ACF00151.1	V	D	I	F	T	C	N	S	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	D	K	V
6. ACF14288.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	V	N	R	K	V
7. ACM03751.1	V	D	V	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	L	N	Q	K	V
8. ACO80999.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
9. ACS86388.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
10. ADE84351.1	V	D	V	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	L	N	Q	K	V
11. ADP72547.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
12. ADQ79509.1	K	K	V	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	L	A	W	L	N	Q	M	V
13. ADY57120.1	V	D	I	F	V	C	N	S	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	K	Q	K	V
14. AEI37863.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	A	G	H	H	K	I	N	I	A	W	V	N	N	K	V
15. AEO47910.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	V	N	Q	K	V
16. AFA47492.1	V	D	I	F	V	C	N	S	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
17. AGK13257.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
18. AHF89180.1	V	D	I	F	V	C	N	S	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
19. AIF53399.1	V	D	I	F	V	C	N	S	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
20. AJQ26365.1	V	D	I	F	V	C	N	S	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
21. ALK10657.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	V	N	N	K	V
22. BAH05371.1	V	D	I	F	V	C	N	S	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	D	Q	K	V
23. BAR99382.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	V	N	N	K	V
24. CCG07115.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
25. CUU43320.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	V	N	N	K	V
26. EDK32424.1	V	D	I	F	V	C	N	S	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	D	Q	K	V
27. AIQ17623.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	I	I	N	I	A	W	I	K	Q	K	V
28. AIQ57937.1	V	D	I	F	V	C	N	S	P	G	F	Q	G	P	S	Q	S	G	G	H	H	I	I	N	I	A	W	I	K	Q	K	V
29. AIQ63607.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	V	N	N	K	V
30. CQR55107.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V
31. AGF56849.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	N	K	V
32. AJA49856.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	T	I	G	W	V	N	Q	K	V
33. AJA53844.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	T	I	G	W	V	N	Q	K	V
34. AHJ12912.1	K	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	H	E	K	V
35. AAZ70500.1	V	D	I	F	V	C	N	A	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	V	D	Q	K	V
36. AKB52430.1	V	D	I	F	V	C	N	A	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	V	D	Q	K	V
37. AKB82412.1	V	D	I	F	V	C	N	A	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	D	Q	K	V
38. AAM04629.1	V	D	I	F	V	C	N	A	P	G	F	G	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	D	Q	K	V
39. AGK17546.1	V	D	I	F	V	C	N	S	P	G	F	A	G	P	S	Q	S	G	G	H	H	K	I	N	I	A	W	I	N	Q	K	V

Supplementary Figure 2c | Multiple sequence alignment of a segment of representative AnFD protein sequences. Position 180 of AnFD (ADU44702) (corresponds to the residue position 201 of *R. palustris* NifD) is highlighted with a red box.

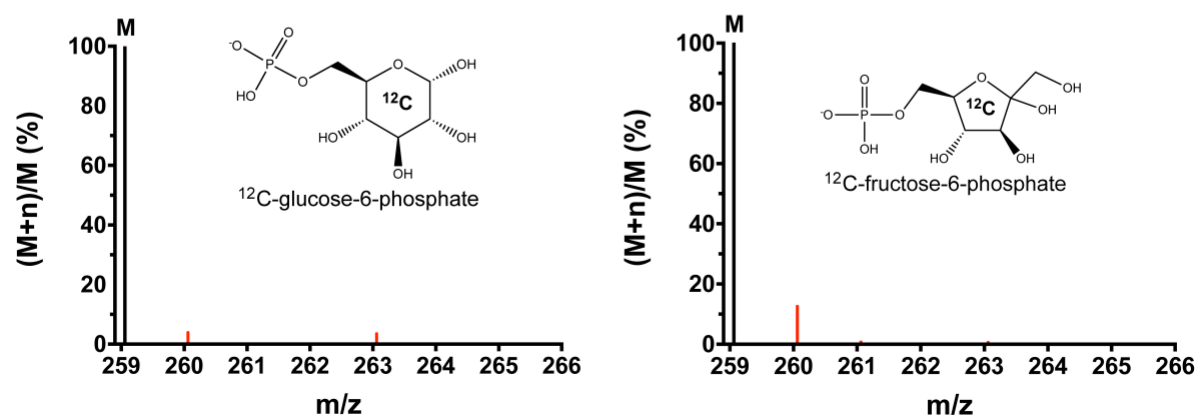


Supplementary Figure 3 | Complete SDS-PAGE gel used to analyze AnfDGK protein purified from *R. palustris* strain CGA7554 and AnfH protein purified from *A. vinelandii* strain DJ1255. The SDS-PAGE gel shown in Fig. 2a is a clipped version of this figure.

R. palustris* (^{13}C -bicarbonate) + *Methylomonas



$^{12}\text{CH}_4$ + *Methylomonas*



Supplementary Figure 4 | LC-MS data showing that $^{13}\text{CH}_4$ produced by *R. palustris* fed ^{13}C -bicarbonate is incorporated into *Methylomonas* sp. LW13 cells. Compounds consistent with a retention time and fragmentation pattern of ^{13}C -labeled glucose-6-phosphate and ^{13}C -labeled fructose-6-phosphate were detected in *Methylomonas* sp. LW13 cells. The data shown are one representative sample of four biological repetitions of this experiment.

Supplementary Table 1 | Taxonomic distribution of all forms of nitrogenases in 4571 complete archaeal and bacterial genomes. The total number of genomes for each phylum along with the number of genomes within that phylum that encode for nitrogenases are shown.

Domain	Phylum	Total genomes	Total genomes encoding nitrogenase				Total nitrogenase homologs			
			AnfD	VnfD	NifD	Total	AnfD	VnfD	NifD	Total
ARCHAEA	Crenarchaeota	60	0	0	0	0	0	0	0	0
	Thaumarchaeota	11	0	0	0	0	0	0	0	0
	Candidatus Korarchaeota	1	0	0	0	0	0	0	0	0
	Nanoarchaeota	2	0	0	0	0	0	0	0	0
	Euryarchaeota	156	2	11	48	48	2	11	48	61
BACTERIA	Acidobacteria	8	0	0	0	0	0	0	0	0
	Actinobacteria	495	0	0	4	4	0	0	4	4
	Aquificae	16	0	0	0	0	0	0	0	0
	Armatimonadetes	1	0	0	0	0	0	0	0	0
	Bacteroidetes	169	1	0	2	2	1	0	2	3
	Caldiserica	1	0	0	0	0	0	0	0	0
	candidate division NC10	1	0	0	0	0	0	0	0	0
	Candidatus									
	Saccharibacteria	4	0	0	0	0	0	0	0	0
	Chlamydiae	89	0	0	0	0	0	0	0	0
	Chlorobi	11	1	0	11	11	1	0	11	12
	Chloroflexi	25	0	0	4	4	0	0	4	4
	Chrysiogenetes	1	0	0	0	0	0	0	0	0
	Cloacimonetes	1	0	0	0	0	0	0	0	0
	Cyanobacteria	87	0	1	20	20	0	0	21	21
	Deferribacteres	4	0	0	2	2	0	0	2	2
	Deinococcus-Thermus	22	0	0	0	0	0	0	0	0
	Dictyoglomi	2	0	0	0	0	0	0	0	0
	Elusimicrobia	3	0	0	0	0	0	0	0	0
	Fibrobacteres	3	0	0	0	0	0	0	0	0
	Firmicutes	1013	11	8	70	70	11	8	70	89
	Fusobacteria	19	0	0	1	1	0	0	1	1
	Gemmatimonadetes	1	0	0	0	0	0	0	0	0
	Ignavibacteriae	2	0	0	0	0	0	0	0	0
	Nitrospirae	8	0	0	2	2	0	0	2	2
	Planctomycetes	7	0	0	0	0	0	0	0	0

	Proteobacteria	2102	15	1	188	188	15	1	193	209
	Spirochaetes	64	0	0	2	2	0	0	2	2
	Synergistetes	4	0	0	0	0	0	0	0	0
	Tenericutes	142	0	0	0	0	0	0	0	0
	Thermodesulfobacteria	4	0	0	1	1	0	0	1	1
	Thermotogae	25	0	0	0	0	0	0	0	0
	Verrucomicrobia	7	1	0	3	3	1	0	3	4
EUKARYA	Apicomplexa	1	0	0	0	0	0	0	0	0
	Arthropoda	2	0	0	0	0	0	0	0	0
	Ascomycota	10	0	0	0	0	0	0	0	0
	Chlorophyta	2	0	0	0	0	0	0	0	0
Total		4586	31	20	358	358	31	20	364	415

Supplementary Table 2 | Strains and plasmids used.

Strain or plasmid	Genotype or phenotype	Source
<i>R. palustris</i> strains		
CGA009	Wild type; <i>hupV</i> mutant; spontaneous frameshift (4-bp deletion) in <i>hupV</i>	45
CGA010	Wild type; <i>hupV</i> repaired derivative of CGA009; 4 bp inserted in <i>hupV</i>	46
CGA766	CGA009 <i>AnifH nifD::Tn5 ΔanfA</i> ; expresses wild-type V nitrogenase	24
CGA7661	CGA766 in which the <i>vnfD</i> ^{V57AH180Q} mutation was introduced at its native locus using allelic exchange; expresses variant V nitrogenase	This study
CGA755	CGA009 <i>AnifH ΔvnfH</i> ; expresses wild-type Fe-only nitrogenase	24
CGA7551	CGA755 in which the <i>anfD</i> ^{V57AH180Q} mutation was introduced at its native locus using allelic exchange; expresses variant Fe-only nitrogenase	This study
CGA7552	CGA009 <i>AnifHDK ΔvnfH</i>	This study
CGA7553	CGA009 <i>AnifHDK ΔvnfHDKG</i> ; expresses wild-type Fe-only nitrogenase	This study
CGA7554	CGA755 in which the <i>anfD::his</i> was introduced at its native locus using allelic exchange; used for the purification of His-tagged AnfDGK complex	This study
<i>A. vinelandii</i> strains		
DJ	Wild type	47
DJ1255	DJ <i>ΔnifDK ΔvnfK::Km^R</i>	This study
<i>R. rubrum</i> UR2	Wild type; spontaneous Sm ^R derivative of ATCC11170	48
<i>R. capsulatus</i> SB 1003	Wild type	49
<i>Methylomonas</i> sp. LW13	Wild type	30
<i>E. coli</i> S17-1	<i>thi pro hdsR hdsM⁺ recA</i> ; chromosomal insertion of RP4-2 (Tc::Mu K _m ::Tn7)	50
Plasmids		
pJQ200SK	Gm ^R , <i>sacB</i> ; mobilizable suicide vector	51
pJQ- <i>anfD</i> ^{V57AH180Q}	Gm ^R ; <i>anfD</i> ^{V57AH180Q} coding sequence cloned into the PstI site of pJQ200SK	This study
pJQ- <i>vnfD</i> ^{V57AH180Q}	Gm ^R ; <i>vnfD</i> ^{V57AH180Q} coding sequence cloned into the PstI site of pJQ200SK	This study
pJQ- <i>anfD::his</i>	Gm ^R ; <i>anfD</i> coding sequence with an his8x tag encoded at the C terminus cloned into the PstI site of pJQ200SK	This study
pJQ- <i>ΔnifHDK</i>	Gm ^R ; in-frame <i>ΔnifHDK</i> cloned into PstI site of pJQ200SK	This study
pJQ- <i>ΔvnfDGK</i>	Gm ^R ; in-frame <i>ΔvnfDGK</i> cloned into PstI site of pJQ200SK	This study

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