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SUPPLEMENTARY INFORMATION

ARTICLE NUMBER: 16055 | DOI: 10.1038/NMICROBIOL.2016.55

Phenotypic heterogeneity driven by nutrient limitation promotes growth in fluctuating environments - Supplementary Information

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Sequence information for probes used for smFISH

nifH-targeted smFISH probes:

GCGCATTGACGCATGGTCAT
GATACCGCCTTTACCGTAAA
TCTGCGTGGTGGTGGATTTA
ATCTCCGCCAGCGCGGCGAC
GACGATCATCACTTTCTTAC
AGTCCGCCTTCGGATCGCAG
GCGTGCAGAATCAGACGGGT
CATAATGGTGTTCTGTGCTT
AGCCGACTTCCGCGGCCATC
TCGAGTTCGAGGTCCTCGAC
GTAGCCAATTTGCAGCACGT
ATTCCGCGCAGCGCACATCG
ACGCCTGGCTCCGGGCCGCC
CACGCCGCGTCCCGCGCAGC
CAAGAAAGTTGATCGCCGTG
TCCTCGTAGGCGCCTTCTTC
ATAGAACACGAAATCGAGAT
AGACCACGTCGCCGAGCACG
ATCGGCATGGCGAAGCCGCC
CTCCTGGGCTTTGTTTTCGC
CGCCGGAGCAGACGATGTAG
GCCGCGTACATCGCCATCAT
GATCCCTTTGGAGATATTGT
TGCCGGATTTGGCGTATTTA
ATCAGGCCGCCGAGGCGCAC
GTCGGTCTGACGTGAGTTAC
CAATAATCAGTTCGTCTTCA
GTACCGAGCTTTTCCGCCAG
GGGCACAAAGTGGATCATCT
CGCGCTGCACGATGTTGTCG
ACCGTCATGCGGCGGATCTC
ACAGGCGGGGTCGTACTCGA
TGCGGTATTCGTTGGCCTGT
TTGACGATCTTCTGCGCCAG
CGGCACCACTTTCATGGTGT
GCTCATCCATGGTGCAGGGC
AACTCCATCAGCAGCGATTC
GTCTTCCTCTTCCATGATGC
CGGTTTTGCCAATGATGCTG
CAGGCCGCGTTTTCTTCGGC

glnK-targeted smFISH probes:

TAATGGCCGCAGCTAACACG
GTCCCCTCCAGTCAGAATTC
ACCACGGTAACCAGCTTCAT
TTCCAGTTTGAATGGTTTGA
AAGATAATCGTTCACGCACA
GTCAGGCCCTGAATACCCAT
GAACCCTTTAACTTCAGTGA
CCGCGTGGCCTTTCTGACGG
TATTCGGCGCCGCGATAGAG
TTTCGGTAGAAAATTGACGC
CAATCGCGACATCAATCTTC
ACTTCGTCCAGCTGATCGTC
CGCTTTACTGATGACATCGA
CGCCAATCTTTCCGGTGTAC
CAGCTCGGCGACAAAGATTT
CGGTACGGATGCGAATCACG
AGTGCGGCTTCGTCAGATTC

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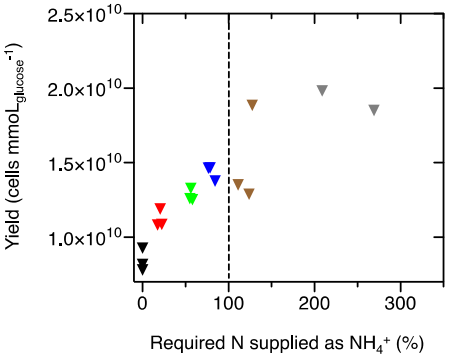

Supplementary Figure 1. Biomass yield expressed in cells per mmol glucose in chemostats with increasing NH_4^+ supply levels as shown in Figure 1. The symbols share the colour code with the schematic representation in Figure 1a.

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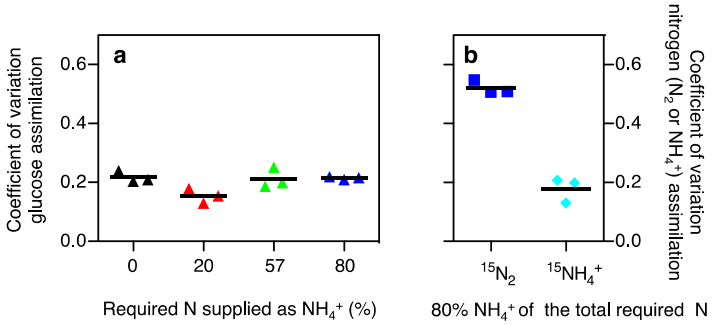

Supplementary Figure 2. Phenotypic heterogeneity in glucose and NH_4^+ assimilation. **(a)** The coefficient of variation in glucose assimilation at different NH_4^+ supply levels comparable to Figure 2f. Statistical analysis was performed with a 1-Way-ANOVA (p=0.029). **(b)** The coefficient of variation in NH_4^+ assimilation (cyan diamond) as compared to N_2 fixation (blue square, taken from Figure 2f) at NH_4^+ supply with 80% of the total required N. Statistical analysis was performed with an unpaired t-test (p=0.0002). Data originates from replicated chemostat populations.

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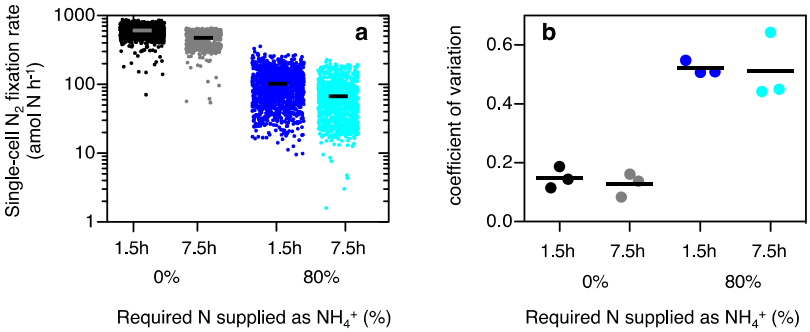

Supplementary Figure 3. Phenotypic heterogeneity in N_2 fixation at different NH_4^+ supply levels and different incubation times with $^{15}N_2$. NH_4^+ was supplied at 0% (black and grey) and 80% (blue and cyan) of the total required N. Incubations were run for 1.5 h (black and blue, taken from Figure 2f) and 7.5 h (grey and cyan). **(a)** Single-cell N_2 fixation rates (circles) and the average (horizontal bars) calculated from the single-cell NanoSIMS data for replicate chemostat populations (replicates and number of cells measured are shown in Supplementary Table 1). **(b)** The corresponding coefficients of variation in N_2 fixation activity for each replicate chemostat population (circles) as shown in **(a)**. The horizontal bar displays the mean. Statistical analysis between groups with different incubation times at the same NH_4^+ supply regime were performed with an unpaired t-test ($p_{0\%(1.5h:7.5h)}$=0.523 & $p_{80\%(1.5h:7.5h)}$=0.892).

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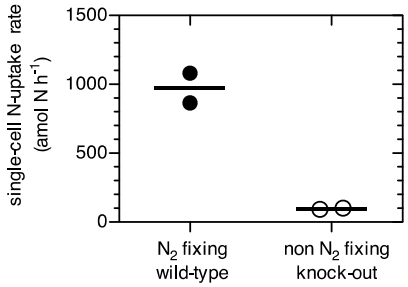

Supplementary Figure 4. Single-cell N-uptake rates of N_2 fixing wild-type *K. oxytoca* cells (black circles) and cells of the non N_2-fixing strain *K. oxytoca* UNF107::rpsL1Δ107Δgnd-his-nif(pUA66::P_{rpsM}::*gfp*::*km*) (open circles) incubated in a mixed chemostat in the presence of $^{15}N_2$ and without supply of external NH_4^+. N_2-fixing wild-type cells apparently leak $^{15}NH_4^+$ produced from $^{15}N_2$ that is being taken up by *K. oxytoca* UNF107. The uptake rate of NH_4^+ produced from N_2 per cell makes up about 10% of the uptake rate of N_2 directly to wild-type cells.

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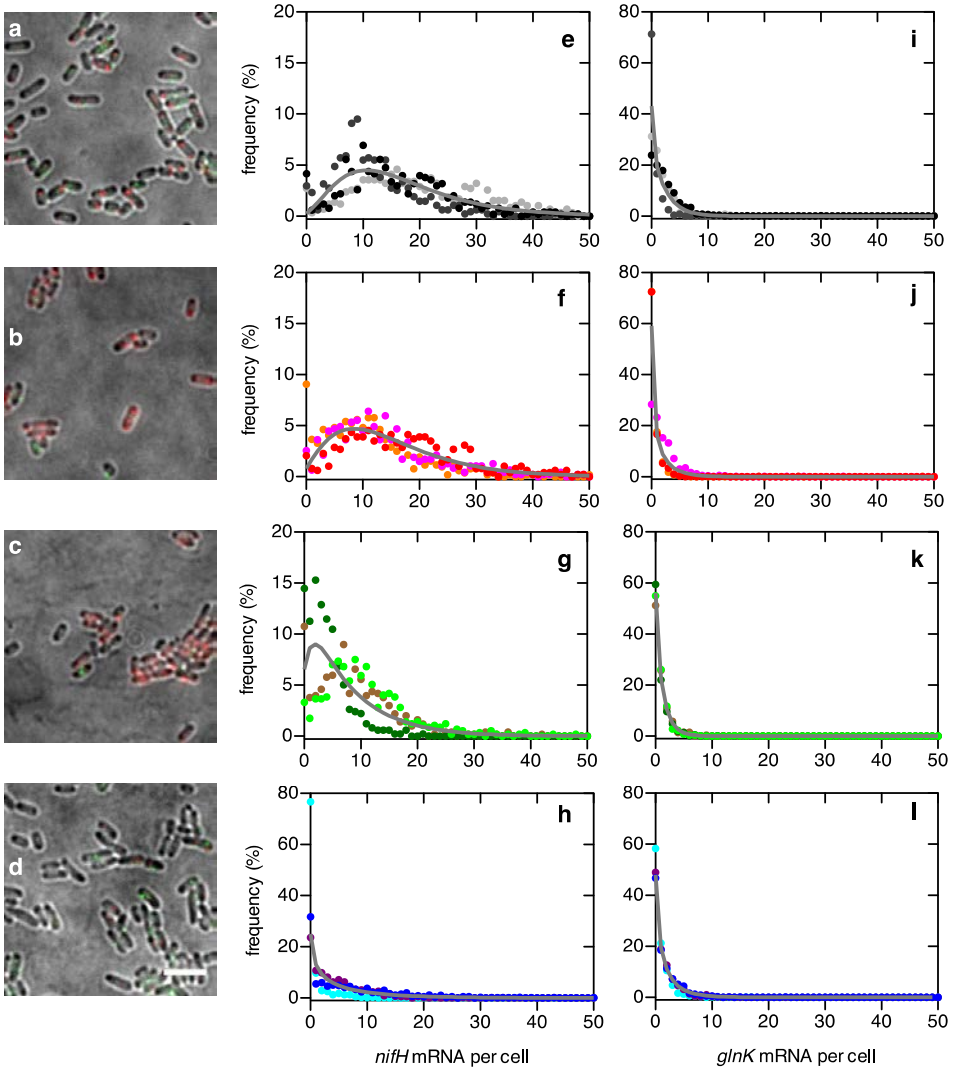

Supplementary Figure 5. Micrographs of *nifH* and *glnK* mRNA expression measured with smFISH in single *K. oxytoca* cells grown in chemostats supplied with 0% **(a)**, 29% **(b)**, 60% **(c)**, and 86% **(d)** NH_4^+ of the total required N and the corresponding histograms for *nifH* **(e-h)** and *glnK* **(i-l)** expression. We analysed approximately 500 cells in each of the 3 biological replicates per experimental condition. The experimental data from the 3 replicated chemostat populations (filled circles) and the average fit to a negative binominal distribution (grey line) are shown in histograms. Non-expressing cells in **(e)** and **(f)** were excluded from the fit. The population displayed with cyan circles in panels **h & l** was removed from the analysis for reasons described in the caption of Figure 2. The scale bar in **(a-d)** corresponds to 4 µm. A threshold was applied to the green (41 of 255) and the red (10 of 255) channel to remove background signal.

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Supplementary Table 1

NanoSIMS measurements: data values and number of analysed cells

	Relates to Figure	Number of analysed cells	Mean single-cell N_2 fixation rate amol N h^{-1}	Coefficient of variation N_2 fixation
No NH_4^+	2e; SF** 2a; SF 3	270	617	0.115
($^{15}N_2$, ^{13}C-glucose,1.5 h)*		317	682	0.144
		414	529	0.187
No NH_4^+	SF 3	244	339	0.162
($^{15}N_2$, 7.5 h)*		223	499	0.138
		323	572	0.084
20% NH_4^+	2e; SF 2a	504	493	0.129
($^{15}N_2$, ^{13}C-glucose,1.5 h)*		375	472	0.157
		414	398	0.143
57% NH_4^+	2e; SF 2a	265	275	0.225
($^{15}N_2$, ^{13}C-glucose,1.5 h)*		461	269	0.266
		513	186	0.277
80% NH_4^+	2e; SF 2a&b; SF 3	337	119	0.507
($^{15}N_2$, ^{13}C-glucose,1.5 h)*		443	77	0.548
		395	112	0.509
80% NH_4^+	SF 3	169	109	0.442
($^{15}N_2$, 7.5 h)*		316	54	0.450
		423	61	0.643
80% NH_4^+	SF 2b	262	579***	0.199****
($^{15}NH_4^+$, 1.5 h)*		256	466***	0.208****
		316	542***	0.131****

*type of isotopically labelled substrate and incubation time
**SF (Supplementary Figure)
***NH_4^+ assimilation rate
**** coefficient of variation in NH_4^+ assimilation

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Supplementary discussion

Supplementary discussion on the relevance of NH_4^+ leakage and re-uptake

We performed an experiment to test how much of fixed NH_4^+ leaks from cells and is then being taken up by cells that do not fix N_2. We constructed a reporter strain (*K. oxytoca* UNF107::rpsL1Δ107Δgnd-his-nif[pUA66::P_{rpsM}::*gfp*::*km*], denoted in short UNF107) based on UNF107rpsL1Δ107(gnd-his-nif-deletion)[1] by transforming the strain with plasmid pUA66::P_{rpsM}::*gfp*::*km*[2] (*gfp* under the control of the *E. coli rpsM* promoter and kanamycin resistance). We grew *K. oxytoca* wild-type cells in a chemostat without NH_4^+ supply as described in the Methods section. Further, we grew *K. oxytoca* UNF107 in a parallel chemostat under NH_4^+ limitation (20 mM Glucose and 2 mM NH_4Cl in feed medium). The feed medium for UNF107 contained in addition: 40 mg L^{-1} histidine (UNF107 is his-auxothroph) and 50 mg L^{-1} Kanamycin (to stabilize the plasmid). After both chemostats reached steady state, we pulsed the chemostat that contained the wild-type cells with citrate buffer-washed $^{15}N_2$ (50%; Sigma Lot MBB0968V, see Supplementary discussion) and incubated the chemostat for 1.75 h. Simultaneously, we washed and concentrated *K. oxytoca* UNF107 in a anaerobic glove box as described in the Methods section.

The concentrated cell suspension of *K. oxytoca* UNF107 was added to the chemostat with the wild-type cells to reach a relative abundance of 5 % after the wild-type cells were incubated 1.75 h with $^{15}N_2$. The pre-incubation was done to allow for the removal of any residual $^{15}NH_3$ supplied with the $^{15}N_2$ gas and to facilitate the equilibration of the external NH_4^+ pool with $^{15}NH_4^+$ that leaked from $^{15}N_2$ fixing cells. The mixed cell suspension was incubated for additional 1.75 h in the presence of $^{15}N_2$ before cells were exposed for 5 min to the air (to allow for the maturation of Gfp in UNF107) followed by washing in PBS and fixation in 3.7% paraformaldehyde as described in the Methods section. Cells were filtered on polycarbonate membrane filters, imaged and marked with a laser micro-dissection microscope, and subjected to NanoSIMS analysis as described in the Methods section. UNF107 cells were distinguished from wild-type cells based on their Gfp-fluorescence. The experiment was performed in duplicate. We analysed 500 and 521 wild-type cells and 25 and 45 UNF107 cells per repeat, respectively.

The results show that the wild-type cells fixed 973 amol N h^{-1} (±152 amol N h^{-1}; SD, N=2) into their biomass, while UNF107 incorporated 96 amol N h^{-1} (±5 amol N h^{-1}; SD, N=2) into their biomass (Supplementary Fig. 4). The data demonstrates that the uptake of leaked NH_4^+ per cell makes up about 10 % of the total fixed N under N_2 fixing conditions. This indicates that leakage and re-uptake does not strongly affect our estimates of heterogeneity in N_2 fixation.

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Supplementary discussion that ^{13}C-glucose assimilation is a proxy for growth

The pulse-chase/pulse experiment is based on the assumption that glucose assimilation is a good proxy for growth. To support this assumption, we concomitantly labelled chemostats with ^{13}C-glucose and determined single-cell, ^{13}C-carbon-based specific growth rates (calculated with equations 4-6) of chemostat-grown cells. We find that the ^{13}C-carbon-based specific growth rates averaged across all NH_4^+ supply levels (0.095 ± 0.01 h^{-1}; mean ± SEM, N=13) are similar to the set dilution rate of the chemostat (i.e. 0.1 h^{-1}).

Further, the data of the pulse-chase/pulse experiment indicates that ^{13}C-carbon assimilation is a good proxy to assess growth also after a switch to NH_4^+-depletion, because the single-cell, carbon-based growth rates averaged across all 4 experimental runs (0.093 ± 0.008 h^{-1}; mean ± SEM; N=4 as calculated from data in Fig. 3a,b) are in the same range as the population growth rates (0.08 ± 0.01 h^{-1}; mean ± SEM; N=4) determined from OD measurements during the experiment. Populations do not have a considerable lag-time under the conditions of the experiment, because they already contain the enzymes for N_2 fixation that have been produced during NH_4^+-limited growth before the switch (Fig. 3b). This experiment also indicates that most carbon-assimilation after the switch is linked to growth and not to production of carbon storage compounds, because nitrogen and carbon are assimilated at a ratio (Fig 3b; Deming regression slope = 2.9 ± 0.15 amol C per amol N; mean ± SEM, N=2) that is not above the C:N ratio of the biomass in NH_4^+-free chemostats (4.7 ± 0.1; mean ± SEM, N=2; assessed from elemental analyser data).

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Supplementary discussion on the purity of $^{15}N_2$ gas stocks

It has recently been reported that commercially available $^{15}N_2$ gas stocks could be contaminated with $^{15}NH_3$[3]. We calculated the potential effect of the contamination by calculating the ratio of $^{15}NH_3/^{15}N\text{-}N_2$ in the new biomass based on the new biomass formed during the incubation time, the $^{14}NH_4^+$ supplied from the feed during the incubation, the amount of $^{15}NH_3$ supplied during the pulse, the isotopic labelling% of $^{15}N_2$, and by assuming that cells would consume $^{15}NH_3$ and $^{14}NH_4^+$ before engaging in N_2 fixation. The calculation showed that a contamination of 6 µmol NH_3 per mole N_2 would result in a $^{15}NH_3/^{15}N\text{-}N_2$ ratio $\leq$ 0.01 across different NH_4^+ supply treatments. Dabundo et al. (2014) reports that gas provided by Cambridge Isotopes is relatively pure with values ranging between 0.014-0.052 µmol NH_3 per mole N_2 across different Lots. We conclude that the pulse-chase/pulse experiments conducted with gas provided by Cambridge Isotopes are not affected by the contamination.

In contrast, Dabundo et al. (2014) reported that gas provided by Sigma (Lot MBB0968V, as used by us) is contaminated with 1900 µmol NH_3 per mole N_2. This contamination could theoretically affect the N_2 fixation measurements. Therefore, we removed NH_3 from gas provided by Sigma by incubating 72 mL gas at an overpressure of 0.8 bar with 20 mL anoxic citrate buffer (0.1M, pH3) in a 60 mL sealed serum flask for 2 h on a shaker (200 rpm) at room temperature. 20 mL of the purified gas was withdrawn from the serum flask and was used in the pulse labelling experiments. We confirmed the purity of the gas incubated with citrate buffer by equilibrating it with 10 mL 0.1x phosphate buffered saline (pH 7.4; citrate buffer cannot be used since it interferes with the NH_4^+ assay) in a 36 mL closed serum flask on a shaker (200 rpm) at room temperature. Subsequently, we measured the NH_4^+ concentration in the liquid phase using the sensitive orthophthaldialdehyde assay[4]. We found that the remaining contamination is about 1.7 $\pm$ 1.5 (N=4) µmol NH_3 per mole N_2. We conclude that our cleaning procedure reduces the $^{15}NH_3$ contamination in the gas provided by Sigma to levels that will not affect our results.

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Supplementary references

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