Cell-to-cell Heterogeneity in Growth Rate and Gene Expression

in Methylobacterium extorquens AM1

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Running title: Single Cell Analysis in Methylobacterium extorquens AM1

Key Words: Methylobacterium extorquens AM1, methanol dehydrogenase, promoter activity, heterogeneity, single cells
Cell-to-cell heterogeneity in gene expression and growth parameters was assessed in the facultative methylotroph, *Methylobacterium extorquens* AM1. A transcriptional fusion between a well-characterized methylotrophy promoter ($P_{mxaF}$) and $gfp_{uv}$ was used to assess single cell gene expression. Using a flow-through culture system and laser scanning microscopy, data on fluorescence and cell size were obtained over time through several growth cycles on cells grown on succinate or methanol. Cells were grown continuously with no discernable lag between divisions with high cell-to-cell variability observed for cell size at division (2.5-fold range), division time, and growth rates. When individual cells were followed over multiple division cycles, no direct correlation was observed between growth rate before a division and subsequent growth rate, or between cell size at division and subsequent growth rate. The cell-to-cell variability for GFPuv fluorescence from the $P_{mxaF}$ promoter was less, with a range on the order of 1.5-fold. Fluorescence and growth rate was also followed during a carbon shift experiment, in which cells growing on succinate were shifted to methanol. Variability of response was observed and growth rate at the time of the shift from succinate to methanol was a predictor of response. Higher growth rates at the time of substrate shift resulted in greater decreases in growth rates immediately after the shift, but full induction of $P_{mxaF}$-$gfp_{uv}$ was achieved faster. These results demonstrate that in *M. extorquens*, physiological heterogeneity at the single-cell level plays an important role in determining population response to this metabolic shift.
Introduction

A growing body of evidence shows that isogenic populations of exponentially-growing microorganisms contain substantial cell-to-cell heterogeneity at both the gene expression and growth rate levels (6, 9, 10, 12-16, 19, 20, 29-31, 36, 39). Cell-to-cell heterogeneity in gene expression has been shown to arise from fluctuations in the global gene expression machinery of the cell, which has been termed variously “extrinsic noise”, “global noise”, or “gene expression capacity” (9, 12, 27, 29, 30). In some cases the source of the variation has been shown to be cell-to-cell differences in transcription, mRNA stability, and/or translation (5, 27). Two recent studies have also measured mRNA in individual cells (13, 19). The results of these studies suggest that cell-to-cell variation of gene expression is not due to fluctuations in low copy mRNA numbers, but rather, appears to be due to variations at the bulk mRNA stability and/or translational level.

Significant cell-to-cell variations have also been reported for generation times and growth rate. It was shown as early as 1932 that bacteria and yeast cells exhibit a 2-3 fold variation in individual cell division time, and that the previous division time does not influence the subsequent one, in other words, that the variation appears stochastic (15). More recent studies with *Escherichia coli* found a similar range of division times (36), and studies of growth rate in yeast also found a broad spread (9).

Although cell-to-cell variation in gene expression and growth rate have been suggested to generate phenotypic diversification (1, 3, 7, 17, 18, 34), very little is known concerning the connection between stochasticity in gene expression and resultant phenotypic diversity. One prediction is that gene expression might correlate with growth rate, and in the study cited above in yeast (9), a positive correlation was obtained between output of the mating pheromone...
response pathway in yeast and growth rate (increase in cell volume). However, similar studies have not yet been reported in bacteria.

Further information suggests that even minor subpopulations of bacteria in a significantly different physiological state from the population average, for instance, growing very slowly, can play a major role in population response especially under stressful conditions (1, 3, 7, 17, 18, 34). It has been suggested that cell-to-cell phenotypic heterogeneity generates physiologically-distinct subpopulations that are resistant to stress (7).

We are interested in how various parts of metabolism are integrated at the transcript, protein, and flux level in the facultative methylotroph, Methylobacterium extorquens AM1. This bacterium has two strongly contrasting modes of metabolism, growth on multi-carbon compounds, which is energy-limited, and growth on one-carbon compounds, which is limited by reducing power (37). In addition, growth on one-carbon compounds involves high flux through the toxic intermediate formaldehyde, raising the possibility of fluctuating stress conditions (8, 24). Our studies involve perturbing the metabolic state, either by stress or by changing growth substrate, and following metabolic response at the global level. Therefore, in this system it is important to understand cellular heterogeneity within these populations and ultimately to link physiological state to phenotypic response. In this study, we have begun to characterize heterogeneity at the individual cell level in M. extorquens AM1 by simultaneously monitoring expression of GFPuv from a well-characterized methylotrophy promoter (PmxaF; 21, 40) and measuring change in cell size over time in a flow-through system coupled to laser scanning microscopy. We have monitored these parameters in both stable growth conditions and during response to change in substrate from a multi-carbon compound (succinate) to a one-carbon compound (methanol).
Materials and Methods

Bacterial strains and growth conditions. *M. extorquens* AM1 strains were grown in batch culture at 28°C in minimal salts media supplemented with either 0.3% (v/v) methanol or 0.4% succinate as a growth substrate (2, 38). The strains and plasmids used for this study are listed in Table 1. As appropriate, antibiotics were added: 50 µg/ml rifamycin and 10 µg/ml tetracycline.

Construction of vectors for the deletion of flagellar gene clusters. Allelic exchange vectors were constructed from pCM184 (22). Fragments (~600bp) flanking the ΔmotA-fliN-fliM-fliG, ΔmotC-motB, and ΔpomA-motB flagellar gene clusters were PCR amplified and inserted into pCR2.1 (Invitrogen, Carlsbad, CA) to make pXG1, pXG2, pTS63, pTS64, pTS67, and pTS68. The ΔmotA-fliN-fliM-fliG mutant insertion vector was generated by inserting the ~560bp *Bgl*II-*Nde*I upstream fragment from pXG1 into pCM184 to make pXG3. The ~600bp *Sac*I-*Sac*II downstream flank from pXG2 was inserted into pXG3 to make pXG4. The ΔmotC-motB mutant insertion vector was constructed by inserting the ~630bp *Bgl*II-*Not*I upstream fragment from pTS63 into pCM184 to make pTS65, and then inserting the ~630bp *Apa*I-*Sac*I downstream fragment from pTS64 into pTS65 to make pTS66. The ΔpomA-motB mutant insertion vector was made by inserting the ~740bp upstream fragment from pTS67 into pCM184 to make pTS69, and then inserting the ~670bp downstream fragment from pTS68 into pTS69 to make pTS70.

Construction of the non-motile mutant, TSX. Flagellar gene cluster mutations were sequentially introduced using the insertion vectors described above (pXG4, pTS66, and pTS70) by electroporation (35) into *M. extorquens* AM1 and screening for kanamycin resistance (33 µg/ml) and tetracycline sensitivity (22). All mutations were confirmed by PCR analysis. Antibiotic markers in mutants were removed using the *cre-lox* system using pCM157 as
described (22, 23). Motility of *M. extorquens* AM1 strain TSX was assessed by visual observation with a Zeiss Axioplan microscope using a 100x 1.3 N.A. objective (Thornwood, NY). The first two constructions maintained motility (TS66 and TS70), while the final construction (TSX) showed no detectible motility.

**Fluorimetry analysis.** Fluorescence measurements were carried out with a Shimadzu RF-5301PC fluorimeter (Columbia, MD). GFPuv excitation was conducted at 405nm and emissions monitored at 509nm with an emission slit width of 5:5.

**Flow system setup for microscopy.** A continuous flow system was assembled to facilitate microscope experiments (Fig. 1).

*Design:* The tubing and adapters used were all obtained from Upchurch Scientific (Oak Harbor, WA). All tubing used was 1/16” OD (0.75mm ID) PEEK tubing. One-liter Pyrex bottles were used for a media reservoir and waste. The bottle caps for the reservoir and waste were modified to house a 0.2 µm syringe filter and to accommodate the intake or output lines. The intake line was weighted with an inline solvent filter housing to insure the intake stayed submerged. Fluid control was conducted with a Global FIA Milligat pump (Fox Island, WA) controlled using LabView (National Instruments, Austin TX). A gas equilibration system was constructed to equilibrate the medium with air before it entered the culture chamber. This system consisted of a 250 ml Pyrex bottle with a modified cap that had four holes drilled into it. Two holes housed the inlet and outlet lines and the other two holes allowed the interior of the bottle to stay equilibrated with atmospheric gasses. The inlet and outlet lines were connected to 50 ft of 0.51mm ID x 0.94 mm OD silastic tubing (VWR, West Chester PA) within the pyrex bottle. A luer inline check valve was used for the injection of cell samples into the culture chamber. A Bioptechs FCS2 closed system chamber (Butler, PA) was used for cell culturing, with a 0.5 mm gasket generating
a volume of 350 µl. For carbon shift experiments, a 0.5 cm channel was cut into a solid 0.5 mm
gasket to reduce mixing of medium containing the two substrates. For all experiments, the cover
slip for the FCS2 was treated with a 0.01% (w/v) Poly-L-Lysine solution (Sigma Aldrich, St
Louis MO) such that cells could be anchored in place by their flagella (4). The slides were
immersed in the solution for 10 minutes and allowed to cure in air at 37°C for an hour.
Temperature control of medium prior to entry into the culture chamber was accomplished by
immersion of the medium reservoir and gas equilibration system in a water bath. Temperature
control of the FCS2 chamber was maintained with an objective heater and a chamber heater
(Bioptechs, Butler PA). For carbon shift experiments, two sets of a medium reservoir, pump,
and gas equilibration system were connected to the rest of the flow system using a 4-port switch
valve.

System prep and maintenance: Prior to experiments, the flow system was primed by flowing
medium at 10 µl/sec for 30 minutes to eliminate any air bubbles. All experiments were
conducted at a flow rate of 1 µl/sec. For cleaning and sterilization, the system was flushed with
10% hypochlorite for 30 minutes, followed by dH₂O for at least 2 hrs, all at a flow rate of 10
µl/sec. The entire flow system was then autoclaved.

Microscopy: Microscopy experiments were conducted on a Zeiss LSM 510 META using a
100x 1.45 N.A. objective (Thornwood, NY). All experiments were conducted using minimal
medium supplemented with rifamycin and a carbon source. Cells were injected into the FCS2
chamber and allowed to settle onto the Poly-L-Lysine-treated glass slide for 10 minutes. Once
the cells were attached, 10-15 locations were marked using the microscope’s software and
monitored for the duration of the experiment. GFPuv excitation was obtained with a 488 nm
argon laser at 1% power and emissions were detected through a 505 nm longpass filter in
channel 3. For the analysis of growth, images were acquired every ten minutes. However, for
the observation of fluorescence content per cell, images were taken every 30 minutes at 1% laser
power to minimize photobleaching effects. Microscope experiments were conducted for up to 96
hours. Zeiss LSM 510 META imaging software (version 3.2, SP2) was used for image analysis
and data were imported into Excel.

Results

Flow-through system to observe individual tethered cells.

To microscopically observe single cells of *M. extorquens* AM1 growing on methanol or
succinate, a flow-through culture system for the Zeiss LSM 510 META microscope was
designed and set up as described in Materials and Methods. To facilitate long-term observations
of large numbers of individual cells in this flow-through system, cells were attached to a poly-L-
lysine-coated glass slide by their polar flagella. A non-motile mutant was necessary to facilitate
long-term observations. This mutant in *M. extorquens* AM1 (TSXCM174) was constructed by
deleting three clusters of genes required for the flagellum motor function. This strain could not
be distinguished from the corresponding motile strain in bulk culture with regard to growth rate,
dynamics of the growth curve, and gene expression from the *mxaF* promoter-*gfp*$_{uv}$ fusion (23)
used in this study (data not shown). In the flow-through system, attached cells were observed
over several division times, and at division, the daughter cells were swept away by the flow
before they could attach. Using this system, it was possible to monitor the duration of time
between cell divisions, size of the cells over time (growth rate), and GFP$_{uv}$ fluorescence
intensity at each time point for actively growing individual attached cells. In this growth
condition with methanol and succinate as a carbon source, cells grew well and showed no
discernable lag between divisions.

**Distribution of cell doubling times.**

To determine the range and distribution of cell doubling time values, cells were observed
in the flow-through growth system during growth with either succinate or methanol. In many
cases, cells were observed for multiple divisions. The time between each division was
determined and the data are presented in Fig. 2 as doubling times. Variability was high, ranging
from 2.5 and 2.6 fold, respectively, for the two growth conditions. The doubling times ranged
between 1.9 and 4.6 hours on succinate (mean doubling time 3.12 +/- 0.55 hours) and between
2.6 and 6.6 hours on methanol (mean doubling time 3.73 +/- 0.63 hours). These means are
significantly lower compared to those obtained for batch cultures (~4 and ~5.5 hours,
respectively). From the data available, the distribution of division rates did not appear to follow
either Gaussian or lognormal distribution, but rather a more random pattern was observed (Fig.
2). The cells at the highest and lowest generation times made up a few percent of the total
population.

**Distribution of cell size and growth rates.**

Growth dynamics was followed for a group of individual tethered cells over the duration
of time sufficient for multiple consecutive divisions, monitoring cell size as a function of time.
For individual cells followed over multiple divisions, significant variability was observed for cell
length before and after each division, for the growth rate (increase in length/time) between
different divisions, and for the duration of time between the divisions. No obvious correlation
existed between size of cell at division and subsequent growth rate, or between previous and
subsequent growth rates (data not shown). Fig. 3 shows an example of one such set of results.
This cell was followed over several growth periods and divisions, and significant variability was observed from one division to the next in terms of cell size at division and of growth rate. For example, the growth period marked B has a 33% higher growth rate than the previous growth period, marked A. In addition, the cell length at the division preceding growth period A is 20% greater than the cell length at the end of growth period A.

**Distribution of fluorescence from promoter-GFPuv fusion.**

In order to analyze gene expression from a promoter of interest, a transcriptional fusion between a well-characterized methylotrophy promoter, $P_{mxaF}$ (the promoter for the methanol dehydrogenase operon) and $gfp_{uv}$ (23) was inserted into the chromosome of the non-motile strain in a standard insertion site for this organism (see Methods). The intensity of GFPuv-based fluorescent emissions (in relative fluorescence units, or RFU) from single cells containing this $P_{mxaF}$-$gfp_{uv}$ fusion was measured in both succinate- and methanol-grown cells and normalized to cell size as RFU/µm² (average RFU/pixel of a whole cell). Fluorescence intensity per µm² of individual cells was variable in cells from both growth conditions (1.6 and 1.7 fold, respectively), but the variability was not as great as growth rate. Calculated from approximately 1000 cells, the mean relative fluorescence for a cell grown on succinate was 1993 +/- 468 RFU/µm², with a range of 1202-2084 RFU/µm², and 3075 +/- 243 RFU/µm² on methanol, with a range of 1649-2610 RFU/µm². These means are similar to previous results showing that the difference in expression of this promoter in succinate- and methanol-grown bulk cultures is about 1.5 to 2-fold (21, 40).

**Relationship of fluorescence to growth rate and cell size.**

In order to determine whether fluorescence resulting from expression from the $mxaF$ promoter correlated with growth rate or cell size, these parameters were measured from...
individually in a single dataset from experiments in which cells were grown in the flow-through system either on methanol or on succinate. The data collected from 25 individual cells at different time points are shown in Fig. 4. When RFU/µm² was plotted against these two parameters, no correlation was observed for either parameter for methanol-grown cells. For succinate-grown cells, no correlation was observed for growth rate, but a small positive correlation ($R^2 = 0.26$) was found for cell size.

**Response during carbon shift.**

In order to assess whether variability in gene expression and/or growth rate influenced response of individual cells during the transition from succinate growth to methanol growth, these parameters were measured in individual cells during a shift from succinate to methanol as growth substrate. Relative fluorescence as a function of time was observed for approximately 1000 random cells in each experiment for up to 20 hours before the shift and up to 20 hours after the shift, and 25 individual cells were followed throughout the transition. The data from one of the succinate to methanol shift experiments are shown in Fig. 5. The range of fluorescence per µm² in individual cells was about 1.6-fold both before and after the shift (Fig. 5A). However, when the dynamics of individual cells were tracked throughout the experiment, the patterns of those cells also showed significant variability during the experiment, both before and after the shift (Fig. 5B). Variability was observed not only in the patterns of fluorescence with time, but also in the total increase in fluorescence of the $mxaF$ promoter and in the time required to achieve full induction. While fluorescence emissions from individual cells were variable, the difference in intensity between succinate and methanol growth could be easily discerned and was in keeping with the earlier results from batch culture (21, 40).
Growth rate was also measured for the same 25 cells noted above immediately before the shift, immediately after the shift, and throughout the remainder of the post-shift adaptation period. Again, significant variability was observed for individual cells, with a range on the order of 2-fold for this dataset. Immediately after the shift, all cells showed a change in growth rate, and the magnitude of that change was also variable.

The dataset for these 25 cells undergoing the transition from succinate to methanol was analyzed for correlations with regard to the following parameters: growth rates and RFU/µm² immediately before and after the shift, change in growth rate immediately after the shift, total increase in fluorescence after the shift, and the time until the cells exhibited full induction of \( \text{P}_{\text{mxaF}-\text{gfp}} \). Each of these variables was compared to each other, to assess correlations and trends. Only two trends were observed (Fig. 6). Cells growing faster before the switch to methanol achieved full induction of the \( \text{mxaF} \) promoter faster than slower growing cells (Fig. 6A), and the cells that showed faster induction were also those that exhibited a greater decline in growth rate immediately after the carbon shift (Fig. 6B). These trends suggested that the cells growing faster before the switch should exhibit a greater decline in growth rate immediately after the carbon shift, and that correlation was confirmed (Fig 6C).

**Discussion**

In this study, cell-to-cell heterogeneity was assessed in \( \text{M. extorquens AM1} \), comparing expression from a promoter-\( \text{gfp} \) fusion to growth parameters. A flow-through system was designed to grow cells in a culture chamber placed under the microscope, to allow data acquisition of individual cells over multiple divisions. Most single-cell analysis to date has involved cells imbedded in soft agar (3, 15, 24, 32), which involves microcolony formation and
the possible development of chemical and physical gradients. Such environmental heterogeneity might alter intrinsic biological heterogeneity. The system used in this study involving a commercial flow-through chamber, attachment by flagella, and a non-motile mutant allows observation of individual cells maintained in a constant environment. In this system, cells grew significantly faster than in batch cultures, with means on the order of 25-35% faster, especially during growth on methanol. Although the reason for this is not known, the flow-through system does not allow buildup of wastes or cell signaling molecules, which could contribute to this difference. Other parameters measured in this system correlated well with population-based culture data when population means were calculated from the single cell data, including fluorescence per cell from the $P_{mxaF}$-gfp$^{uv}$ fusion, magnitude of induction of this promoter as measured by fluorescence, and time for induction after the shift from succinate to methanol. These results suggest that this single cell analysis system generates response results comparable to population-based culture systems.

It has previously been reported for E. coli that both growth rate and gene expression are highly variable between cells in isogenic populations (10, 13, 15, 16, 19, 20, 29, 36, 39). We have confirmed a similar level of cell-to-cell heterogeneity for both growth characteristics and expression from the $P_{mxaF}$-gfp$^{uv}$ fusion for a more slowly-growing bacterium, M. extorquens AM1 using the flow-through system noted above. As described previously for division times in bacteria (15), no correlation was obtained between a specific division time and the next division time. In addition, we found no correlation between cell size at division and the immediately previous or subsequent division time or growth rate, or between growth rate and the immediately previous or subsequent division time or growth rate. Such results are consistent with suggestions from the literature cited above that this variability results from stochastic processes in the cell.
This experimental system allowed the assessment of correlations between expression of the mxaF promoter, as judged by the GFPuv reporter, and growth parameters. Several studies have suggested that cell-to-cell variations in gene expression are the result of extrinsic noise, that is, variations in gene expression capacity between cells (11, 27, 29, 30). However, the sources of cell-to-cell variations in growth rate are not known. If they were due to cell-to-cell variations in gene expression capacity, then a correlation would be expected between growth rate and gene expression, as measured by the gfp<sub>uv</sub> reporter construct. For instance, a cell with higher than average gene expression might be expected to also exhibit higher than average growth rate, as noted in a previous study with yeast (9). However, no such correlation was obtained in our study, suggesting that in <i>M. extorquens</i> AM1, the mechanisms generating heterogeneity in expression of this gfp<sub>uv</sub> reporter fusion and growth rate are different. If growth rate is not controlled by overall gene expression capacity, other possibilities are functions such as energy metabolism rate or cell pool parameters, such as the NADH/NAD<sup>+</sup> ratio, the energy charge, amino acid pool levels, etc.

The existence of physiological heterogeneity predicts that individual cells in a population will respond differently to the same environmental perturbation, depending on their physiological state at the time of the perturbation. In that case, the population average would not capture the true response at the cellular level. In order to test this hypothesis directly in <i>M. extorquens</i> AM1, we used the flow-through system to monitor individual cells during the metabolic transition from succinate growth to methanol growth. In <i>M. extorquens</i> AM1, a shift from multi-carbon compounds to one-carbon compounds is a major change in metabolism, shifting from energy-limitation to reducing power-limitation, with over 100 genes involved in methylotrophy (8, 37). Therefore, at the individual cell level this transition might be expected to
depend on parameters such as growth rate and gene expression. Under these conditions, we found that the faster growing cells had an advantage. A trend was observed in which the faster growing cells showed the greatest drop in growth rate immediately after the switch, suggesting they were the most stressed. These same cells also recovered fastest, and induced the *mxaF* promoter to its maximum amount in the shortest time.

These results are in contrast to the example of antibiotic persistence in *E. coli*, in which the mostly slowly growing cells have an advantage over the faster growing cells. However, in antibiotic persistence, most of the cells in the population die, and the slowly growing cells continue to grow slowly for multiple generations (3). In the perturbation described here, viability is maintained and growth is affected only transiently. Under these conditions it is likely that more rapid growth poises the cells to respond more quickly. During this transition to growth on methanol, cultures not only experience transient starvation, they also accumulate formaldehyde due to an initial imbalance between the formaldehyde production and consumption fluxes (8, 24). Thus, it would be expected that faster growing cells would be metabolizing more rapidly and would experience a greater initial drop in growth rate from more rapid starvation, formaldehyde accumulation, or both. However, these cells would also be poised to recover the most quickly, by rapidly inducing methylotrophic pathways and thus allowing formaldehyde detoxification as well as energy extraction and biosynthesis from methanol. As technology to study both gene expression and physiological parameters in large numbers of single cells expands, it will be possible to test this hypothesis to determine the underlying causes for the behavior of individual cells during this transition. However, these results demonstrate that the pre-existing physiological state of individual *M. extorquens* cells does dictate differential response to a shift from multicarbon to methylotrophic growth.
Simulations predict that physiological heterogeneity is an important selective factor for populations subjected to intermittent environmental change (17, 18). *Methylobacterium* strains live on the surface of leaves, utilizing methanol emitted by stomata (33) and can also be found in freshwater and soil environments. This environment is characterized by highly fluctuating methanol concentrations (26), and it is possible that the heterogeneity of response demonstrated in this study contributes to success of the natural populations of *Methylobacterium* under these conditions.

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References


Table 1. *M. extorquens* AM1 strains and plasmids used in this study

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<th>Strain</th>
<th>Description</th>
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<td>Non-motile mutant with <em>katA::</em>(loxP-<em>trmB</em>-P&lt;sub&gt;mxaf&lt;/sub&gt;-gfp&lt;sub&gt;uv&lt;/sub&gt;-<em>T7</em>)</td>
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Figure legends

Figure 1. Flow system used to conduct single cell experiments. A, Media reservoir; B, Global FIA Milligat pump; C, air equilibration system; D, FCS2 cell culture chamber; E, waste.

Figure 2. A, Distribution of doubling times for individual cells grown on succinate (n = 115). B, Distribution of doubling times for individual cells grown on methanol (n = 195).

Figure 3. Length over time for an individual TSXCM174 cell with several successive divisions during growth on methanol. A, Growth rate = 0.55\(\mu\)m/hr. B, Growth rate = 0.73\(\mu\)m/hr

Figure 4. Single cell fluorescence intensity normalized to cell size (RFU/\(\mu\)m\(^2\)) as a function of growth rate (\(\mu\)m/hr) and cell area (\(\mu\)m\(^2\)) during growth on succinate (A, C) and methanol (B, D). R\(^2\) value for panel C is 0.26.

Figure 5. A, Fluorescence over time for approximately 1000 cells (black dots) during a carbon shift experiment from succinate to methanol. Shift was made at approximately 17 hours. B, Fluorescence data from 25 individual single cells tracked from 8 to 30 hrs in the experiment.

Figure 6. A, Growth rate on succinate prior to carbon shift as a function of time for full mxaF response after the transition to methanol. B, Decrease in growth rate after the carbon shift from succinate to methanol as a function of time for full mxaF response after the transition to methanol. C, Decrease in growth rate after the carbon shift from succinate to methanol as a function of the growth rate before the shift.
Fig. 1
Fig. 2

A

B

Frequency

Time (hrs)

Frequency

Time (hrs)
Fig. 3
Fig. 4
Fig. 5

A

B