

Supplemental Material

Dormancy is not necessary or sufficient for bacterial persistence

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Running title: Persister dormancy

Table S1. Primer list.

Forward primer (5'---3')	Reverse primer(5'---3')	Explanation
TCTGGTGGCCGGAAGGCGAAG CGGCATGCATTTACGTTGATGC AGCATTACACGTCTTGA	TGCCTAATGAGTGAGCTAACTCAC ATTAATTGCGTTGCGCCAAGATCC GCAGTTCAACCT	Primers for using Datsenko and Wanner method to knock-out chromosomal <i>LacI</i> including promoter. Primers amplified <i>Kan^R</i> cassette from pKD4 plasmid, and have 40-nt homology regions (bold).
TCTGGTGGCCGGAAGGCGAAG CGGCATGCATTTACGTTGACAC CATCGAATGGTGCAAAA	TCCAGCCTACACAATCGCTCTCAC TGCCCGCTTCCAGTC	Primers for amplifying <i>LacI^q</i> gene from pQE80L plasmid (Qiagen). The forward primer has a 40-nt homology extension (bold) for up-stream sequence of chromosomal <i>LacI</i> gene, and the reverse primer has a 20-nt homology extension (bold) to append <i>Kan^R</i> cassette to the 3' end of <i>LacI^q</i> coding strand.
GAGCGATTGTGTAGGCTGGA	TGCCTAATGAGTGAGCTAACTCAC ATTAATTGCGTTGCGCTTAACGGC TGACATGGGAAT	Primers for amplifying <i>Kan^R</i> cassette from pKD4 plasmid. Forward primer is homologous to reverse primer of <i>LacI^q</i> for overlap extension, and reverse primer has a 40-nt homology extension (bold) for down-stream sequence of chromosomal <i>LacI</i> gene. <i>LacI^q+Kan^R</i> DNA fragment was chromosomally integrated in place of <i>LacI</i> using Datsenko & Wanner method.
ATTAATGTGAGTTAGCTCACTC ATTAGGCACCCAGGCTTCTCG AGAAATCATAAAAAAT	TCCTCGCCCTTGCTCACCATAGTT AATTTCTCCTTTAA	Primers for amplifying <i>T5</i> promoter from pQE-80L plasmid. The forward primer has a 40-nt homology extension (bold) for up-stream sequence of <i>Lac</i> operon, and the reverse primer has a 20-nt homology extension (bold) to append <i>mCherry</i> gene to the 3' end of <i>T5p</i> coding strand.
ATGGTGAGCAAGGGCGAGGA	CGATCCTCATCCTGTCTCTTGATC TTCTACTTGTACAGCTCGTCCATG	Primers for amplifying <i>mCherry</i> gene from pAS08.3 plasmid. Forward primer is homologous to reverse primer of <i>T5p</i> (overlap extension), and reverse primer has a 23-nt homology extension (bold) to append <i>Kan^R</i> gene to the 3' end of <i>mCherry</i> coding strand.
ATCAAGAGACAGGATGAGGAT CG	TGTAGATCGCTGAACTTGTAGGC CTGATAAGCGCAGCGTATCAGGC AATTTCAGAAGAACTCGTCAAGAA GG	Primers for amplifying <i>Kan^R</i> gene. Forward primer is homologous to reverse primer of <i>mCherry</i> for overlap extension, and reverse primer has a homology region (bold) for down-stream sequence of chromosomal <i>Lac</i> operon. <i>T5p+mCherry+Kan^R</i> DNA fragment was chromosomally integrated in place of <i>Lac</i> operon using Datsenko & Wanner method.
ATCGAATGGCGCAAAACCT	TTCCAGTCGGGAAACCTGT	Internal primers for <i>LacI</i> to confirm the absence of duplicate gene after knocking out chromosomal <i>LacI</i> .
GGGATCAGGAGGAGAAGATCG		Forward primer for upstream sequence of the <i>LacI</i> gene. This primer and a <i>Kan^R</i> reverse primer (see below) were used to confirm the presence of the kanamycin resistance cassette in the proper chromosomal location.
AGGACAGTCGTTTGCCGTCT	ATCGACAGATTTGATCCAGCG	Internal primers for <i>LacZ</i> to confirm the absence of duplicate gene after knocking out chromosomal <i>Lac</i> operon.
CCGATTTGGCTACATGACATC	AAACAGACCAGATAAATCGTCGC	Internal primers for <i>LacY</i> to confirm the absence of duplicate gene after knocking out chromosomal <i>Lac</i> operon.
GCCAATGACCGAAAGAATAAGA	GAAATAATAGTGCTTATCCCGGTC	Internal primers for <i>LacA</i> to confirm the absence of duplicate gene after knocking out chromosomal <i>Lac</i> operon.
CGGTAGTGGGATACGACGATAC		Forward primer for upstream sequence of the <i>Lac</i> operon. This primer and <i>Kan^R</i> or <i>mCherry</i> reverse primer were used to confirm the presence of the kanamycin resistance cassette or <i>mCherry</i> gene in the proper chromosomal location.
	ATGATGGATACTTTCTCGGCAGGAG	<i>Kan^R</i> reverse primer

Table S2. Persister Frequencies.

Persister frequencies of mCherry expressing cells		
	Ampicillin	Ofloxacin
A*	4.47E-05 ± 1.17E-05	6.93E-05 ± 9.33E-06
B*	5.80E-05 ± 1.59E-05	9.60E-05 ± 1.33E-05
C*	4.27E-03 ± 4.81E-04	7.40E-03 ± 6.43E-04
Persister frequencies of RSG stained cells		
A	7.19E-04 ± 1.55E-04	1.52E-03 ± 1.03E-04
B	2.05E-05 ± 4.99E-06	5.00E-05 ± 8.29E-06
C	2.00E-05 ± 4.24E-06	3.80E-05 ± 1.17E-05
D	2.30E-05 ± 7.14E-06	3.65E-05 ± 6.95E-06
Persister frequencies of RSG stained-mCherry expressing cells		
NGI	3.93E-03 ± 3.33E-04	7.40E-03 ± 5.77E-04
NGII	3.67E-03 ± 7.51E-04	5.07E-03 ± 8.11E-04
GI	6.93E-05 ± 1.57E-05	9.93E-05 ± 1.64E-05
GII	5.33E-05 ± 9.96E-06	9.53E-05 ± 1.34E-05

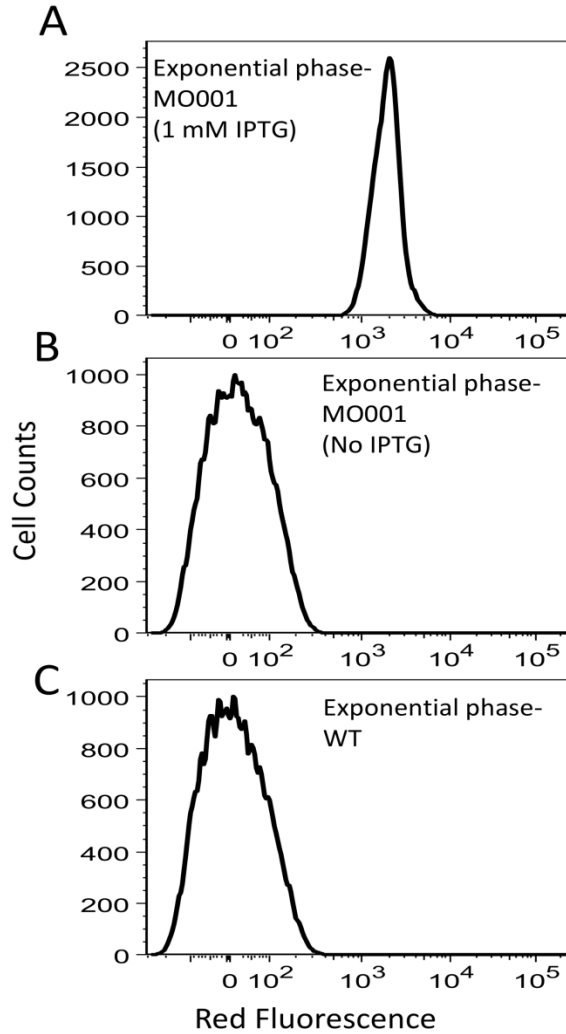


Figure S1. MO001 background fluorescence is as low as that of the parent strain (without *mCherry*). MO001 was incubated without IPTG during the overnight and both with and without IPTG during the exponential growth (A, B), and fluorescence intensities were measured by flow cytometry. Exponential wild-type cells were used to determine the background fluorescence (C).

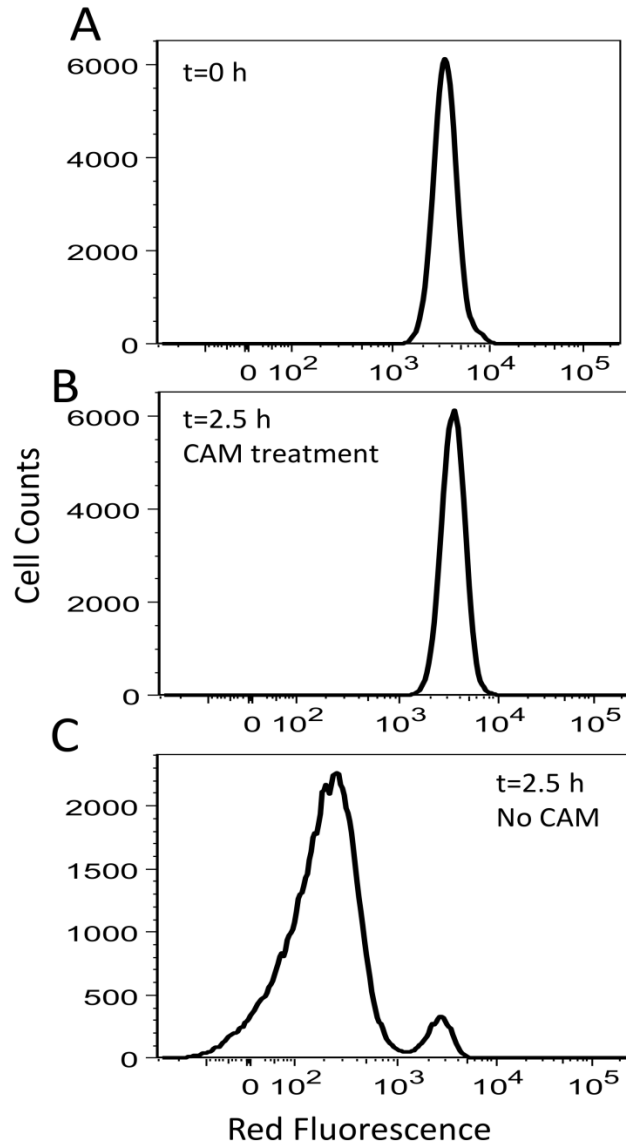


Figure S2. mCherry was not degraded during the time-frame of the experiment. After accumulation of mCherry protein during the overnight culture (t=0 h) (A), cells were inoculated in fresh media (without IPTG) with 50 μ g/mL chloramphenicol (B) or without chloramphenicol (C). Exponential-phase samples (2.5 h after the inoculation) were analyzed by flow cytometry.

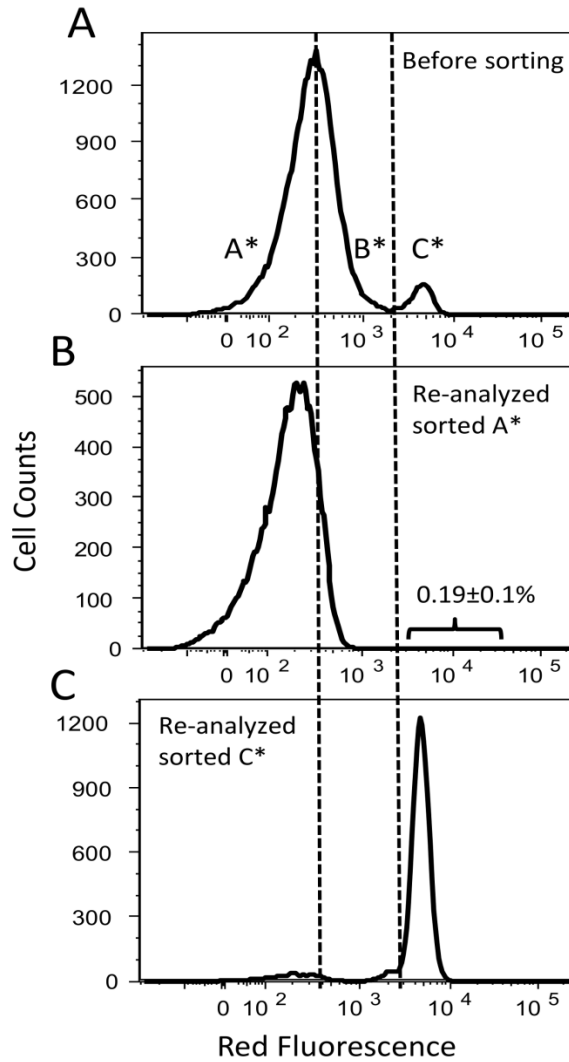


Figure S3. The number of non-growing cells that were improperly sorted into the fastest-growing quantile was measured. After sorting approximately 5×10^5 cells from the A* or C* regions (A), the cells from A* and C* were separately reanalyzed by the same sorter (B and C, respectively). It was found that $0.19 \pm 0.1\%$ of cells in A* were non-growing cells.

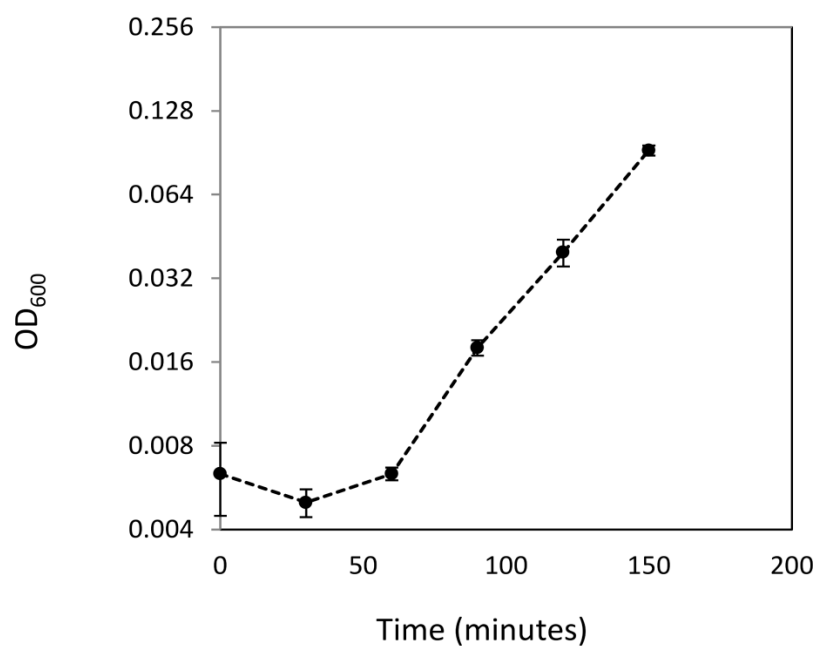


Figure S4. Growth of MO001 cells. After inoculation of overnight culture in fresh media (1:1000), OD₆₀₀ was monitored every 30 minutes, and the growth rate was calculated using exponential fit between 60 to 150 minutes time points.