The importance of lag time extension in determining bacterial resistance to antibiotics

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Supplementary Information

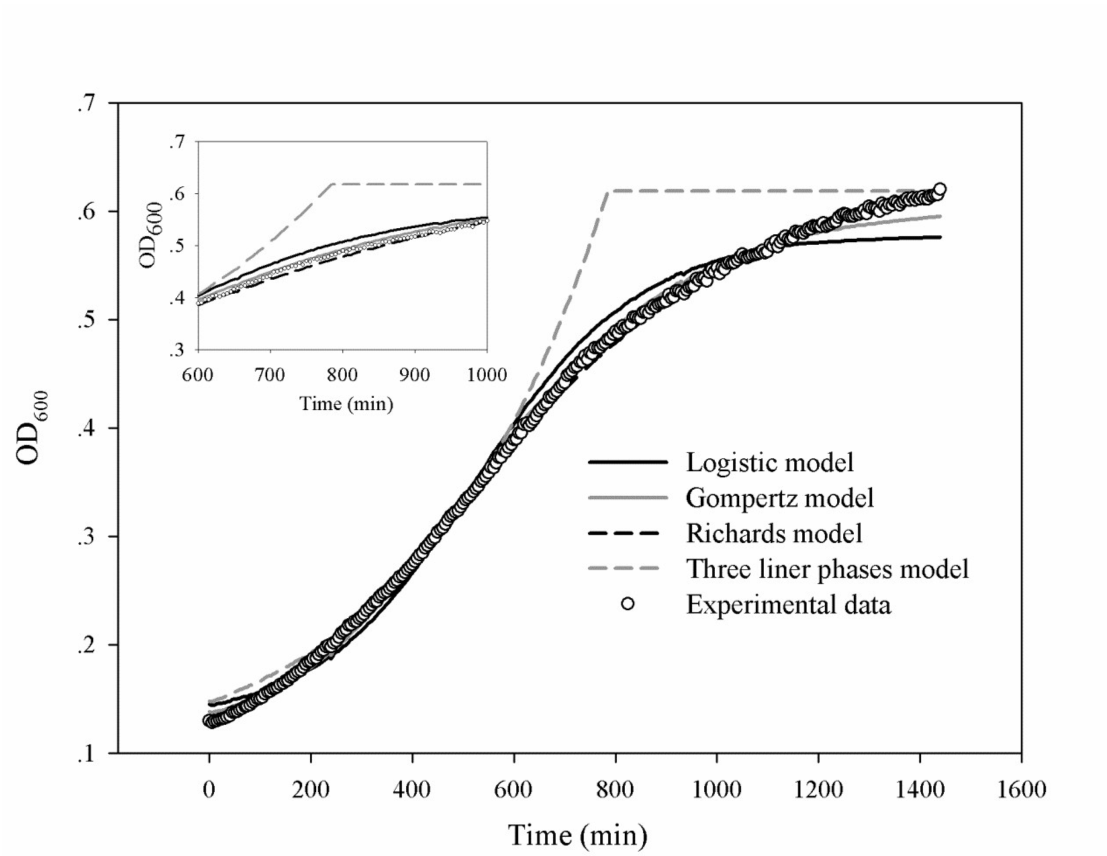

Figure S-1. Performance of different models fitting the experimental growth curve

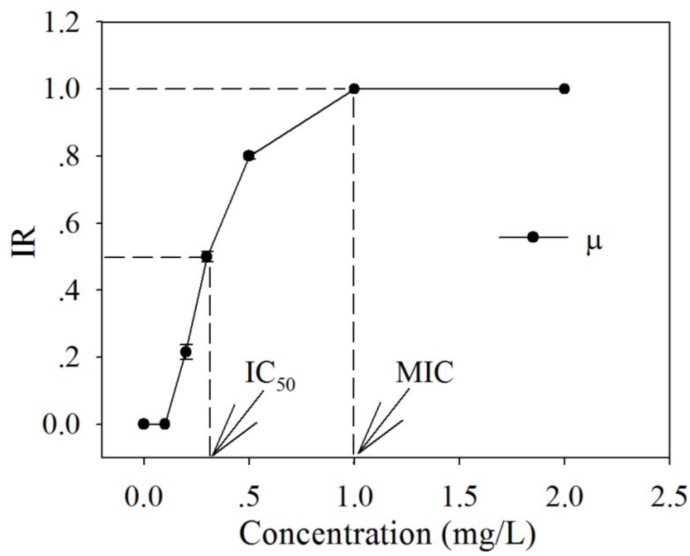

Figure S2. IC_{50} and MIC obtained from an exampled inhibition curve of *Pseudomonas putida* under CIP.

Table S1 Bacterial culture conditions *

Name	Culture medium	Culture temperature
Escherichia coli	Peptone 10 g/L, beef extract 3 g/L, NaCl 5 g/L	37℃
Acinetobacter sp.		
Pseudomonas aeruginosa	Peptone10 g/L, beef extract 10 g/L, glucose 10 g/L, NaCl 5 g/L	30℃
pseudomonas putida		
Comamonas denitifier	Peptone 5 g/L, yeast extract 2 g/L, beef extract 1 g/L, NaCl 5 g/L	30℃
Alcaligenes faecalis		
Microlunatus phosphovorus	Peptone 0.5 g/L, glucose 0.5 g/L, Monosodium glutamate 0.5 g/L, yeast extract 0.5 g/L, KH_2PO_4 0.44 g/L, $(NH_4)_2SO_4$ 0.1 g/L, $MgSO_4 \cdot 7H_2O$ 0.1g/L	35℃
Corynebacterium glutamicum	Peptone 10 g/L, yeast extract 5 g/L, glucose 5 g/L, NaCl 5 g/L	30℃
Sludge bacteria	Peptone 8 g/L, meat extract 5.5 g/L, urea 1.5 g/L, NaCl 0.35 g/L, $CaCl \cdot 2H_2O$ 0.2 g/L, $MgSO_4 \cdot 7H_2O$ 0.1 g/L, K_2HPO_4 1.4 g/L	30℃

* Bacteria were cultured on a shaker (150 rpm) to the exponential phase (OD_{600}=0.6-0.8)

Table S2. The maximum specific growth rate and lag time of pure strains under different antibiotics *

	Escherichia coli			*Pseudomonas aeruginosa*			*Pseudomonas putida*			*Alcaligenes faecalis*			*Acinetobacter sp.*			*Corynebacterium glutamicum*			*Comamonas denitifier*			*Microlunatus phosphovorus*		
	Con. mg/L	μ min^{-1}	λ min	Con. mg/L	μ min^{-1}	λ min	Con. mg/L	μ min^{-1}	λ min	Con. mg/L	μ min^{-1}	λ min	Con. mg/L	μ min^{-1}	λ min	Con. mg/L	μ min^{-1}	λ min	Con. mg/L	μ min^{-1}	λ min	Con. mg/L	μ min^{-1}	λ min
AMO	0	0.013	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	1	0.0125	23	0.005	0.0032	35	5	0.0059	87.5	0.05	0.0038	95	5	0.0075	85	0.1	0.012	55	2	0.005	38	0.1	0.007	235
	2	0.0065	20	0.01	0.003	40	10	0.0057	81.3	0.1	0.0025	90	10	0.0076	89	0.2	0.0085	60	5	0.0024	40	0.5	0.0041	210
	3	0.0032	26	0.05	0.0025	38	50	0.004	145	0.5	0.0019	88	50	0.0076	78	0.5	0.002	56	8	0.0015	35	1	0.0022	237
	5	0	/	0.1	0	/	100	0.0038	128	1	/	/	100	0.0073	80	1	0	/	10	0	/	2	0	/
AMP	0	0.013	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	1	0.0128	25	0.005	0.0034	54	10	0.0055	119	0.05	0.0029	102	5	0.0079	76	0.1	0.012	55	5	0.0058	35	0.5	0.0065	225
	2	0.0062	28	0.01	0.0033	30	20	0.0051	92.6	0.1	0.0025	70	10	0.0074	79	0.5	0.008	62	10	0.0031	35	0.8	0.004	230
	3	0.0035	25	0.05	0.0022	41	50	0.0049	103	0.2	0.0018	82	50	0.006	85	1	0.0025	58	20	0.001	35	1	0.0025	215
	5	0	/	0.1	0	/	100	0.0044	83.8	0.5	0	/	100	0.0055	75	2	0	/	25	0	/	2	0	/
TET	0	0.013	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	0.5	0.012	25	0.005	0.0032	30	0.05	0.0057	137	0.5	0.0039	103	0.5	0.0072	90	0.1	0.0085	58	0.05	0.0063	35	0.05	0.0073	193
	0.8	0.0092	23	0.01	0.0021	35	0.1	0.0053	199	1	0.0031	290	1	0.0065	95	0.2	0.0057	56	0.1	0.0062	38	0.1	0.0042	239
	1	0.0065	25	0.05	0.0007	44	0.5	0.0032	867	2	0.0025	450	2	0.004	85	0.5	0.0023	69	0.2	0.004	60	0.2	0.0021	209
	2	0	/	0.1	0	/	1	0	/	5	0	/	5	0	/	1	0	/	0.5	0	/	0.5	0	/
CHL	0	0.013	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	0.5	0.0062	25	0.005	0.0025	33	0.05	0.0037	167	0.1	0.0038	112	0.1	0.0075	85	0.05	0.0085	60	0.05	0.0055	48	0.005	0.0065	218
	1	0.0035	21	0.01	0.0015	44	0.1	0.0038	932	0.5	0.0029	367	0.5	0.0058	115	0.1	0.0059	58	0.1	0.0032	70	0.01	0.0042	234
	2	0.0016	25	0.05	0.0002	30	0.2	0.0028	968	0.8	0.002	465	1	0.0027	409	0.2	0.0028	55	0.2	0.002	95	0.02	0.0022	194
	3	0	/	0.1	0	/	0.5	0	/	1	0	/	2	0	/	0.5	0	/	0.5	0	/	0.05	0	/

	0	0.013	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	0.1	0.0096	24	0.1	0.0037	44	10	0.0048	125	0.5	0.0033	126	1	0.0072	80	5	0.0012	53	1	0.005	40	1	0.0063	215
KAN	0.5	0.0076	22	0.5	0.0027	30	50	0.0036	114	1	0.0026	114	5	0.0056	71	10	0.011	66	2	0.0038	38	2	0.0058	237
	1	0.0068	25	1	0.0011	32	80	0.0028	126	2	0.0018	95	8	0.0038	75	50	0.058	54	5	0.0015	35	5	0.0041	215
	2	0	/	2	0	/	100	0.002	118	5	0	/	10	0	/	80	0	/	10	0	/	10	0	/
	0	0.013	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	0.01	0.009	24	0.05	0.0039	38	0.5	0.0055	97	0.05	0.0026	84	0.5	0.0054	70	0.05	0.011	55	0.5	0.0045	38	0.1`	0.0083	221
NOR	0.02	0.0072	28	0.1	0.004	53	1	0.006	105	0.1	0.0017	70	1	0.005	68	0.1	0.0083	57	1	0.031	35	0.5	0.0042	225
	0.05	0.0045	24	0.2	0.0016	40	5	0.0026	90	0.2	0.0008	90	2	0.0039	73	0.2	0.007	55	2	0.0018	35	1	0.0018	237
	0.1	0	/	0.5	0	/	10	/	/	0.5	/	/	5	0	/	0.5	0	/	5	0	/	2	0	/
	0	0.013	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	0.01	0.013	30	0.05	0.0032	46	0.1	0.0048	148	0.5	0.0037	95	0.05	0.0079	65	0.5	0.012	58	0.05	0.0055	38	0.1	0.0079	205
OFL	0.02	0.076	28	0.1	0.0028	32	0.5	0.0028	153	1	0.0033	87	0.1	0.004	75	1	0.0075	63	0.1	0.004	35	0.2	0.0066	211
	0.05	0.062	25	0.2	0.0017	39	1	0.001	135	2	0.002	85	0.2	0.0025	70	2	0.0058	56	0.2	0.0032	36	0.5	0.0041	225
	0.1	0	/	0.5	0	/	2	/	/	5	0	/	0.5	0	/	5	0	/	0.5	0	/	1	0	/
	0	0.013	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	0.005	0.012	24	0.05	0.0028	37	0.05	0.0042	125	0.005	0.0035	90	0.005	0.0076	75	0.5	0.0085	58	0.05	0.0049	34	0.05	0.0042	235
ENR	0.01	0.0088	24	0.1	0.0049	648	0.1	0.0028	132	0.01	0.003	95	0.01	0.0075	76	1	0.0061	65	0.1	0.0045	40	0.1	0.0026	221
	0.02	0.0063	24	0.2	0.0018	1050	0.2	0.0008	143	0.02	0.0026	88	0.02	0.0038	80	2	0.0032	60	0.2	0.003	36	0.2	0.0015	228
	0.05	0	/	0.5	0	/	0.5	0	/	0.05	0	/	0.05	0	/	5	0	/	0.5	0	/	0.5	0	/
	0	0.013	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	0.001	0.0128	32	0.05	0.002	26	0.05	0.0052	126	0.05	0.002	86	0.05	0.007	110	1	0.0084	80	1	0.0056	40	0.5	0.005	218
CIP	0.003	0.0087	30	0.1	0.0037	987	0.1	0.0042	127	0.1	0.0021	88	0.1	0.005	155	2	0.0072	120	2	0.004	55	1	0.0039	227
	0.005	0.0062	25	0.2	0.0016	1060	0.5	0.0032	124	0.2	0.0018	95	0.2	0.0039	260	5	0.0057	198	5	0.0031	70	2	0.0021	205
	0.01	0	/	0.5	0	/	1	0	/	0.5	/	/	0.5	0	/	10	0	/	10	0	/	5	0	/
ERY	0	0.013	24	0	0.0034	35	0	0.057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221

	0.05	0.001	24	0.05	0.0034	40	10	0.0055	110	1	0.0035	95	1	0.0078	80	0.5	0.011	50	1	0.0062	38	1	0.0054	221
	0.1	0.0065	21	0.1	0.0026	35	20	0.0052	136	5	0.002	91	2	0.005	73	1	0.0062	48	5	0.0037	35	5	0.0042	331
	0.2	0.0032	28	0.2	0.0015	30	50	0.0039	120	8	0.0016	90	5	0.0032	68	2	0.0032	44	8	0.0028	36	8	0.0025	450
	0.5	0	/	0.5	0	/	100	0.0329	133	10	0	/	10	0	/	5	/	/	10	0	/	10	0	/
	0	0.0132	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	0.05	0.0125	25	0.1	0.0026	38	10	0.0055	130	0.5	0.0027	84	1	0.0076	79	0.05	0.0083	60	1	0.0064	35	0.1	0.0078	245
CLA	0.1	0.0097	29	0.5	0.0018	44	20	0.0049	103	1	0.0021	394	5	0.0082	85	0.1	0.0058	50	5	0.0045	40	0.5	0.0042	440
	0.2	0.0066	27	1	0.0004	30	50	0.003	124	2	0.0018	500	10	0.004	79	0.2	0.003	58	8	0.003	38	1	0.003	612
	0.5	0	/	2	0	/	100	0.0014	230	5	0	/	15	0	/	0.5	0	/	10	0	/	5	0	/
	0	0.013	24	0	0.0034	35	0	0.0057	121	0	0.0038	93	0	0.0079	76	0	0.012	55	0	0.0063	35	0	0.0083	221
	0.05	0.012	21	1	0.0035	38	5	0.0061	104	5	0.0038	90	5	0.0079	76	10	0.0073	94	5	0.0063	35	1	0.0083	221
LIN	0.1	0.0089	24	5	0.0024	40	10	0.0059	125	10	0.0038	95	10	0.0079	76	20	0.0062	118	10	0.0062	41	5	0.004	215
	0.2	0.0062	25	8	0.0016	72	50	0.0059	123	50	0.0038	93	50	0.0079	76	50	0.0018	190	50	0.0062	35	10	0.0035	247
	0.5	0	/	10	0	/	100	0.006	131	100	0.0035	90	100	0.0065	76	60	0	/	100	0.0062	39	20	0	/

* Note: all the data were the average of three parallel experiments, and the ratios of standard deviations/average were all < 5%.

Table S3. The maximum specific growth rate and lag time of bacterial communities under different antibiotics*

	Bacterial community(North)			*Bacterial community(South)*		
	Con. mg/L	μ min^{-1}	λ min	Con. mg/L	μ min^{-1}	λ min
AMO	0	0.0121	23	0	0.0099	55
	5	0.0124	23	5	0.0113	66
	10	0.0131	20	10	0.0108	57
	50	0.0138	26	50	0.0108	59
	100	0.0131	23	100	0.0105	60
AMP	0	0.0121	23	0	0.0099	55
	5	0.0123	16	5	0.0106	46
	10	0.0123	14	10	0.0100	47
	50	0.0120	11	50	0.0115	52
	100	0.0121	14	100	0.0082	55
TET	0	0.0121	23	0	0.0099	55
	5	0.0097	67	5	0.0078	78
	10	0.0084	86	10	0.0064	115
	50	0.0067	290	50	0.0041	206
	100	0.0060	597	100	0.0047	566
CHL	0	0.0121	23	0	0.0099	55
	5	0.0086	86	5	0.0066	138
	10	0.0084	148	10	0.0069	218
	50	0.0106	493	50	0.0082	508
	100	0.0104	601	100	0.0042	682
KAN	0	0.0121	23	0	0.0099	55
	5	0.0109	51	5	0.0078	56
	10	0.0103	68	10	0.0080	60
	50	0.0099	137	50	0.0086	144
	100	0.0103	168	100	0.0106	190
NOR	0	0.0121	23	0	0.0099	55
	5	0.0091	50	5	0.0071	131
	10	0.0087	74	10	0.0063	142
	50	0.0093	190	50	0.0066	213
	100	0.0090	280	100	0.0071	259
OFL	0	0.0121	23	0	0.0099	55
	5	0.0079	67	5	0.0079	150
	10	0.0075	126	10	0.0065	152
	50	0.0097	364	50	0.0073	406
	100	0.0085	479	100	0.0078	519
ENR	0	0.0121	23	0	0.0099	55
	5	0.0081	93	5	0.0064	150
	10	0.0080	128	10	0.0061	182

	50	0.0080	252	50	0.0051	210
	100	0.0082	345	100	0.0068	434
	0	0.0121	23	0	0.0099	55
	5	0.0074	98	5	0.0081	160
CIP	10	0.0071	113	10	0.0078	175
	50	0.0081	193	50	0.0064	254
	100	0.0085	287	100	0.0072	293
	0	0.0121	23	0	0.0099	55
	5	0.0105	34	5	0.0089	51
ERY	10	0.0100	49	10	0.0082	61
	50	0.0088	78	50	0.0070	86
	100	0.0080	118	100	0.0071	121
	0	0.0121	23	0	0.0099	55
	5	0.0112	36	5	0.0095	49
CLA	10	0.0105	40	10	0.0084	41
	50	0.0080	83	50	0.0073	82
	100	0.0070	123	100	0.0061	177
	0	0.0121	23	0	0.0099	55
	5	0.0125	21	5	0.0109	56
LIN	10	0.0111	21	10	0.0104	71
	50	0.0096	38	50	0.0091	85
	100	0.0097	47	100	0.0090	107

* Note: all the data were the average of three parallel experiments, and the ratios of standard deviations/average were all < 5%.

Table S4. IC_{50}, MIC and LE_λ at IC_{50} of different bacteria under 12 kinds of antibiotics*

Antibiotics	unit (mg/L)	Escherichia coli	Pseudomonas aeruginosa	Pseudomonas putida	Alcaligenes faecalis	Acinetobacter sp.	Corynebacterium glutamicum	Comamonas denitifier	Microlunatus phosphovorus	Extract Community 1	Extract Community 2
AMO	IC_{50}	2	0.08	>100	0.5	>100	0.3	4	0.5	>100	>100
	MIC	5	0.1	>100	1	>100	1	10	2	>100	>100
	LE_λ	1	1	1	1	1	1	1	1	1	1
AMP	IC_{50}	2	0.08	>100	0.2	>100	0.8	10	0.8	>100	>100
	MIC	5	0.1	>100	0.5	>100	2	25	2	>100	>100
	LE_λ	1	1	1	1	1	1	1	1	1	1
TET	IC_{50}	1	0.03	0.5	3	2	0.2	0.3	0.1	>100	>100
	MIC	2	0.1	1	5	5	1	0.5	0.5	>100	>100
	LE_λ	1	1	6	5	1	1	2	1	25	10
CHL	IC_{50}	0.5	0.02	0.2	0.8	0.8	0.1	0.1	0.02	>100	>100
	MIC	3	0.05	0.5	1	2	0.5	0.5	0.05	>100	>100
	LE_λ	1	1	8	5	3	1	2	1	25.5	12
KAN	IC_{50}	1	0.8	80	2	8	20	3	5	>100	>100
	MIC	2	2	>100	5	10	80	10	10	>100	>100
	LE_λ	1	1	1	1	1	1	1	1	7	3.4
NOR	IC_{50}	0.03	0.2	5	0.1	2	0.3	1	0.5	>100	>100
	MIC	0.1	0.5	10	0.5	5	0.5	5	2	>100	>100
	LE_λ	1	1	1	1	1	1	1	1	11	4.7
OFL	IC_{50}	0.05	0.2	0.5	2	0.1	2	0.2	0.5	>100	>100
	MIC	0.1	0.5	2	5	0.5	5	0.5	1	>100	>100
	LE_λ	1	1	1	1	1	1	1	1	21	9
ENR	IC_{50}	0.02	0.2	0.1	0.03	0.02	1	0.1	0.05	>100	>100

	MIC	0.05	0.5	0.5	0.05	0.05	5	0.5	0.5	>100	>100
	LE_λ	1	30	1	1	1	1	1	1	15	8
CIP	IC_{50}	0.004	0.2	0.3	0.2	0.2	5	5	1	>100	>100
	MIC	0.01	0.5	1	0.5	0.5	10	10	5	>100	>100
	LE_λ	1	30	1	1	3.5	3.6	2	1	12.5	5.3
ERY	IC_{50}	0.1	0.1	>100	5	3	1	6	5	>100	>100
	MIC	0.5	0.5	>100	10	10	5	10	10	>100	>100
	LE_λ	1	1	1	1	1	1	1	1.5	5	2.2
CLA	IC_{50}	0.2	0.6	50	2	10	0.1	8	0.5	>100	>100
	MIC	0.5	2	>100	5	15	0.5	10	5	>100	>100
	LE_λ	1	1	1	6	1	1	1	2	5.5	3
LIN	IC50	0.2	8	>100	>100	>100	20	>100	5	>100	>100
	MIC	0.5	10	>100	>100	>100	60	>100	20	>100	>100
	LE_λ	1	2	1	1	1	2	1	1	2	2

* If the IC_{50}>100 mg/L, the value of LE_λ was the lag time extension at 100 mg/L.

Protocols.

Time-lapse tracking of bacterial growth. The microfluidic device as described previously [1] was used to observe the growth of sludge bacterial cells (South) in real time. Culture medium with or without 100 mg/L tetracycline was introduced into the two separate channels of the device. Time-lapse images of cells below each channel were recorded from the onset of experiments. This enabled tracking of the lag times of living cells.

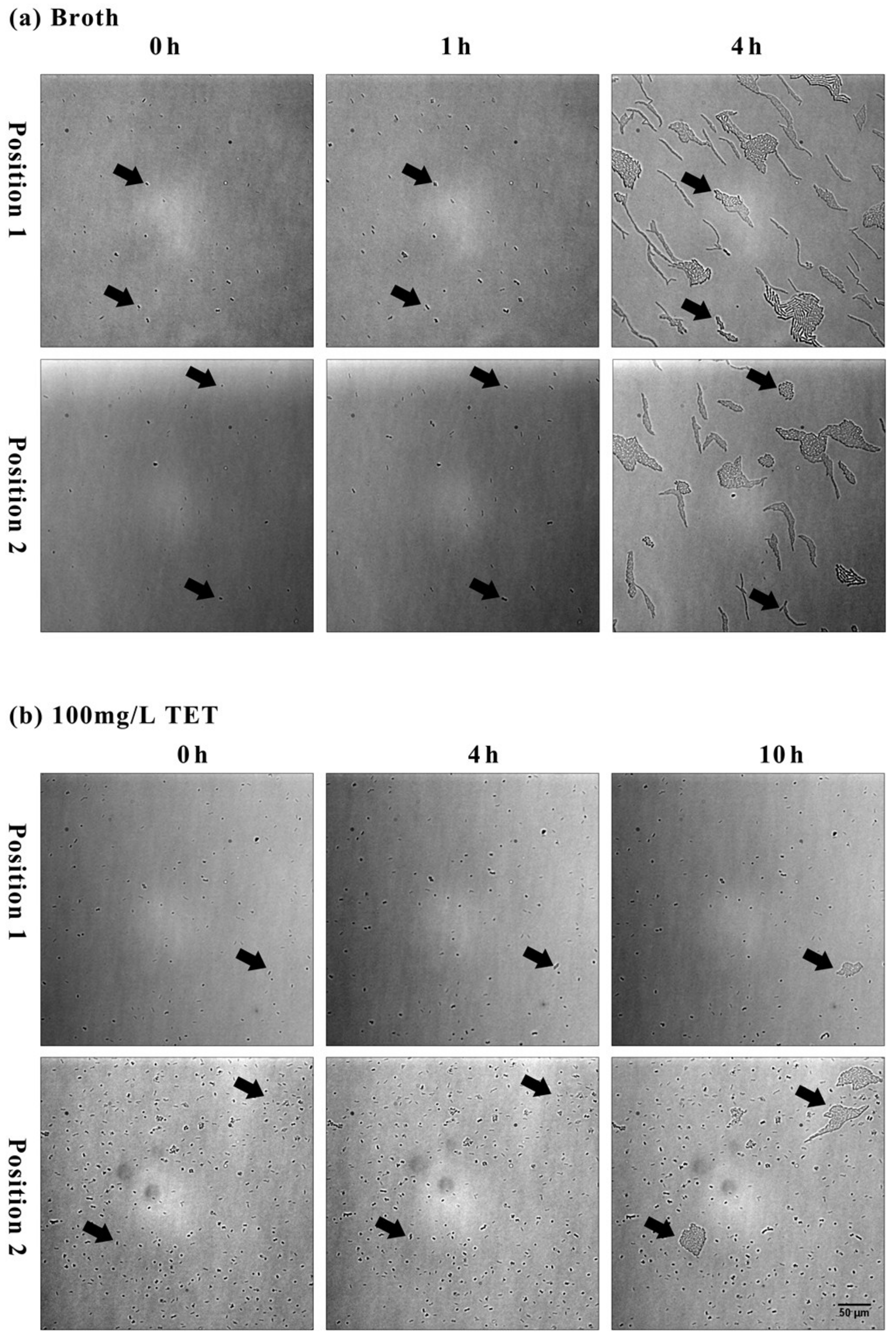

Figure S-3: Time-lapse images (bright field) of sludge bacterial cells in (a) broth alone and in (b) broth containing 100 mg/L tetracycline (TET). The tracks of two positions randomly chosen for each condition are shown. Arrows were used to track the growth from the same cells. (a) In the absence of tetracycline, no obvious growth was seen within the first hour, which agrees well with the calculated lag time for this condition. By hour 4, almost all the cells had formed colonies. (b) In the presence of 100 mg/L TEF, hardly any cells had exhibited growth by hour 4. However, a few live cells (as indicated by arrows) had been able to form colonies by hour 10, indicating their lag times were significantly extended when in 100 mg/L tetracycline.

Reference

[1] B. Li, Y. Qiu, A. Glidle, D. McIlvenna, Q. Luo, J. Cooper, H. Shi and H. Yin, *Analytical Chemistry*, 2014, **86**, 3131-3137.