

Supplemental Material

Hha and CspD toxins and Hfq are involved in persister formation through MqsR in *Escherichia coli*

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Supplemental Table S1. Bacterial strains, plasmids, and primers used in this study. f indicates forward primer and r indicates reverse primer.

Strains, plasmids, primers	Genotype/relevant characteristics or sequence	Source
<i>E. coli</i> K-12 strains		
BW25113	<i>lacI^f rrnB_{T14} ΔlacZ_{WJ16} hsdR514 ΔaraBAD_{AH33} ΔrhaBAD_{LD78}</i>	[23]
BW25113 <i>mqsR</i>	K-12 BW25113 Δ <i>mqsR</i> Ω Km ^R	[23]
BW25113 <i>mqsR mqsA</i>	K-12 BW25113 Δ <i>mqsR</i> Δ <i>mqsA</i> Ω Km ^R	[10]
BW25113 <i>hha</i>	K-12 BW25113 Δ <i>hha</i> Ω Km ^R	[23]
BW25113 <i>clpX</i>	K-12 BW25113 Δ <i>clpX</i> Ω Km ^R	[23]
BW25113 <i>clpP</i>	K-12 BW25113 Δ <i>clpP</i> Ω Km ^R	[23]
BW25113 <i>lon</i>	K-12 BW25113 Δ <i>lon</i> Ω Km ^R	[23]
BW25113 <i>yjz</i>	K-12 BW25113 Δ <i>yjz</i> Ω Km ^R	[23]
BW25113 <i>cspD</i>	K-12 BW25113 Δ <i>cspD</i> Ω Km ^R	[23]
BW25113 <i>hokA</i>	K-12 BW25113 Δ <i>hokA</i> Ω Km ^R	[23]
BW25113 <i>hfq</i>	K-12 BW25113 Δ <i>hfq</i> Ω Km ^R	[23]
Plasmids		
pCA24N	Cm ^R ; <i>lacI^f</i> , pCA24N	[24]
pCA24N- <i>mqsR</i>	Cm ^R ; <i>lacI^f</i> , pCA24N P _{T5-lac} :: <i>mqsR</i> ⁺	[24]
pCA24N- <i>hha</i>	Cm ^R ; <i>lacI^f</i> , pCA24N P _{T5-lac} :: <i>hha</i> ⁺	[24]
pCA24N- <i>clpX</i>	Cm ^R ; <i>lacI^f</i> , pCA24N P _{T5-lac} :: <i>clpX</i> ⁺	[24]
pCA24N- <i>clpP</i>	Cm ^R ; <i>lacI^f</i> , pCA24N P _{T5-lac} :: <i>clpP</i> ⁺	[24]
pCA24N- <i>lon</i>	Cm ^R ; <i>lacI^f</i> , pCA24N P _{T5-lac} :: <i>lon</i> ⁺	[24]
pCA24N- <i>yjz</i>	Cm ^R ; <i>lacI^f</i> , pCA24N P _{T5-lac} :: <i>yjz</i> ⁺	[24]
pCA24N- <i>cspD</i>	Cm ^R ; <i>lacI^f</i> , pCA24N P _{T5-lac} :: <i>cspD</i> ⁺	[24]
pCA24N- <i>hokA</i>	Cm ^R ; <i>lacI^f</i> , pCA24N P _{T5-lac} :: <i>hokA</i> ⁺	[24]
pCA24N- <i>hfq</i>	Cm ^R ; <i>lacI^f</i> , pCA24N P _{T5-lac} :: <i>hfq</i> ⁺	[24]
pBS(Kan)	Km ^R ; cloning vector	[26]
pBS(Kan)- <i>mqsR</i>	Km ^R ; pBS(Kan) P _{lac} :: <i>mqsR</i> ⁺	[10]
pBS(Kan)- <i>mqsA</i>	Km ^R ; pBS(Kan) P _{lac} :: <i>mqsA</i> ⁺	[10]
pBS(Kan)- <i>mqsR-mqsA</i>	Km ^R ; pBS(Kan) P _{lac} :: <i>mqsR</i> ⁺ - <i>mqsA</i> ⁺	[10]
Primers for qRT-PCR		
<i>mqsR</i>	f: 5'-CCTGTTAAATGCAGATGAGTTAGGT-3' r: 5'-CTTAAACGAGACGATCAGTACGTCA-3'	
<i>hha</i>	f: 5'-GAATTCTTGCAGGTTGATACCTCTA-3' r: 5'-GACCAATAAACTCAGGGATAAGACC-3'	
<i>cspD</i>	f: 5'-GAAAAGGGTACTGTTAAGTGGTTCA-3' r: 5'-GGACATCAAACCTGAACGGATTGTCC-3'	
<i>hokA</i>	f: 5'-CACGCTTTTATTTTTCACCTGGATG-3' r: 5'-CTTCAATTTGCAGGCTAAAAACGC-3'	
<i>hfq</i>	f: 5'-TTAAGCTGCAAGGGCAAATC-3' r: 5'-GGTTTCTTCGCTGTCCTGTT-3'	

Km^R and Cm^R are kanamycin and chloramphenicol resistance, respectively.

Supplemental Table S2. Partial list of differentially expressed genes for planktonic cells of BW25113 *hfq* vs. BW25113 grown to a turbidity of 0.5 at 600 nm in LB medium at 37°C, adjusted to a turbidity of 1, and exposed to 100 µg/mL ampicillin for 2 h.

Class	Gene	b number	Fold change	Description
Metabolism	<i>entC</i>	b0593	-3.5	isochorismate hydroxymutase 2, enterochelin biosynthesis
	<i>fumC</i>	b1611	4.0	fumarase C, fumarate hydratase Class II, isozyme
	<i>narP</i>	b2193	3.7	nitratenuitrite response regulator (sensor NarQ)
	<i>lrhA</i>	b2289	4.0	NADH dehydrogenase transcriptional regulator, LysR family
	<i>yghJ</i>	b2974	-19.7	putative endoglucanase
	<i>yghK</i>	b2975	-58.0	putative permease
	<i>glcB</i>	b2976	-22.5	malate synthase G
	<i>glcG</i>	b2977	-20.7	hypothetical protein
	<i>glcF</i>	b2978	-23.2	glycolate oxidase iron-sulfur subunit
	<i>glcD</i>	b2979	-14.9	glycolate oxidase subunit D
	<i>glcC</i>	b2980	-28.9	transcriptional activator for <i>glc</i> operon
	<i>yghO'</i>	b2981	-180.3	hypothetical protein
	<i>tdcR</i>	b3119	-4.9	threonine dehydratase operon activator protein
	<i>gadA</i>	b3517	-3.5	glutamate decarboxylase isozyme
	<i>aceB</i>	b4014	4.3	malate synthase A
	<i>aceA</i>	b4015	3.5	isocitrate lyase
	Motility	<i>csgD</i>	b1040	-3.5
<i>csgB</i>		b1041	-3.7	minor curlin subunit precursor, similar to CsgA
<i>flgA</i>		b1072	-4.0	flagellar biosynthesis, assembly of basal-body periplasmic P ring
<i>flgB</i>		b1073	-4.9	flagellar biosynthesis, cell-proximal portion of basal-body rod
<i>cheW</i>		b1887	-7.0	positive regulator of CheA protein activity
<i>cheA</i>		b1888	-5.3	sensory transducer kinase for chemotaxis
<i>fliA</i>		b1922	-4.6	flagellar biosynthesis, alternative sigma factor 28
<i>fliC</i>		b1923	-19.7	flagellar biosynthesis, flagellin, filament structural protein
<i>fliD</i>		b1924	-8.0	flagellar biosynthesis, filament capping protein
Stress	<i>dnaK</i>	b0014	2.5	chaperone Hsp70, DNA biosynthesis
	<i>dnaJ</i>	b0015	2.3	chaperone with DnaK; heat shock protein
	<i>clpP</i>	b0437	2.5	ATP-dependent proteolytic subunit of <i>clpA-clpP</i> serine protease
	<i>clpX</i>	b0438	2.1	ATP-dependent specificity component of <i>clpP</i> serine protease
	<i>lon</i>	b0439	2.1	DNA-binding, ATP-dependent protease La, heat shock K-protein
	<i>sfnC</i>	b0531	-3.7	putative chaperone
	<i>cspH</i>	b0989	-4.0	cold shock-like protein
	<i>cspG</i>	b0990	-3.5	homolog of Salmonella cold shock protein
	<i>ymgA</i>	b1165	-4.9	hypothetical protein
	<i>cspF</i>	b1558	-3.5	cold shock protein homolog
	<i>emrY</i>	b2367	-3.5	multidrug resistance protein Y
	<i>yqiH</i>	b3047	-4.0	putative periplasmic pilus chaperone
	<i>yhcA</i>	b3215	-3.5	putative chaperone
	<i>slp</i>	b3506	-5.7	outer membrane protein induced after carbon starvation
	<i>cspA</i>	b3556	-3.5	cold shock protein 7.4, transcriptional activator of <i>hns</i>
Transport	<i>oppA</i>	b1243	4.6	oligopeptide transport, periplasmic binding protein
	<i>oppB</i>	b1244	5.7	oligopeptide transport permease protein
	<i>oppC</i>	b1245	4.9	homolog of Salmonella oligopeptide transport permease protein
	<i>oppD</i>	b1246	4.3	homolog of Salmonella oligopeptide ABC transport system
	<i>oppF</i>	b1247	4.0	homolog of Salmonella oligopeptide ABC transport system
	<i>proV</i>	b2677	-4.0	transport system for glycine, betaine and proline
	<i>proW</i>	b2678	-4.0	high-affinity transport system for glycine betaine and proline
	<i>dppA</i>	b3544	3.7	dipeptide transport protein
	Outer membrane	<i>sbmA</i>	b0377	7.0
<i>yaiW</i>		b0378	4.6	required for swarming phenotype, probable lipoprotein

Regulator	<i>ybfM</i>	b0681	19.7	putative outer membrane porin protein	
	<i>ybfN</i>	b0682	7.0	novel verified lipoprotein, function unknown	
	<i>hha</i>	b0460	2.0	haemolysin expression modulating protein	
	<i>appY</i>	b0564	-3.7	regulatory protein affecting <i>appA</i> and other genes	
	<i>ybdO</i>	b0603	-4.0	putative transcriptional regulator LYSR-type	
	<i>hns</i>	b1237	2.5	DNA-binding protein, pleiotropic regulator	
	<i>ygeK</i>	b2855	-4.3	putative 2-component transcriptional regulator	
	<i>tdcA</i>	b3118	-3.5	transcriptional activator of <i>tdc</i> operons	
	<i>fis</i>	b3261	1.9	site-specific DNA inversion stimulation factor	
	<i>cadC</i>	b4133	-3.5	transcriptional activator of <i>cad</i> operons	
	<i>gadY</i>	b4452	-3.7	sRNA regulator of <i>gadAB</i> transcriptional activator GadX mRNA	
	<i>micC</i>	b4427	-3.7	MicC sRNA regulator of OmpC translation	
	Uncharacterized	<i>dksA</i>	b0145	2.0	transcriptional regulator of rRNA
		<i>ybcN</i>	b0547	-4.3	hypothetical protein
		<i>ybcV</i>	b0558	-3.5	putative an envelop protein
<i>ybiJ</i>		b0802	3.7	hypothetical protein	
<i>ycfS</i>		b1113	5.7	hypothetical protein	
<i>yncJ</i>		b1436	4.0	hypothetical protein, predicted secreted protein	
<i>yebE</i>		b1846	4.0	hypothetical protein	
<i>ypeC</i>		b2390	3.7	hypothetical protein	
<i>yqaD</i>		b2654	-4.6	hypothetical protein	
<i>yqaC</i>		b2655	-4.0	hypothetical protein	
<i>yqeH</i>		b2846	-3.5	hypothetical protein	
<i>yqeK</i>		b2849	-4.6	hypothetical protein	
<i>ygeF</i>		b2850	-3.5	hypothetical protein	
<i>ygeG</i>		b2851	-3.5	hypothetical protein	
<i>ygeH</i>		b2852	-4.9	putative invasion protein	
<i>ygeI</i>		b2853	-3.7	hypothetical protein	
<i>ygeJ</i>		b2854	-4.6	hypothetical protein, putative murein hydrolase	
<i>yhcN</i>		b3238	3.7	hypothetical protein, periplasmic protein	
<i>yhiK</i>		b3489	-4.3	hypothetical protein	
<i>yhiL</i>		b3490	-3.7	hypothetical protein	
<i>yhiM</i>	b3491	-4.6	hypothetical protein		
<i>yhjW</i>	b3546	3.7	hypothetical protein		

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. Effect of isogenic mutations on persister cell production. BW25113 *clpX*/pCA24N-*clpX*, BW25113 *clpP*/pCA24N-*clpP*, BW25113 *lon*/pCA24N-*lon*, and BW25113 *yjz*/pCA24N-*yjz* were grown to a turbidity of 0.5 in LB medium at 37°C, contacted with 1 mM IPTG for 2 hr for induction, adjusted to a turbidity of 1, and exposed to 100 µg/mL ampicillin for 2 h in LB. Persister data are the average of two independent cultures, and one standard deviation is shown. The asterisk indicates statistical significance determined using a Student's *t*-test ($p < 0.05$).

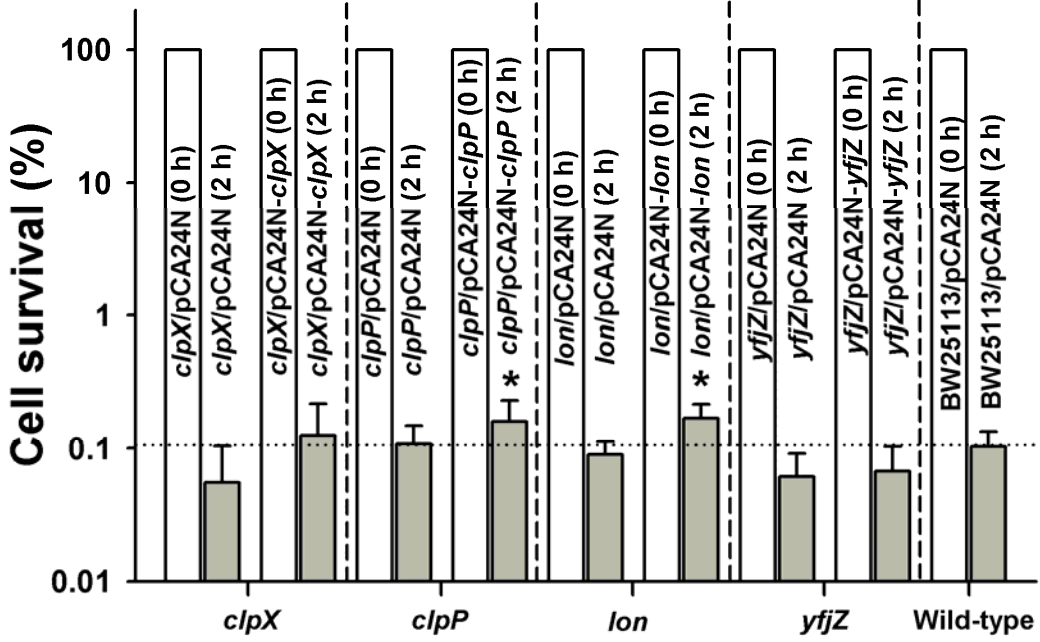


Figure S1