

Supplementary Material for:

**Hydroxyurea induces a stress response that alters DNA replication and
nucleotide metabolism in *Bacillus subtilis***

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Running title: Metabolic stress response to hydroxyurea

Key words: Hydroxyurea, Tn-seq, *Bacillus subtilis*, Ribonucleotide reductase, genome instability

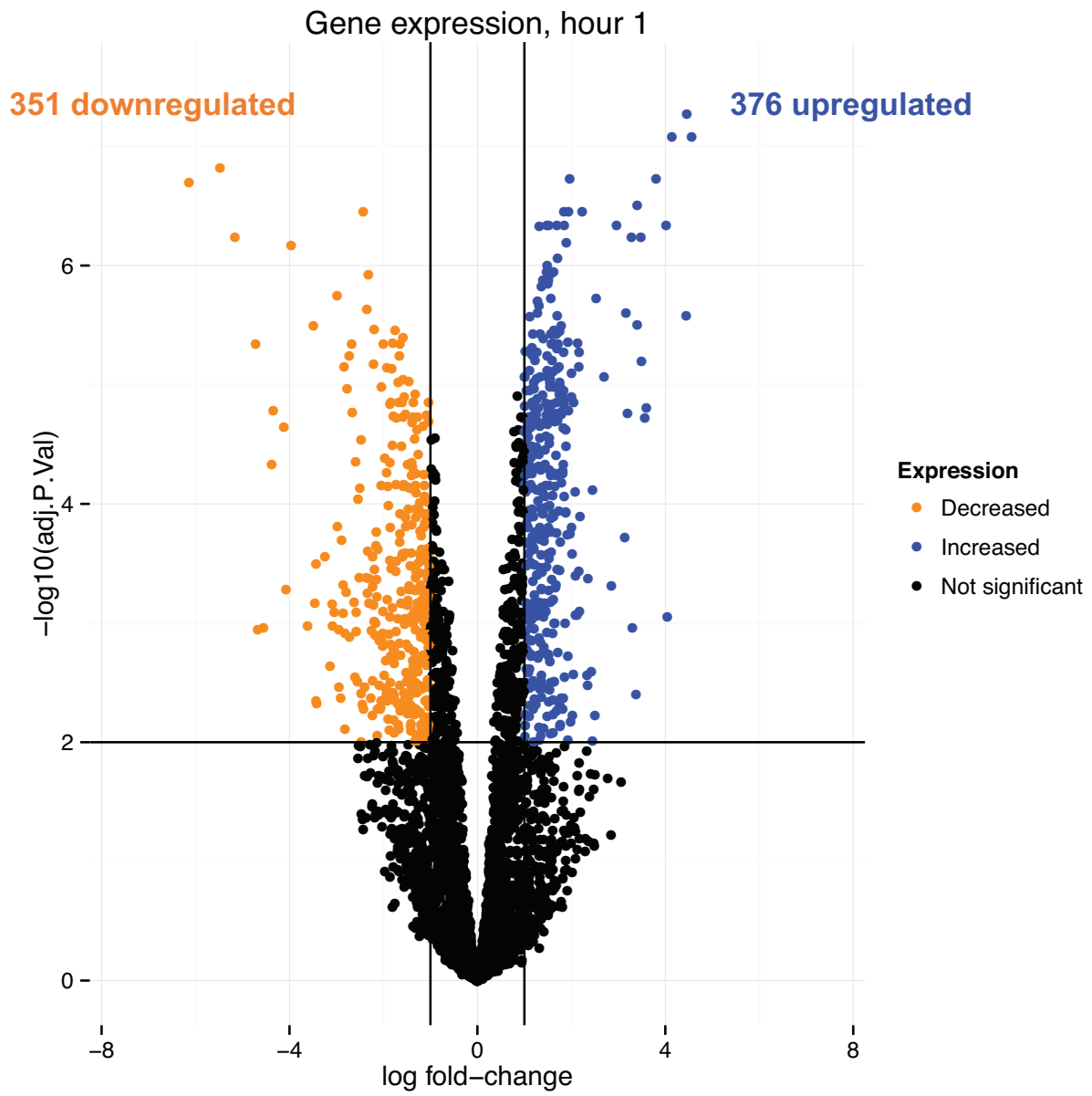


Figure S1. Differential gene expression after 1 hour in hydroxyurea.

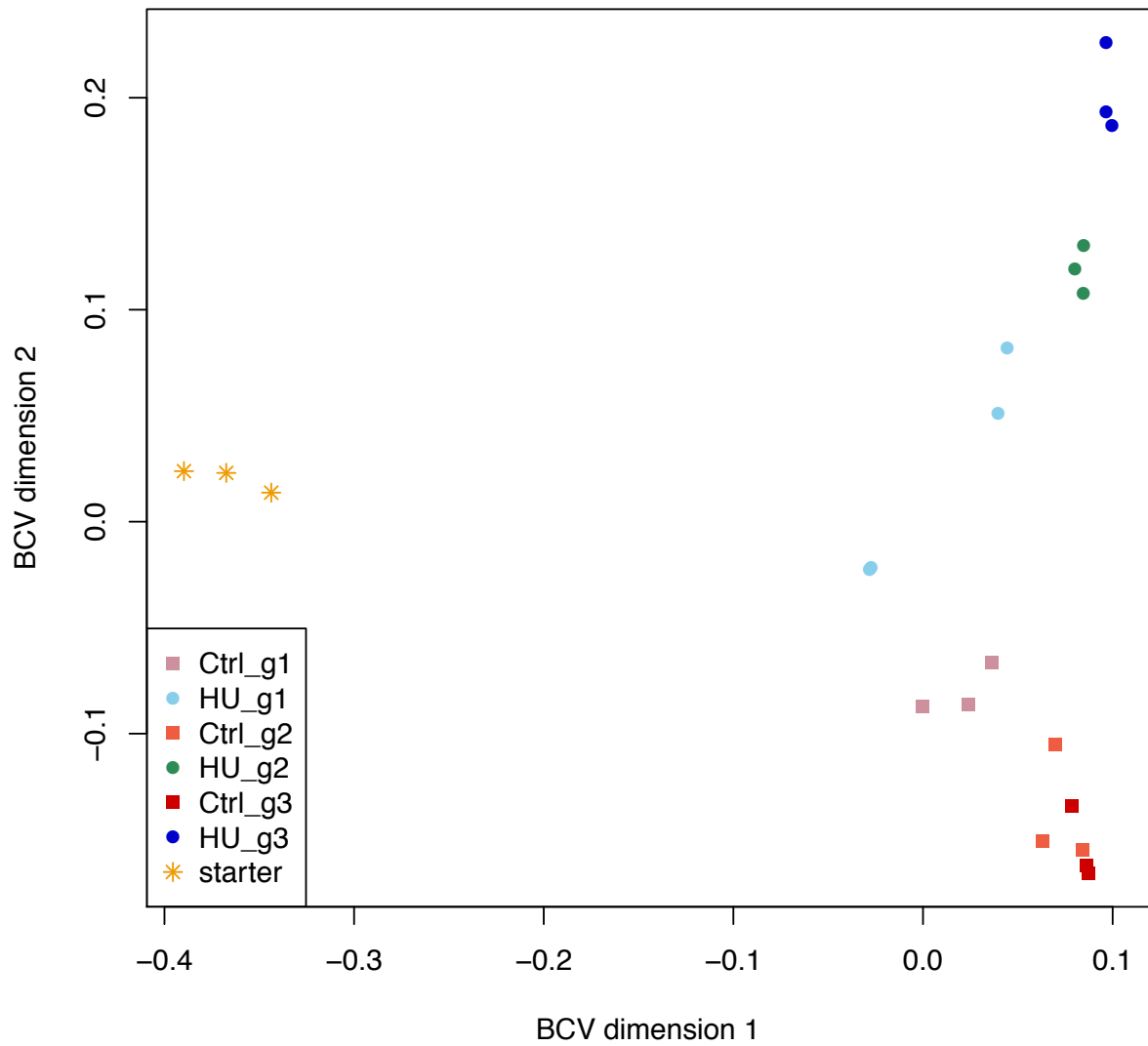


Figure S2. PCA analysis of Tn-seq fitness data showing clustering of the growth period replicates.

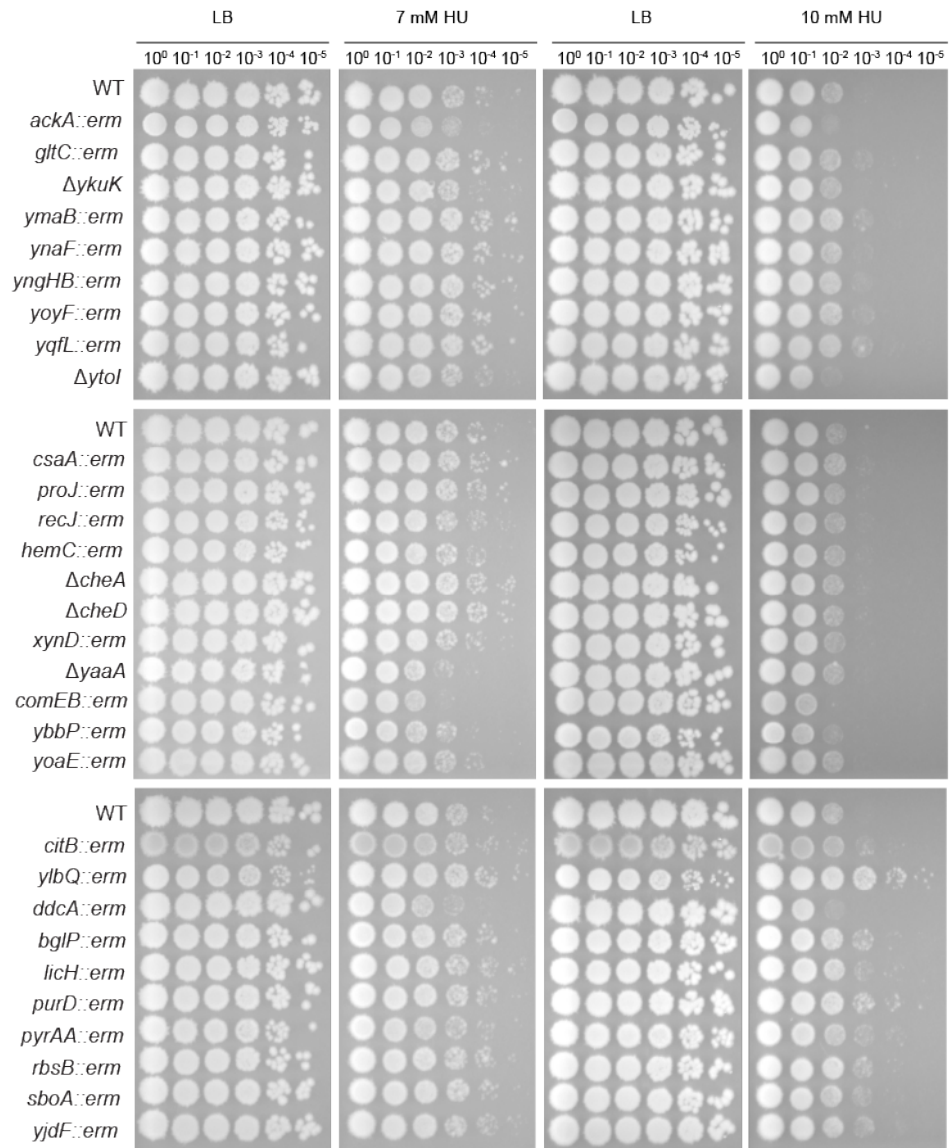


Figure S3. Tn-seq and RNA-seq hits confer a range of phenotypes on hydroxyurea. Top hits from Tn-seq (growth 2 and 3) and RNA-seq were deleted and tested for sensitivity on HU. First row of plates were top hits from Tn-seq (growth 2 and 3), the second row of plates were top hits from Tn-seq (growth 2 or 3) and RNA-seq (either hour 1 or 2), and the third row of plates are from RNA-seq.

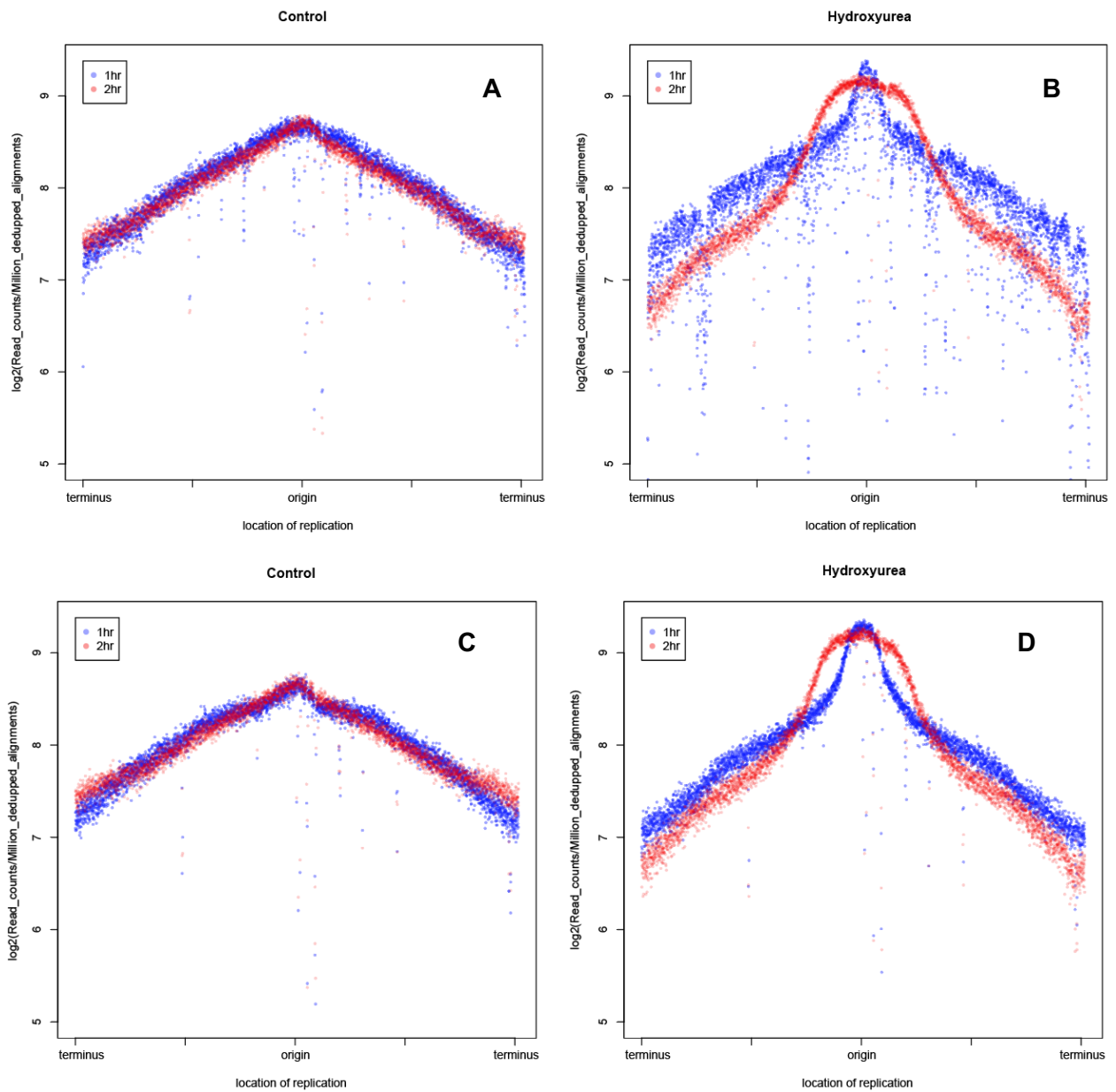


Figure S4. Hydroxyurea causes slowed DNA replication fork progression. Left panels show WT grown with vehicle control at one and two hours. The right panels show the replication profile of cells following challenge with HU for one or two hours. One-hour data are shown in blue and the two-hour are shown in red. The data presented here represents the two independent replicates combined in the experiment shown in Figure 4. Replicate #1 is represented by A and B and replicate #2 by C and D.

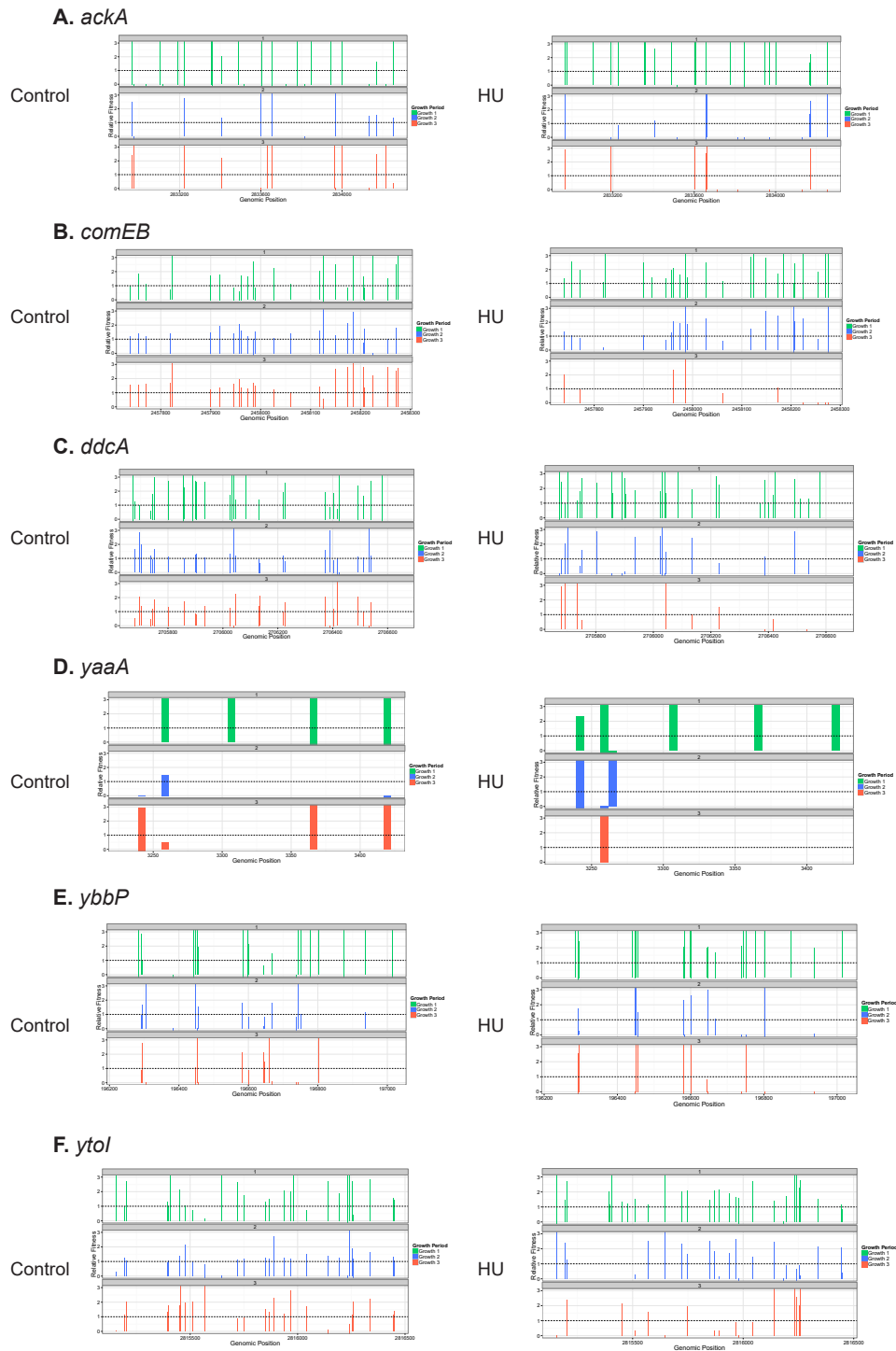


Figure S5. Insertion maps for top validated genes in control treated (left) and HU treated (right)

Table S1. RNA-seq results growth 1 hour (excel spreadsheet)

Table S2. RNA-seq results growth 2 hours (excel spreadsheet)

Table S3. Tn-seq growth period 2 (excel spreadsheet)

Table S4. Tn-seq growth period 3 (excel spreadsheet)

Table S5. Summary of Tn-seq and RNA-seq gene hits evaluated

Gene	Deletion phenotype	Relevance	Putative function	Category
<i>ackA::erm</i>	sensitive	Tn-seq growth 2 & 3	Acetate kinase	ATP generation
<i>gltC::erm</i>	WT	Tn-seq growth 2 & 3	Transcriptional regulator of glutamate synthesis	Regulator (carbohydrate metabolism)
$\Delta ykuK$	sensitive	Tn-seq growth 2 & 3	Unknown, possibly related to RNase H	Unknown
<i>ymaB::erm</i>	WT	Tn-seq growth 2 & 3	Nudix hydrolase	mRNA cleavage
<i>ynaF::erm</i>	WT	Tn-seq growth 2 & 3	Unknown	Unknown
<i>yngHB::erm</i>	WT	Tn-seq growth 2 & 3	Acyl-CoA carboxylase	Leucine utilization
<i>yoyF::erm</i>	WT	Tn-seq growth 2 & 3	Unknown	Unknown
<i>yqfL::erm</i>	WT	Tn-seq growth 2 & 3	Modulator of CcpN	Regulator (Carbohydrate metabolism)
$\Delta ytol$	sensitive	Tn-seq growth 2 & 3	Transcriptional regulator	Regulator (unknown)
<i>csaA::erm</i>	WT	Tn-seq growth 2	Protein-secretion chaperone	Protein secretion
<i>proJ::erm</i>	WT	Tn-seq growth 2	Glutamate 5-kinase	Osmotic stress
<i>recJ::erm</i>	WT	Tn-seq growth 2	Exonuclease	DNA replication
<i>hemC::erm</i>	WT	Tn-seq growth 3	Hydroxymethylbilane synthase	Heme synthesis
$\Delta cheA$	WT	Tn-seq growth 2 & 3, RNA-seq hour 1 (up)	Two-component sensor kinase for chemotaxis	Chemotaxis
$\Delta cheD$	WT	Tn-seq growth 2 & 3, RNA-seq hour 1 (up)	Chemoreceptor deaminase	Chemotaxis
<i>xynD::erm</i>	WT	Tn-seq growth 2 & 3, RNA-seq hour 1 (up)	Arabinoxylan arabinofuranohydrolase	Catabolism

<i>ΔyaaA</i>	sensitive	Tn-seq growth 2 & 3, RNA-seq hour 1 (down)	Ribosome assembly factor	Ribosomal
<i>comEB::erm</i>	sensitive	Tn-seq growth 2 & 3, RNA-seq hour 2 (down)	dCMP deaminase, late competence	Nucleotide synthesis
<i>ΔybbP</i>	sensitive	Tn-seq growth 3, RNA-seq hour 1 (down)	Diadenylate cyclase	Second messenger synthesis
<i>yoaE::erm</i>	WT	Tn-seq growth 2, RNA-seq hour 2 (up)	Formate dehydrogenase	ATP generation
<i>citB::erm</i>	resistant	Tn-seq growth 2, RNA-seq hour 1, 2 (down)	Aconitase	TCA cycle
<i>ylbQ::erm</i>	resistant	Tn-seq growth 2, RNA-seq hour 1, 2 (down)	2-dehydropantoate 2-reductase	Antimicrobial peptide synthesis
<i>ΔddcA</i>	sensitive	Tn-seq growth 3, RNA-seq hour 1, 2 (down)	DNA damage checkpoint agonist	DNA repair
<i>bglP::erm</i>	resistant	RNA-seq hour 1, 2 (down)	Beta-glucoside permease	Phosphotransferase system
<i>lich::erm</i>	WT	RNA-seq hour 1, 2 (down)	6-phospho-beta- glucosidase	Phosphotransferase system
<i>purD::erm</i>	resistant	RNA-seq hour 1, 2 (up)	Phosphoribosylglyci namide synthetase	Purine synthesis
<i>pyrAA::erm</i>	WT	RNA-seq hour 1, 2 (up)	Carbamoyl- phosphate synthetase	Pyrimidine biosynthesis
<i>rbsB::erm</i>	WT	RNA-seq hour 1, 2 (down)	Ribose ATP transporter	Phosphotransferase system
<i>sboA::erm</i>	WT	RNA-seq hour 1, 2 (up)	Subtilisin A	Antimicrobial peptide synthesis
<i>yjdB::erm</i>	WT	RNA-seq hour 1, 2 (down)	Unknown	Unknown

Table S6. Strains used in this work

Strain	Genotype	Origin or construction
KJW1	PY79	(1)
KJW5	<i>recA::recA-GFP</i> (spec ^R)	(2)
KJW7	<i>tagC::tagC-GFP</i> (spec ^R)	(3)
KJW41	<i>comEB::erm</i>	BKE25580 into PY79
KJW47	$\Delta yaaA$	BKE00030 into PY79, <i>erm</i> removed
KJW49	$\Delta ytol$	BKE29270 into PY79, <i>erm</i> removed
KJW51	$\Delta ykuK$	BKE14110 into PY79, <i>erm</i> removed
KJW53	$\Delta cheA$	BKE16430 into PY79, <i>erm</i> removed
KJW55	$\Delta cheD$	BKE16460 into PY79, <i>erm</i> removed
KJW71	$\Delta rnhC$	JRR84(5-7)
KJW125	<i>proJ::erm</i>	BKE18470 into PY79
KJW127	<i>yoaE::erm</i>	BKE18570 into PY79
KJW129	<i>citB::erm</i>	BKE18000 into PY79
KJW131	<i>hemC::erm</i>	BKE28150 into PY79
KJW133	<i>ybbP::erm</i>	BKE01750 into PY79
KJW135	<i>ddcA::erm</i>	BKE28540 into PY79
KJW137	<i>ymaB::erm</i>	BKE17400 into PY79
KJW139	<i>yqfL::erm</i>	BKE25240 into PY79
KJW141	<i>recJ::erm</i>	BKE27620 into PY79
KJW143	<i>gltC::erm</i>	BKE18460 into PY79
KJW145	<i>yoyF::erm</i>	BKE19669 into PY79
KJW147	<i>yngHB::erm</i>	BKE18239 into PY79
KJW149	<i>ynaF::erm</i>	BKE17540 into PY79
KJW268	$\Delta polA$	(4, 5)
KJW325	$\Delta uvrA$	PEB307 (4)

Table S6. Continued Strains used in this work

Strain	Genotype	Origin or construction
KJW329	<i>ylbQ::erm</i>	BKE 15110 into PY79
KJW350	<i>csaA::erm</i>	BKE19040 into PY79
KJW353	<i>ackA::erm</i>	BKE29470 into PY79
KJW355	<i>xynD::erm</i>	BKE18160 into PY79
KJW396	<i>purD::erm</i>	BKE06530 into PY79
KJW398	<i>yjdB::erm</i>	BKE12030 into PY79
KJW402	<i>rbsB::erm</i>	BKE35960 into PY79
KJW404	<i>sboA::erm</i>	BKE37350 into PY79
KJW406	<i>lich::erm</i>	BKE38560 into PY79
KJW407	<i>bglP::erm</i>	BKE39270 into PY79
KJW494	<i>recU::erm</i>	PEB125 (6)
KJW495	$\Delta recA$	PEB214 (6)
KJW541	<i>amyE::Pspac-nrdIEF-ymaB</i> (spec ^R)	(8)
KJW548	<i>amyE::Pspac-nrdIEF-ymaB</i> (spec ^R), $\Delta polA$	gDNA from KJW541 transformed into KJW268
KJW550	<i>amyE::Pspac-nrdIEF-ymaB</i> (spec ^R), $\Delta uvrA$	gDNA from KJW541 transformed into KJW325
KJW552	<i>amyE::Pspac-nrdIEF-ymaB</i> (spec ^R), $\Delta rnhC$	gDNA from KJW541 transformed into KJW71
KJW554	<i>amyE::Pspac-nrdIEF-ymaB</i> (spec ^R), <i>recU::erm</i>	gDNA from KJW541 transformed into KJW494
KJW738	$\Delta ybbP$	Cre-lox cure of <i>erm</i> cassette by pDR244 transformation into KJW133
KJW740	$\Delta ddcA$	Cre-lox cure of <i>erm</i> cassette by pDR244 transformation into KJW135

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