Supporting Information

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Table S1. Cluster analysis of genes differentially expressed between the L and S lineages within and across generations

	Cluster (no. of genes)					
	e1 (367)	e2 (133)	e3 (96)	e4 (80)	e5 (57)	e6 (27)
Cellular functions	Carbohydrate catabolism	Iron transport	Glyoxylate cycle	Glycerol metabolism	Enterobactin biosynthesis	Glutamate biosynthesis
	Flagella biogenesis	Enterobactin biosynthesis	Nitrogen metabolism	Sulfur metabolism	Nonribosomal peptide biosynthesis	Aspartate biosynthesis
	Amino sugar biosynthesis	RNA modification	Entner–Doudoroff pathway		Iron transport	Polysaccharide catabolism
	Taxis	Nucleobase, nucleoside, and nucleotide interconversion	Glutamate biosynthesis		Nucleobase, nucleoside, and nucleotide interconversion	
	Fatty acid oxidation	Purine biosynthesis				
	Peptidoglycan metabolism	Response to temperature				
	Amino acid catabolism					
	Glycerol metabolism					
	Methylglyoxal metabolism					
Regulators	*crp, csiR, cusR, fadR, flhC, flhD, fucR, gutM, lrp, rpiR, srlR*	*fur, nadR, purR*	*fruR, glnG, gntR, iclR, nac, phoP, rpoN*	*cysB, mlc, glpR, nagC, nanR*	*fur*	*malT*

Columns show cellular functions and regulators that are overrepresented in the six gene expression clusters e1–e6. The number of genes in each cluster is shown after the cluster label. In addition, 37 genes showed variable expression between evolved samples but did not cluster. Many other genes showed no significant differences between samples or significant differences only between the ancestral and evolved samples, and those genes were not included in the cluster analysis. Cellular functions correspond to the Gene Ontology cellular process categories (1); regulators were identified using RegulonDB (2).

1. Riley M, et al. (2006) *Escherichia coli* K-12: A cooperatively developed annotation snapshot—2005. *Nucleic Acids Res* 34:1–9.
2. Gama-Castro S, et al. (2008) RegulonDB (version 6.0): Gene regulation model of *Escherichia coli* K-12 beyond transcription, active (experimental) annotated promoters and Textpresso navigation. *Nucleic Acids Res* 36(Database issue):D120–D124.

Table S2. Cluster analysis of differential growth abilities across environmental conditions between the L and S lineages within and across generations

Cluster (no. of conditions)				
g1 (18)	g2 (9)	g3 (9)	Not clustered (12) or nonsignificant (3)*	No growth (43)
1% NaCl	Acetic acid	α-D-glucose	4% (vol/vol) NaCl	3-Methyl glucose
1% sodium lactate	α-D-lactose	D-gluconic acid	Aztreonam	8% (vol/vol) NaCl
D-fructose	D-galacturonic acid	D-glucose-6-PO_4	Dextrin	Acetoacetic acid
D-fructose-6-PO_4	D-glucuronic acid	D-sorbitol	D-maltose	α-Hydroxy-butyric acid
D-galactose	D-threhalose	L-lactic acid	D-saccharic acid	α-Keto-butyric acid
D-mannitol	Glycerol	Methyl pyruvate	D-serine	α-Keto-glutaric acid
D-mannose	L-alanine	*N*-acetyl-neuraminic acid	Glucuronamide	β-Hydroxy-D,L-butyric acid
D-melibiose	Mucic acid	P-hydroxy-phenylacetic acid	Guanidine HCl	β-methyl-D-glucoside
Fusidic acid	*N*-acetyl-D-glucosamine	Sodium butyrate	L-aspartic acid	Bromo-succinic acid
Glycyl-L-proline			L-glutamic acid	Citric acid
Inosine			L-malic acid	D-arabitol
L-serine			L-rhamnose	D-aspartic-acid
pH 5			*N*-acetyl-β-D-mannosamine	D-cellobiose
pH 6			*N*-acetyl-D-galactosamine	D-fucose
Rifamycin SV			Niaproof4	D-lactic acid methyl ester
Tetrazolium violet				D-malic acid
Troleandomycin				D-raffinose
Vancomycin				D-salicilin
				D-serine
				D-turanose
				Formic acid
				γ-amino-butyric acid
				Gelatin
				Gentobiose
				L-arginine
				L-fucose
				L-galactonic acid
				Lactone
				L-histidine
				Lincomycin
				Lithium chloride
				L-pyroglutamic acid
				Minocycline
				Nalidixic acid
				Pectin
				Potassium tellurite
				Propionic acid
				Quinic acid
				Sodium bromate
				Stachyose
				Sucrose
				Tetrazolium blue
				Tween 40

*The three nonsignificant environments were aztreonam, D-mannitol, and glucuronamide. Aztreonam showed a difference only between the ancestral and evolved bacteria and thus was excluded from this analysis, which focused on differences between the L and S lineages within and across generations.