

Table S1. Bacterial strains used for this study.

species	strain	relevant genotype	source
<i>S. Typhimurium</i>	14028s	wild type	ATCC
<i>S. Typhimurium</i>	KAM30	$\Delta dgaR::kan$	this study
<i>S. Typhimurium</i>	KAM68	$\Delta dgaR$	this study
<i>S. Typhimurium</i>	KAM44	$\Delta dgaAB::kan$	this study
<i>S. Typhimurium</i>	KAM71	$\Delta dgaAB$	this study
<i>S. Typhimurium</i>	KAM117	$\Delta dgaE::kan$	this study
<i>S. Typhimurium</i>	KAM123	$\Delta dgaE$	this study
<i>S. Typhimurium</i>	KAM118	$\Delta dgaF::kan$	this study
<i>S. Typhimurium</i>	KAM124	$\Delta dgaF$	this study
<i>S. Typhimurium</i>	KAM126	$\Delta dgaF\Delta eda::kan$	this study
<i>S. Typhimurium</i>	KAM1262	$\Delta dgaF\Delta eda$	this study
<i>S. Typhimurium</i>	KAM111	$\Delta eda::kan$	this study
<i>S. Typhimurium</i>	KAM106	Δeda	this study
<i>S. Typhimurium</i>	KAM121	$\Delta dgaEF::kan$	this study
<i>S. Typhimurium</i>	KAM122	$\Delta dgaEF$	this study
<i>S. Typhimurium</i>	KAM27	$\Delta STM14_0667::kan$	this study
<i>S. Typhimurium</i>	KAM69	$\Delta STM14_0667$	this study
<i>S. Typhimurium</i>	KAM31	$\Delta STM14_5448::kan$	this study
<i>S. Typhimurium</i>	KAM67	$\Delta STM14_5448$	this study
<i>S. Typhimurium</i>	KAM75	$\Delta STM14_0673 \Delta STM14_0672$ $\Delta dgaAB \Delta STM14_5449$ $\Delta STM14_5450::kan$	this study
<i>S. Typhimurium</i>	KAM81	$\Delta STM14_0673 \Delta STM14_0672$ $\Delta dgaAB \Delta STM14_5449$ $\Delta STM14_5450$	this study
<i>S. Typhimurium</i>	KAM96	$\Delta STM14_0667 \Delta dgaR$ $\Delta STM14_5448::kan$	this study
<i>S. Typhimurium</i>	KAM100	$\Delta STM14_0667 \Delta dgaR$ $\Delta STM14_5448$	this study
<i>S. Typhimurium</i>	ACB01	$\Delta rpoN$	A. Bono
<i>S. Typhimurium</i>	KAM91	$\Delta ptsH::kan$	this study
<i>S. Typhimurium</i>	KAM95	$\Delta ptsH$	this study
<i>S. Typhimurium</i>	KAM131	$\Delta dgaAB \Delta eda$	this study
<i>S. Typhimurium</i>	JE16465	<i>metE205 ara-9 crr891::kan+</i>	J. Escalante-Semerena
<i>S. Typhimurium</i>	DM12321	<i>met+ ptsG4152::Tn10d(Tc)</i>	D. Downs

<i>S. Typhimurium</i>	KAM139	Δ eda/ pLAC22+dgaF	this study
<i>S. Typhimurium</i>	KAM143	Δ eda/ pLAC22	this study
<i>S. Typhimurium</i>	KAM140	Δ dgaAB / pLAC22+dgaABCDEF	this study
<i>S. Typhimurium</i>	KAM145	Δ dgaAB / pLAC22	this study
<i>S. Typhimurium</i>	KAM141	Δ dgaR / pLAC22+dgaABCDEF	this study
<i>S. Typhimurium</i>	KAM146	Δ dgaR / pLAC22	this study
<i>E. coli</i>	DH5 α	<i>fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17</i>	S. Kustu
<i>E. coli</i>	KAM134	DH5 α / pLAC22+dgaABCDEF	this study
<i>E. coli</i>	DH5 α	DH5 α / pLAC22	E. Altman
<i>E. coli</i>	JM101	<i>glnV44 thi-1 Δ(lac-proAB) F'[lacI⁺ZΔM15 traD36 proAB⁺]</i>	S. Kustu
<i>E. coli</i>	KAM142	JM101 / pLAC22+dgaABCDEF	this study
<i>E. coli</i>	KAM150	JM101 / pLAC22	this study
<i>E. coli</i>	BL21	<i>E. coli</i> B F- <i>dcm ompT hsdS</i> (r _B -m _B -) <i>gal [malB⁺]_{K-12}(λ^S)</i>	S. Kustu
<i>E. coli</i>	KAM143	BL21 / pLAC22+dgaABCDEF	this study
<i>E. coli</i>	KAM149	BL21 / pLAC22	this study
<i>E. coli</i>	BL21(DE3)	F ⁻ <i>ompT gal dcm lon hsdS</i> _B (r _B ⁻ m _B ⁻) λ (DE3 [<i>lacI lacUV5-T7 gene 1 ind1 sam7 nin5</i>])	(1)
<i>E. coli</i>	KAM155	BL21/DE3 λ /pACYC184+plyE/pE T21a+dgaE	this study
<i>E. coli</i>	KAM154	BL21/DE3 λ /pACYC184+plyE/pE T21a+dgaF	this study
<i>E. coli</i>	IAI1	Wild type commensal	A. Maurelli and E. Denamur

Table S2. Plasmids used in this study.

plasmid	relevant characteristics	Ref.
pKD46	expresses phage λ recombinase genes γ , β and <i>exo</i> from arabinose-inducible P _{araB} promoter; temperature-sensitive replicon	(2)
pKD4	template plasmid used to generate amplicons for inactivation of target genes in the λ Red system	(2)
pCP20	expresses FLP enzyme for removal of <i>kan</i> gene flanked by FRT sites; temperature-sensitive replicon	(3)
pLAC22	expression vector derived from pBR322 that carries the <i>E. coli lac</i> promoter/operator and <i>lacI</i> ^q	(4)
pLAC22+ <i>dgaABCDEF</i>	carries <i>S. Typhimurium dgaABCDEF</i> under control of <i>lac</i> promoter/operator in pLAC22	this study
pLAC22+ <i>dgaF</i>	carries <i>S. Typhimurium dgaF</i> under control of <i>lac</i> promoter/operator in pLAC22	this study
pET21a	vectors carries an N-terminal T7 Tag sequence and T7 promoter	Novagen
pET21a+ <i>dgaE</i>	carries <i>S. Typhimurium dgaE</i> under control of T7 promoter in pET21a	this study
pET21a+ <i>dgaF</i>	carries <i>S. Typhimurium dgaF</i> under control of T7 promoter in pET21a	this study

Table S3. Primers used in this study.

Sequence	Experiment
5'-ATG AGA CGT ATT GAG ATC GTA CTG GGA GAG TGT GTA GGC TGG AGC TGC TTC-3'	<i>dgaR</i> knock out λ -Red
5'-TTA ACT ATA GAG CAG TTC GTA GAT ATA AAC ATA TGA ATA TCC TCC TTA-3'	<i>dgaR</i> knock out λ -Red
5'-CAT TTT TCG CCA GGC CTT TAA TCA GGA AGG CGA CAC CAT ATG AAT ATC CTC CTT A-3'	<i>dgaAB</i> knock out λ -Red
5'-ATG GCC AAT ATC GTT TTA TGC CGC ATC GAC AGC CGT TGT GTA GGC TGG AGC TGC TTC-3'	<i>dgaAB</i> knock out λ -Red
5'-ATG ACG CCG AAT ATC TAT CAA CAA CTG GGC ATA TGA ATA TCC TCC TTA-3'	<i>dgaE</i> knock out λ -Red
5'-TTA ATC TGC TGC ATG TTC TGC AAT CTC CCT TGT AGG CTG GAG CTG CTT C-3'	<i>dgaE</i> knock out λ -Red
5'-GGC GAT CCG TCA CCT GAG GAA TAA GAT GAC TGT GTA GGC TGG AGC TGC TTC-3'	<i>dgaF</i> knock out λ -Red
5'-AGG GAG ATT GCA GAA CAT GCA GAT TAA CAT ATG AAT ATC CTT A-3'	<i>dgaF</i> knock out λ -Red
5'-CACTAG TAA TCA GGC GAG AGA AGA ATT CCG TGT GTA GGC TGG AGC TGC TTC-3'	<i>eda</i> knock out λ -Red
5'-ATA AGC AAG CGC CAT CGG GCA TTT AAC GGC CAT ATG AAT ATC CTC CTT A-3'	<i>eda</i> knock out λ -Red
5'-CCG TAA AGC AAA CTG TTG AAG TCA CCA ATA AGC TGG TGT GTA GGC TGG AGC TGC TTC-3'	<i>ptsO</i> knock out λ -Red
5'-GGT GAC TGT CAT CAA ATA CCG GGA CGG CGT TGG CAT ATG AAT ATC CTC CTT A-3'	<i>ptsO</i> knock out λ -Red
5'-CCT GTT GGG ATG TGT TAA GAA ACT GGC TGA TAA TGA CAT ATG AAT ATC CTC CTT A-3'	STM14_0673/STM14_0672 knock out λ -Red
5'-ATG ATC AAA TTA GTG CGC ATT GAT TAC CGC CTG CTG TGT GTA GGC TGG AGC TGC TTC-3'	STM14_0673/STM14_0672 knock out λ -Red
5'-CCG GTT GCG AGG CCG CAA ATT TCG GCA TAT TGA CCA CAT ATG AAT ATC CTC CTT A-3'	STM14_5449/STM14_5450 knock out λ -Red
5'-ACG GGT GGA TCA TCG TTT ATT ACA TGG ACA GGT CGC TGT GTA GGC TGG AGC TGC TTC-3'	STM14_5449/STM14_5450 knock out λ -Red
5'-TTG GCG TCT GGA TCT TCG-3'	STM14_0667 knock out overlapping PCR λ -Red
5'-CAG CCT ACA CAA TCG CTC AAG ATG GTG ACT AAT AAT GCT GCG-3'	STM14_0667 knock out overlapping PCR λ -Red
5'-CTG TGT CAC TGA AAA TTG CTT TGA GGT ATG ATT GAG CGA CTG GTC-3'	STM14_0667 knock out overlapping PCR λ -Red
5'-ATC AGG AGT CTA TCT GTA TTC TGC-3'	STM14_0667 knock out overlapping PCR λ -Red
5'-CAT CAT AAT TGC TGA TGT GAT AAT CGT GAG TGTGTA GGC TGG AGC TGC TTC-3'	STM14_5448 knock out λ -Red
5'-TTA AAA CTG TTG ATC TTG CTC GAT AAG TTC	STM14_5448 knock out λ -Red

CAT ATG AAT ATC CTC CTT A-3'	
5'-GGA AGG CTC GCT GAT CCT GA-3'	<i>dgaAB</i> qRT-PCR
5'-CAG GCG GTT AGC ATC CCT TG-3'	<i>dgaAB</i> qRT-PCR
5'-TAT CGA AGA CGG GAT CAA CC-3'	<i>rpoD</i> qRT-PCR
5'-GGA CAA ACG AGC CTC TTC AG-3'	<i>rpoD</i> qRT-PCR
5'-CAT ATG ACG CCG AAT ATC TAT CAA-3'	DgaE purification
5'-AAG CTT ATC TGC TGC ATG TTC TGC-3'	DgaE purification
5'-CAT ATG CAG CAG ATT AAT TTT TAT CG-3'	DgaF purification
5'-AAG CTT CAC CAG CGC TTT GAC TAT C-3'	DgaF purification

Table S4. Distribution of *dga* genes within the domain Bacteria.

genus	<i>dgaABCD</i>	<i>dgaE</i>	<i>dgaF</i>	<i>dgaR</i>	predicted RpoN-dependent promoter sequences
<i>Salmonella</i>	^a 71/86	71/86	71/86	66/86	^b TGGCACARCCTTTGCT
<i>Escherichia</i>	29/149	22/149	22/149	^b 18/149	TGGCACGTCCTTTGCT
<i>Citrobacter</i>	6/8	6/8	6/8	6/8	TGGCACAGCCTTTGCT
<i>Enterobacter</i>	4/14	4/14	4/14	4/14	^b TGGCACGSSSTTTGCT
<i>Enterobacteriaceae</i>	1/1	1/1	1/1	1/1	TGGCATGCGCTTTGCT
<i>Klebsiella</i>	2/25	2/25	2/25	2/25	TGGCACAGCCTTTGCT
<i>Serratia</i>	1/11	1/11	1/11	1/11	TGGCACAGGCATTGCT
<i>Hafnia</i>	1/1	1/1	1/1	0/0	TGGCATGCGCTTTGCT
<i>Enterococcus</i>	57/108	9/108	8/108	56/108	TGGCACACTTATTGCT
<i>Lactobacillus</i>	2/126	2/126	2/126	2/126	TGGCACGAAATTTGCT

^aNumerator denotes the number of genomes for a given genus that possessed the indicated gene(s), while the denominator indicates the number of genomes for the genus in which it was possible to determine which of the *dga* orthologs were present. The data for the analysis were obtained from the Integrated Microbial Genomes database (<http://img.jgi.doe>) on February 20, 2013.

^bPredicted RpoN-dependent promoter sequences upstream of the *dgaA* orthologs. For positions where the sequence varied, R is A or G and S is G or C.

Table S5. Comparison of properties of complete genomes with the *dga* loci.

Characteristic	Complete genome ^a				<i>dga</i> locus ^b			
	ST	EC	EA	EF	ST	EC	EA	EF
% G+C	52.2	50.8	54.8	37.5	55.8	54.1	58.5	39.5
S3 ^c	59.0	55.9	65.7	30.2	67.5	64.2	74.1	36.0
τ^*_{CTAG} ^d	0.21	0.24	0.29	0.89	0.00 ^e	0.70	0.00 ^e	0.89

^a Bacterial strains are abbreviated as follows: ST, *Salmonella enterica* serovar Typhimurium 14028s; EC, *Escherichia coli* IAI1; EA, *Enterobacter aerogenes* KCTC 2190; EF, *Enterococcus faecalis* 62.

^b The *dga* locus encompasses the following genes and intervening intergenic sequences for their respective genomes: STM14_4550 through STM14_4543, ECIAI1_3068 through ECIAI1_3062, EAE_10150 through EAE_10120, and EF62_0067 through EF62_0061.

^c S3 refers to the percent G+C at the third codon position of protein-coding genes.

^d Relative abundance of the CTAG tetranucleotide (see Materials and Methods and ref.(5)).

^e No CTAG tetranucleotide is present in the sequence.

Table S6. Genome signature comparisons.

Sequence		Complete genome ^a				<i>dga</i> locus			
		ST	EC	EA	EF	ST	EC	EA	EF
Complete genome	ST	59	69	63	107	47	60	68	104
	EC		55	74	89	62	61	80	91
	EA			61	110	43	53	56	104
	EF				59	105	98	116	53
PTS locus	ST					-	30	36	92
	EC						-	40	84
	EA							-	109
	EF								-

^a Bacterial strains are abbreviated as follows: ST, *Salmonella enterica* serovar Typhimurium 14028s; EC, *Escherichia coli* IAI1; EA, *Enterobacter aerogenes* KCTC 2190; EF, *Enterococcus faecalis* 62.

Average δ^* -differences between non-overlapping 7 kb segments are shown (multiplied by 1,000). Diagonal entries represent average δ^* -differences between segments from the same genome whereas non-diagonal entries show average δ^* -differences between segments from different genomes or *dga* loci. δ^* -differences within *dga* loci were not assessed because each *dga* locus represents a single 7 kb sequence sample.

Table S7. BLAST comparisons among proteins encoded by the *dga* locus.

DgaR ^a				
Gene	STM14_4550	ECIA11_3068	EAE_10150	EF62_0067
STM14_4550	1907	1674	1580	582
ECIA11_3068		1941	1550	579
EAE_10150			1938	588
EF62_0067				1910

DgaA ^a				
Gene	STM14_4548	ECIA11_3067	EAE_10145	EF62_0066
STM14_4548	285	243	226	91
ECIA11_3067		289	226	95
EAE_10145			286	97
EF62_0066				269

DgaB ^a				
Gene	STM14_4547	ECIA11_3066	EAE_10140	EF62_0065
STM14_4547	326	304	301	172
ECIA11_3066		326	295	169
EAE_10140			327	170
EF62_0065				326

DgaC ^a				
Gene	STM14_4546	ECIA11_3065	EAE_10135	EF62_0064
STM14_4546	496	483	484	333
ECIA11_3065		496	476	329
EAE_10135			495	338
EF62_0064				487

DgaD ^a				
Gene	STM14_4545	ECIA11_3064	EAE_10130	EF62_0063
STM14_4545	571	560	538	350
ECIA11_3064		572	529	352
EAE_10130			568	350
EF62_0063				570

DgaE ^a				
Gene	STM14_4544	ECIA11_3063	EAE_10125	EF62_0062
STM14_4544	752	636	570	287
ECIA11_3063		750	558	286
EAE_10125			751	266
EF62_0062				748

DgaF ^a				
Gene	STM14_4543	ECIA11_3062	EAE_10120	EF62_0061
STM14_4543	504	415	396	207
ECIA11_3062		515	392	200
EAE_10120			507	202
EF62_0061				507

The table shows pairwise BLAST bit scores between protein products of orthologous genes. The data were obtained running the blastp at the NCBI server (<http://blast.ncbi.nlm.nih.gov/>) with default parameters.

^a Protein product assignments based on the results of this study with *S. Typhimurium* 14028s.

Literature Cited

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