# Absence of Global Stress Regulation in *Escherichia coli* Promotes Pathoadaptation and Novel c-di-GMP-dependent Metabolic Capability

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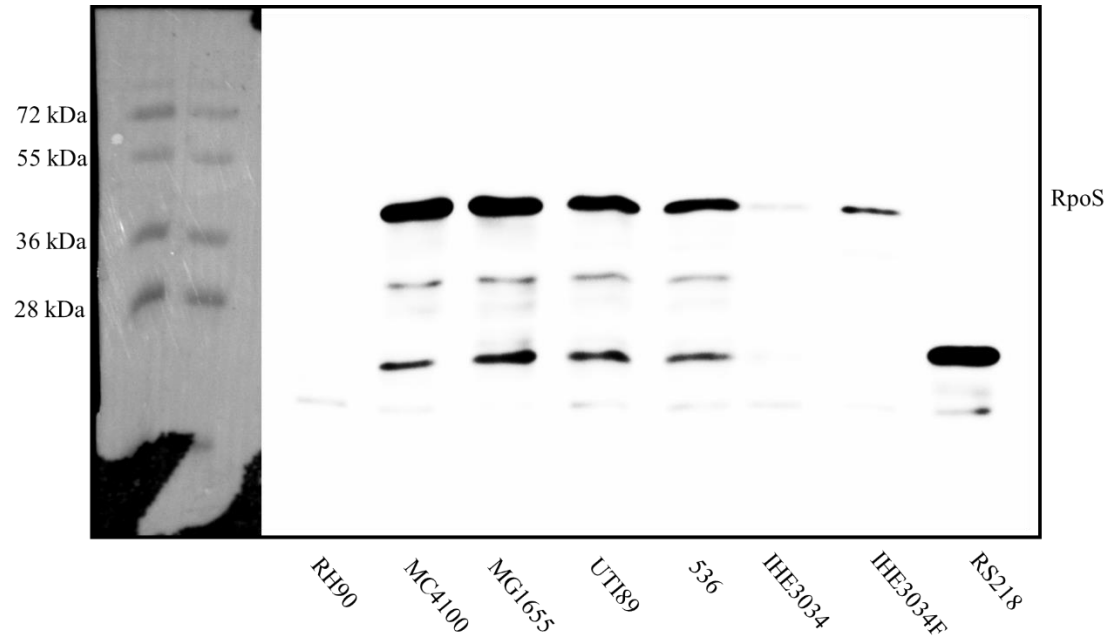
**a**

Fig. S1

**a (extended)**

Fig. S1

**b**


72 kDa
55 kDa
36 kDa
28 kDa
RpoS
RH90
MC4100
MG1655
UTI89
536
IHE3034
IHE3034F
RS218

**c**

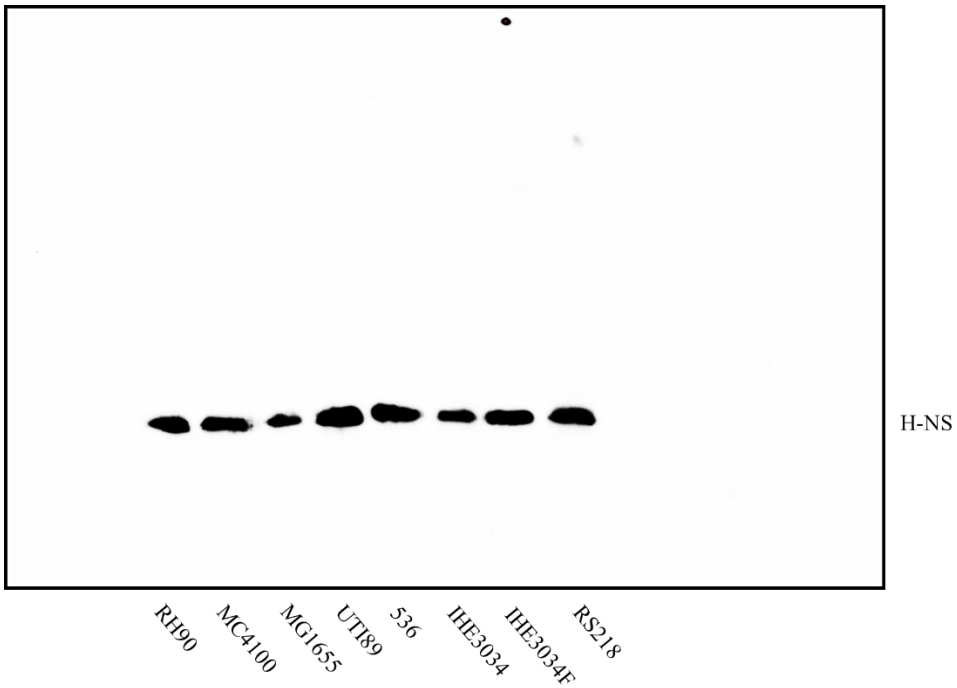

H-NS
RH90
MC4100
MG1655
UTI89
536
IHE3034
IHE3034F
RS218

Fig. S1

**d**

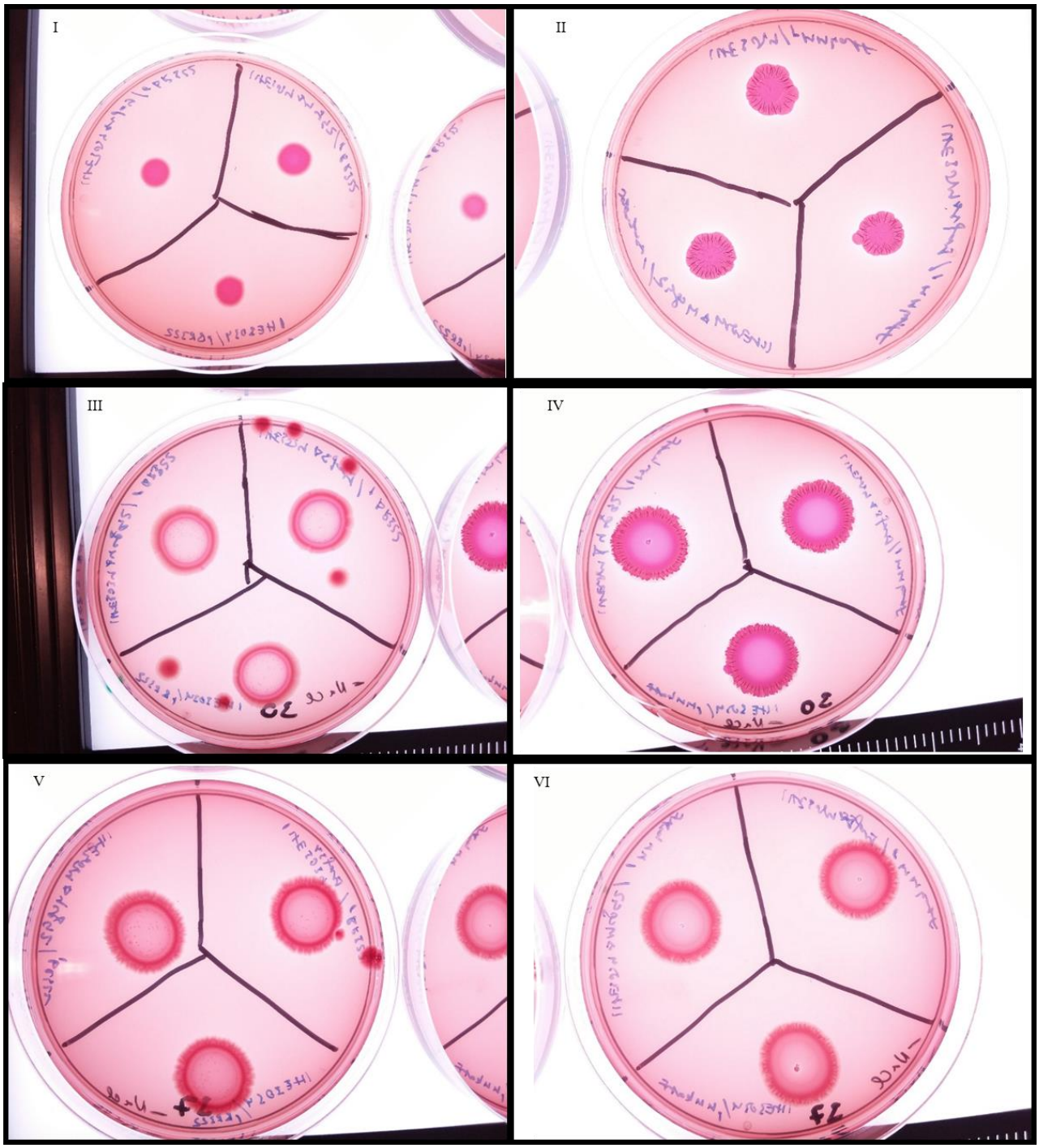



Fig. S1: Properties of the *rpoS* allele in IHE3034.

(a) Multiple sequence alignment of the *rpoS* allelic variants of NMEC compared to the *rpoS* allele of MG1655. Highlighted in yellow are the strains in which *rpoS* gene is turned into a pseudogene, the point mutation that introduced the stop codon shown in bold and underlined. Highlighted in blue are the point mutations that are typical for the *rpoS* allele of IHE3034F which expresses inactive RpoS.

The strains whose *rpoS* allele share the same point mutations of IHE3034F *rpoS* are highlighted in blue too. Common point mutations for all NMEC strains are highlighted in green.

(b) The full membrane of the immunoblot against RpoS present in **Fig.1c**.

(c) The full membrane of the immunoblot against H-NS present in **Fig.1c**.

(d) The full pictures of the Congo Red plates used for the smooth-rugose assay present in **Fig. 1d**.

**a**

>*ycgG2*_K1_IHE3034

ttg-ata-ttt-att-ctg-tta-atc-gcc-tgc-gcc-gct-gcc-ttc-ctg-ctt-gat-agg-tat-ttc-aat-aaa-agc-gca-acg-cct-gaa-gag-atc-ctg-cga-cgg-gct-ata-aat-aat-ggg-gag-atc-gtc-cct-ttt-tac-caa-cct-gtg-gta-aat-ggt-cgg-gaa-ggg-aca-ttg-cgg-gga-gtt-gag-gtg-tta-gcc-cgc-tgg-aaa-caa-cct-cac-ggt-gga-tat-ata-tca-ccc-gcg-gca-ttt-att-cca-ctt-gct-gaa-aaa-tgc-gga-tta-atc-gtt-ccg-ctt-acg-caa-agc-ctg-atg-aat-cag-gtt-gcc-aga-cag-atg-aac-gct-atc-gcg-agt-aaa-ctg-ccg-gaa-ggt-ttt-cat-att-ggg-att-aat-ttt-agc-gcc-tcg-cat-att-att-tcg-ccg-acg-ttt-gtc-gac-gag-tgc-tta-aat-tac-cgt-gac-agt-ttt-acc-cgc-cgc-gat-tta-aac-ctt-gtt-ctg-gaa-gtc-acc-gag-cgt-gag-cca-tta-aat-gtt-gat-gaa-agt-ctg-gtt-cag-cgg-ttg-aac-att-ctg-cat-gaa-aat-ggt-ttt-gtc-atc-gcg-ctg-gat-gat-ttc-ggt-act-ggt-tac-tca-ggg-ctt-tct-tat-ctg-cat-gac-ttg-cat-att-gat-tat-atc-aaa-att-gat-cat-agt-ttc-gtt-ggc-cgc-gtc-aac-gca-gac-cca-gca-tca-acc-cga-att-ctg-gat-tgt-gta-ttg-gat-ctg-gcg-cgt-aaa-ctt-tcg-atc-agt-atc-gtc-gct-gaa-gat-gtc-gaa-acg-aaa-gaa-caa-ttt-gac-tat-ctg-aac-caa-aat-aat-atc-aca-ttt-cag-cag-ggt-tat-tat-ttc-tat-aaa-cct-gtt-aca-tac-atc-gac-ctg-gtc-aag-att-atc-ctt-tct-aaa-ccg-aag-gtg-aag-att-gtg-gtt-gag-tga

**b**

```
YcgG          225 LIFILLIACAAAFLLDRYFNKSATPEEILRRAINNGEIVPFYQPVVNGRE    274
                  :||||||||||||||||||||||||||||||||||||||||||||||||||
YcgG2           1 MIFILLIACAAAFLLDRYFNKSATPEEILRRAINNGEIVPFYQPVVNGRE     50

YcgG          275 GTLRGVEVLARWKQPHGGYISPAAFIPLAEKSGLIVPLTQSLMNQVARQM    324
                  ||||||||||||||||||||||||||||||||.||||||||||||||||||
YcgG2          51 GTLRGVEVLARWKQPHGGYISPAAFIPLAEKCGLIVPLTQSLMNQVARQM    100

YcgG          325 NAIASKLPEGFHIGINFSASHIISPTFVDECLNFRDSFTRRDLNLVLEVT    374
                  ||||||||||||||||||||||||||||||||:|||||||||||||||||
YcgG2         101 NAIASKLPEGFHIGINFSASHIISPTFVDECLNYRDSFTRRDLNLVLEVT    150

YcgG          375 EREPLNVDESLVQRLNILHENGFVIALDDFGTGYSGLSYLHDLHIDYIKI    424
                  ||||||||||||||||||||||||||||||||||||||||||||||||||
YcgG2         151 EREPLNVDESLVQRLNILHENGFVIALDDFGTGYSGLSYLHDLHIDYIKI    200

YcgG          425 DHSFVGRVNADPESTRILDCVLDLARKLSISIVAEGVETKEQLDYLNQNY    474
                  ||||||||||||.|||||||||||||||||||||.||||||.||||||.
YcgG2         201 DHSFVGRVNADPASTRILDCVLDLARKLSISIVAEDVETKEQFDYLNQNN    250

YcgG          475 ITFQQGYYFYKPVTYIDLVKIILSKPKVKVVVE    507
                  ||||||||||||||||||||||||||||:|||
YcgG2          251 ITFQQGYYFYKPVTYIDLVKIILSKPKVKIVVE    283
```

**c**

```
YcgG2          93 MNQVARQMNAIASKLPEGFHIGINFSASHIISPTFVDECLNYRDSFTRRD    142
                  ||||||||||||||||||||||||||||||||||||||||||||||||||
YcgG2_ATG       1 MNQVARQMNAIASKLPEGFHIGINFSASHIISPTFVDECLNYRDSFTRRD     50

YcgG2         143 LNLVLEVTEREPLNVDESLVQRLNILHENGFVIALDDFGTGYSGLSYLHD    192
                  ||||||||||||||||||||||||||||||||||||||||||||||||||
YcgG2_ATG      51 LNLVLEVTEREPLNVDESLVQRLNILHENGFVIALDDFGTGYSGLSYLHD    100

YcgG2         193 LHIDYIKIDHSFVGRVNADPASTRILDCVLDLARKLSISIVAEDVETKEQ    242
                  ||||||||||||||||||||||||||||||||||||||||||||||||||
YcgG2_ATG     101 LHIDYIKIDHSFVGRVNADPASTRILDCVLDLARKLSISIVAEDVETKEQ    150

YcgG2         243 FDYLNQNNITFQQGYYFYKPVTYIDLVKIILSKPKVKIVVE    283
                  |||||||||||||||||||||||||||||||||||||||||
YcgG2_ATG     151 FDYLNQNNITFQQGYYFYKPVTYIDLVKIILSKPKVKIVVE    191
```

**d**

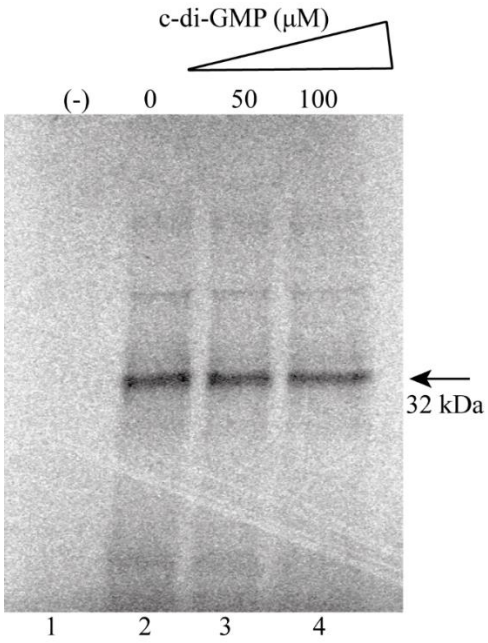
```
YcgG          317 MNQVARQMNAIASKLPEGFHIGINFSASHIISPTFVDECLNFRDSFTRRD    366
                  ||||||||||||||||||||||||||||||||||||||||:||||||||
YcgG2_ATG       1 MNQVARQMNAIASKLPEGFHIGINFSASHIISPTFVDECLNYRDSFTRRD     50

YcgG          367 LNLVLEVTEREPLNVDESLVQRLNILHENGFVIALDDFGTGYSGLSYLHD    416
                  ||||||||||||||||||||||||||||||||||||||||||||||||||
YcgG2_ATG      51 LNLVLEVTEREPLNVDESLVQRLNILHENGFVIALDDFGTGYSGLSYLHD    100

YcgG          417 LHIDYIKIDHSFVGRVNADPESTRILDCVLDLARKLSISIVAEGVETKEQ    466
                  ||||||||||||||||||.|||||||||||||||||||.||||||
YcgG2_ATG     101 LHIDYIKIDHSFVGRVNADPASTRILDCVLDLARKLSISIVAEDVETKEQ    150

YcgG          467 LDYLNQNYITFQQGYYFYKPVTYIDLVKIILSKPKVKVVVE    507
                  .||||||.||||||||||||||||||||||||||:|||
YcgG2_ATG     151 FDYLNQNNITFQQGYYFYKPVTYIDLVKIILSKPKVKIVVE    191
```

Fig. S2.

**e**

>YcgG2_K1_IHE3034
MNQVARQMNAIASKLPEGFHIGINFSASHIISPTFVDECLNYRDSFTRRDLNLVLEVTEREPLNVDES
LVQRLNILHENGFVIALDDFGTGYSGLSYLHDLHIDYIKIDHSFVGRVNADPASTRILDCVLDLARK
LSISIVAEDVETKEQFDYLNQNNITFQQGYYFYKPVTYIDLVKIILSKPKVKIVVE

**f**




**g**

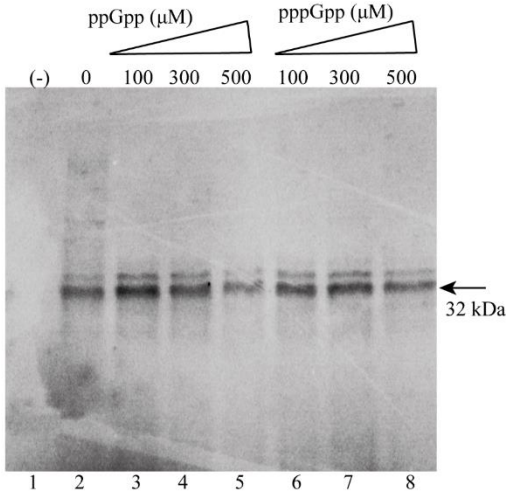



Fig. S2.

**h**


**i**

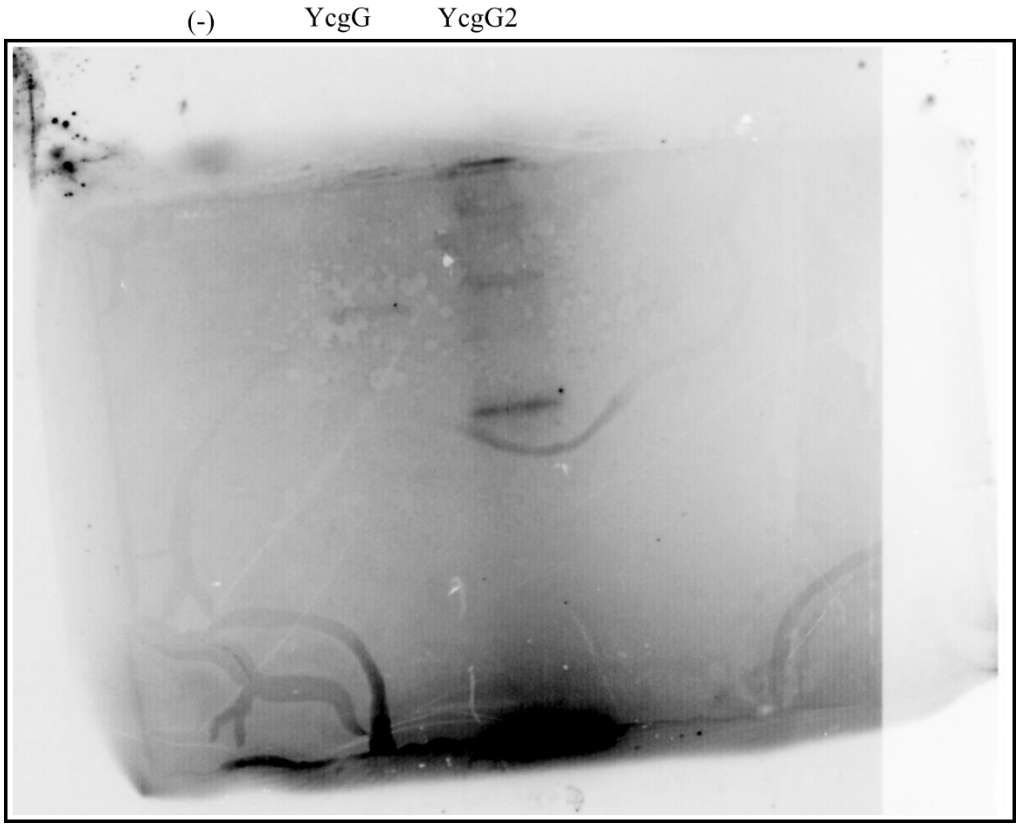
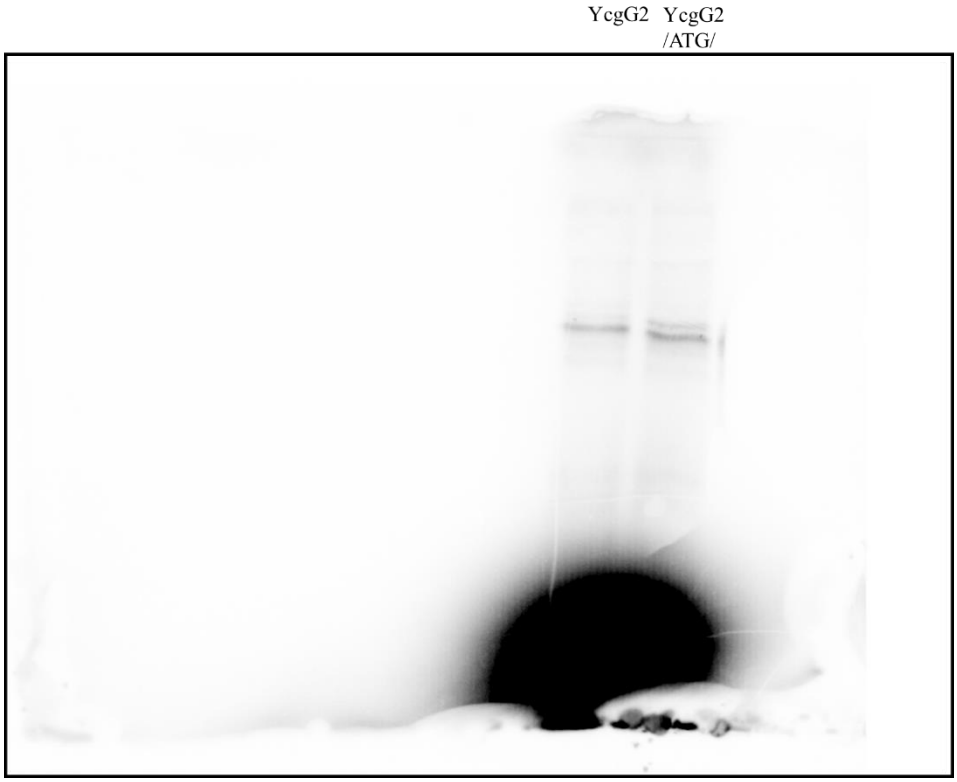



Fig. S2.

**j**




**k**

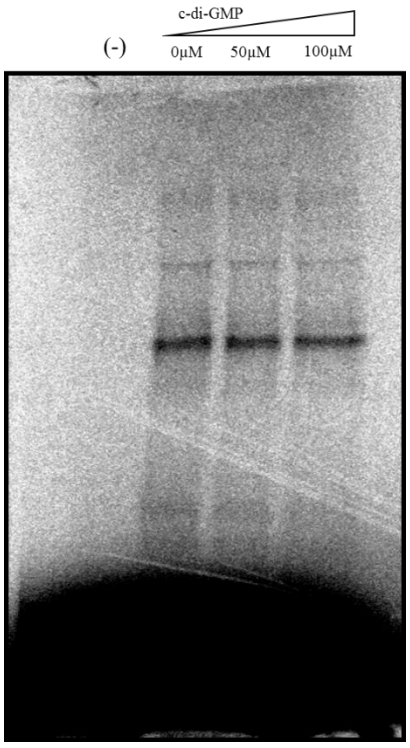



Fig. S2.

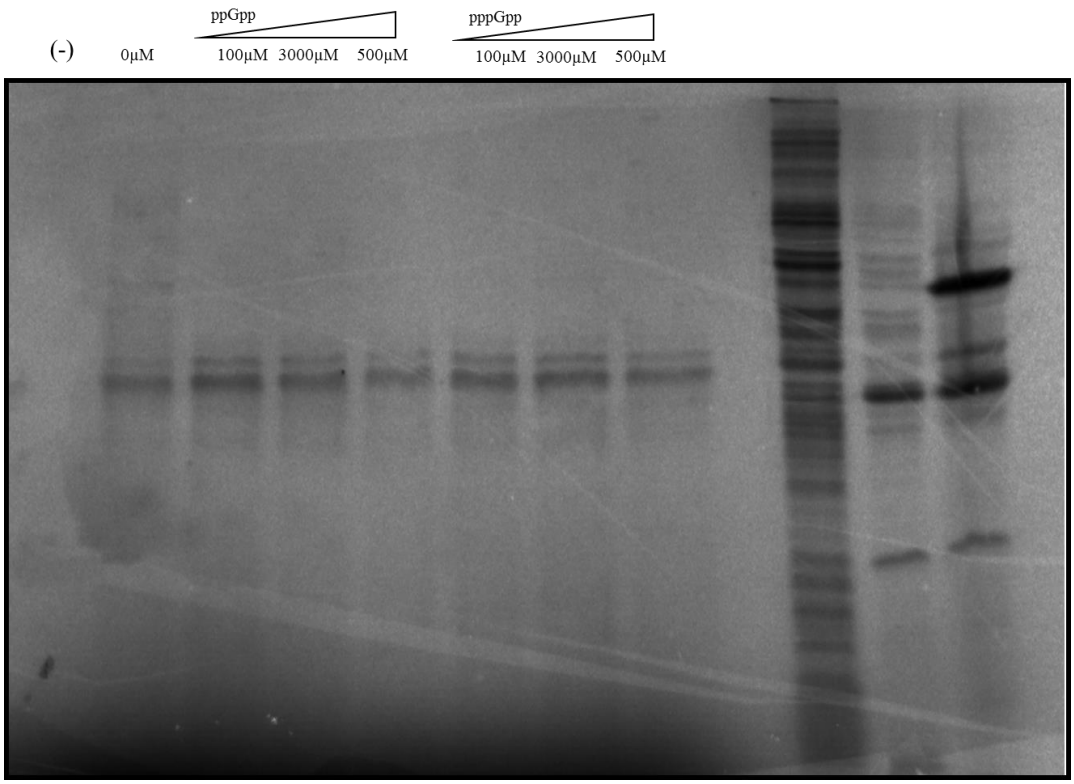



Fig. S2: Analysis of the *ycgG2* gene and its product.

(a) DNA sequence of *ycgG2* split into triplets. The rare start TTG codon is shown in green, the first internal ATG codon – in blue and the stop TGA codon – in red.

(b) Protein alignment of YcgG and Ycg2 showing great similarity between the amino acid residues in the catalytic EAL domain.

(c) Protein alignment between YcgG2 and the protein sequence obtained if the protein is translated from the first internal ATG codon (designated as YcgG2_ATG) showing a 100% match and preservation of the EAL amino acid residues.

(d) Protein alignment between YcgG and YcgG2_ATG showing preservation of the EAL amino acid residues.

(e) Organization of the EAL domain of YcgG2. (Color legend: yellow – residues involved in the EAL domain organization; green – $Mg^{2+}$ - binding residues; blue – c-di-GMP – binding residues; red – glutatamate as a general base catalyst; pink – residues stabilizing the domain).

(f) No effect of c-di-GMP concentrations on YcgG2 translation in *in-vitro* transcription/translation of *ycgG2* under the regulation of T7 promoter (the YcgG2 product shown with an arrow; one representative replica shown of three independent experiments).

(g) No effect of ppGpp and pppGpp alarmones on YcgG2 translation in *in-vitro* transcription/translation of *ycgG2* under the regulation of T7 promoter (the YcgG2 product shown with an arrow; one representative replica shown of three independent experiments).

(h) The full gel of the radioactively labelled YcgG and YcgG2 present in **Fig. 2b** after exposure of 24 hours. The YcgG band was weak and increase of the contrast was applied on the whole picture and then it was used as a main figure.

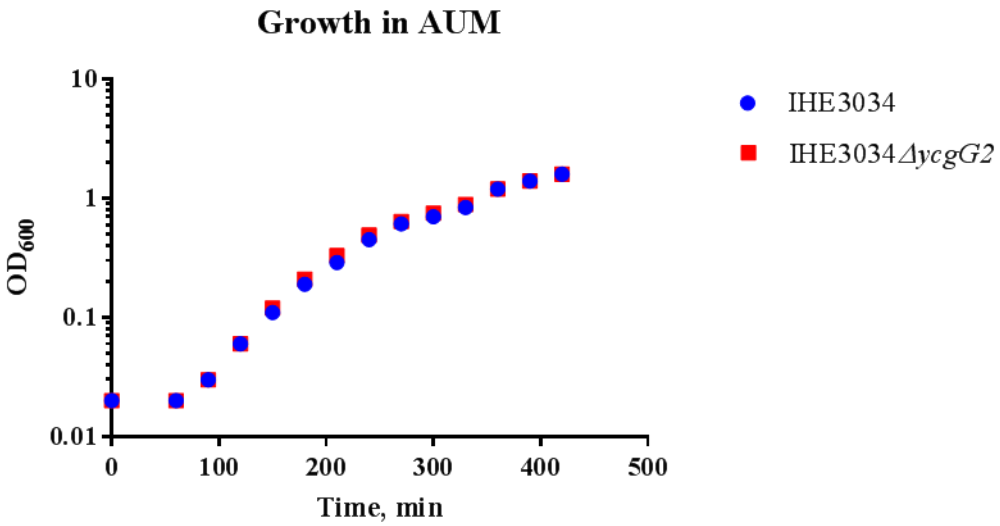
(i) The full gel of the radioactively labelled YcgG and YcgG2 present in **Fig. 2b** after exposure of 5 days.

(j) The full gel of the radioactively labelled YcgG2 and YcgG2-ATG present in **Fig. 2c** after exposure of 24 hours.

(k) The full gel of the radioactively labelled YcgG2 produced in different concentrations of c-di-GMP present in **Fig. S2f** after exposure of 5 days.

(l) The full gel of the radioactively labelled YcgG2 produced in different concentrations of ppGpp and pppGpp present in **Fig. S2g** after exposure of 5 days.

**a**


Growth in AUM
OD600
10
1
0.1
0.01
0
100
200
300
400
500
Time, min
IHE3034
IHE3034ΔycgG2

**b**

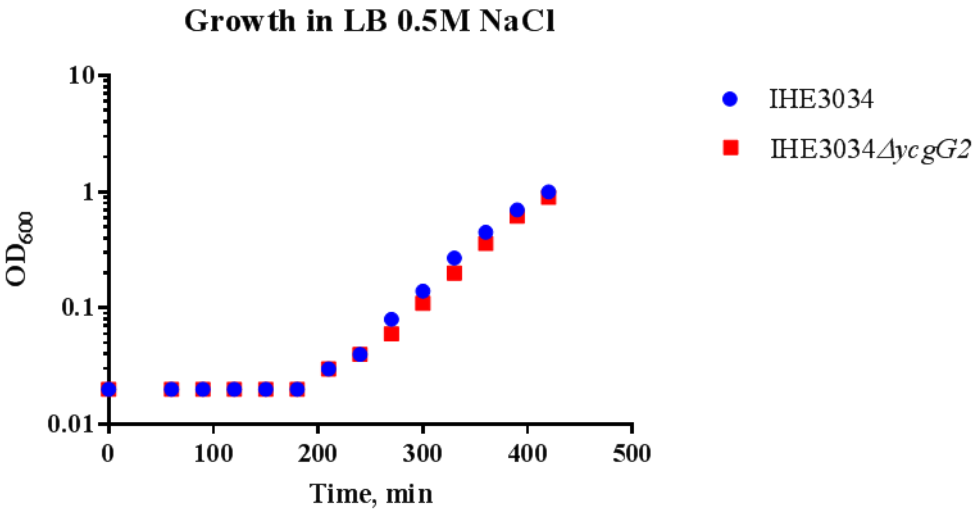
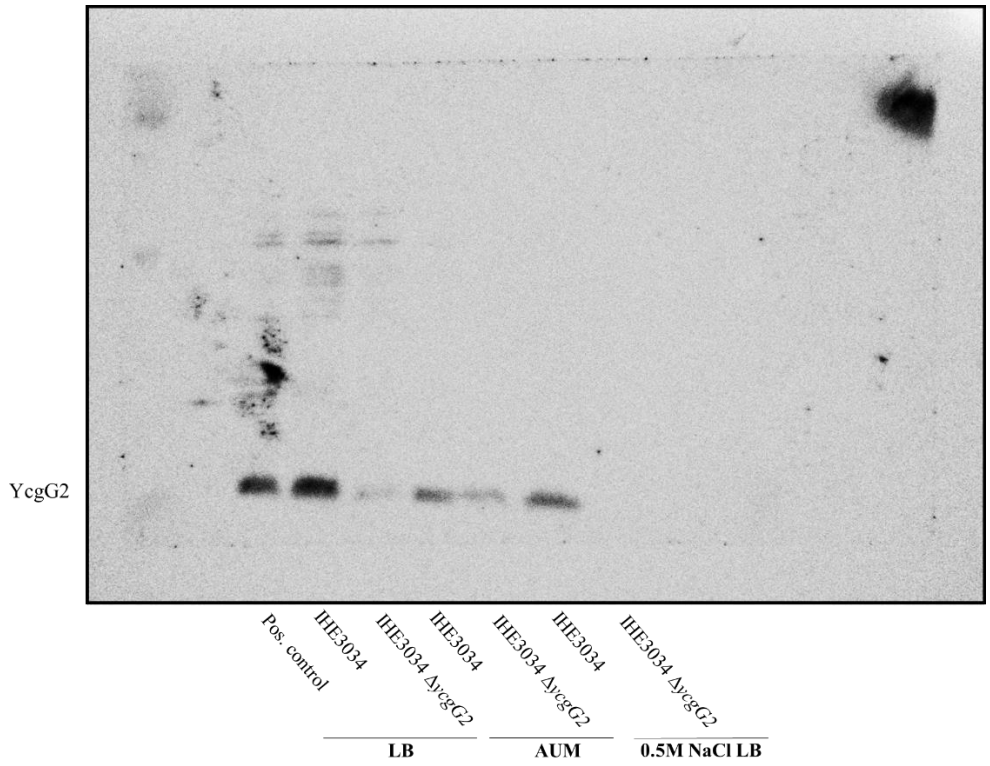

Growth in LB 0.5M NaCl
OD600
10
1
0.1
0.01
0
100
200
300
400
500
Time, min
IHE3034
IHE3034ΔycgG2

Fig. S3

**c**


YcgG2
Pos. control
IHE3034
IHE3034 ΔycgG2
IHE3034
IHE3034 ΔycgG2
IHE3034
IHE3034 ΔycgG2
LB
AUM
0.5M NaCl LB

**d**

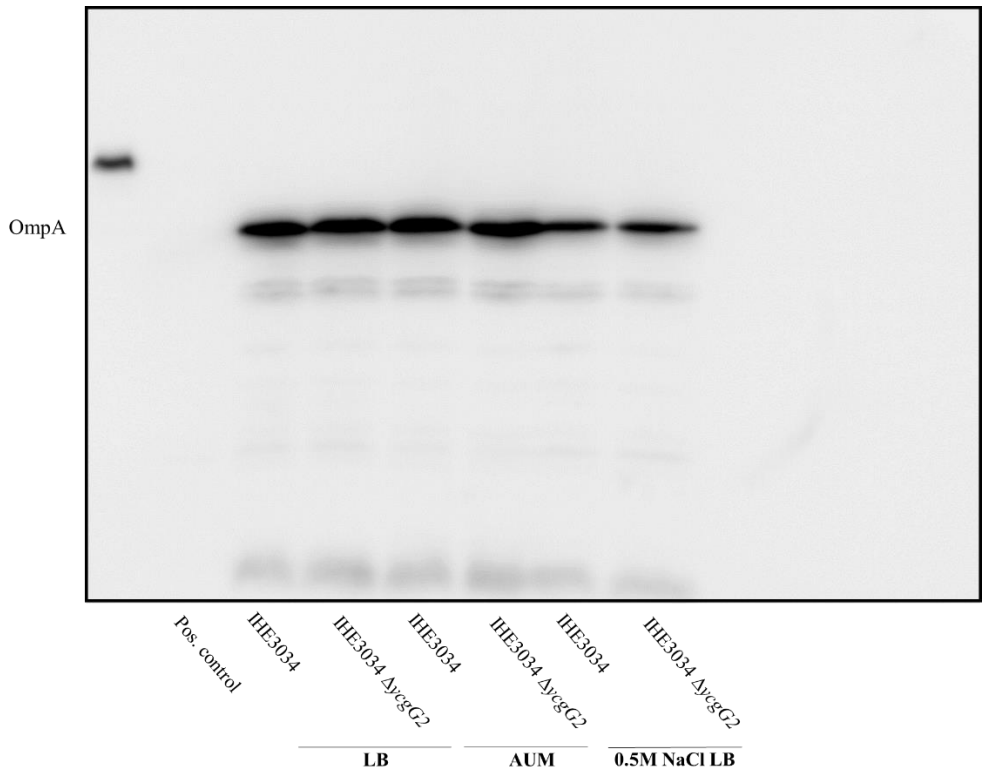
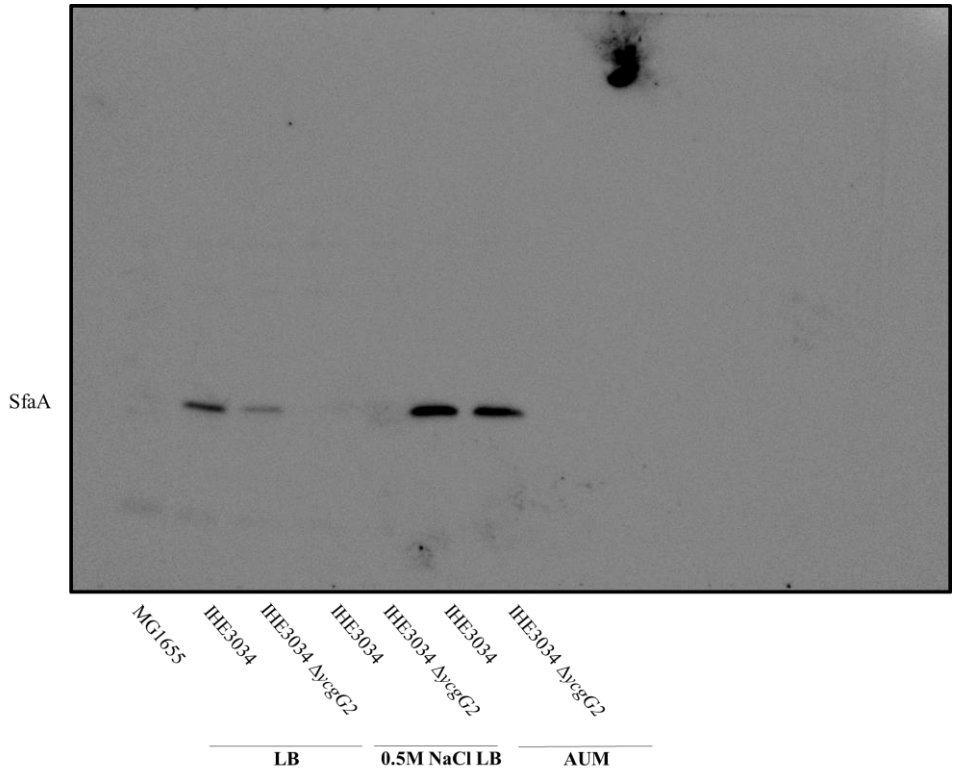

OmpA
Pos. control
IHE3034
IHE3034 ΔycgG2
IHE3034
IHE3034 ΔycgG2
IHE3034
IHE3034 ΔycgG2
LB
AUM
0.5M NaCl LB

Fig. S3

**e**


SfaA
MG1655
IHE3034
IHE3034 ΔycgG2
IHE3034
IHE3034 ΔycgG2
IHE3034
IHE3034 ΔycgG2
LB
0.5M NaCl LB
AUM

**f**

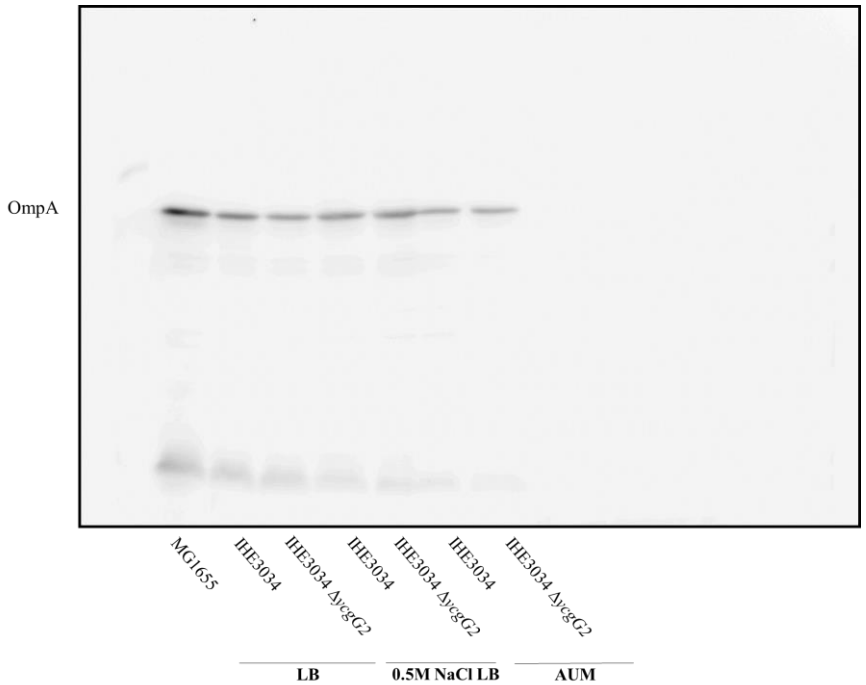
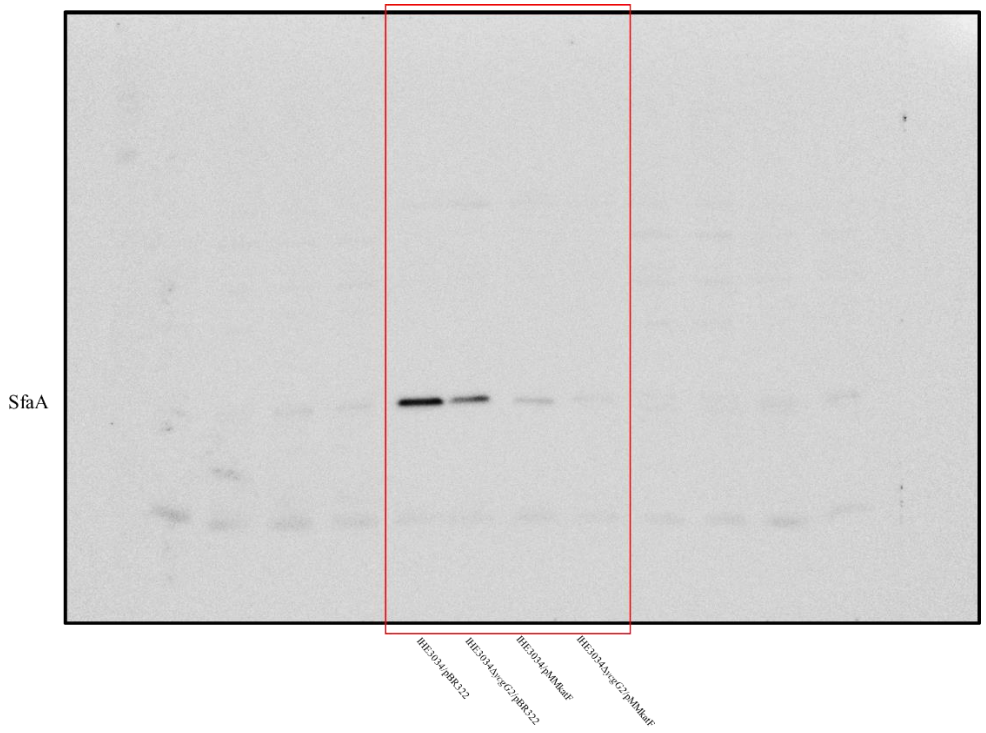

OmpA
MG1655
IHE3034
IHE3034 ΔycgG2
IHE3034
IHE3034 ΔycgG2
IHE3034
IHE3034 ΔycgG2
LB
0.5M NaCl LB
AUM

Fig. S3.

**g**


SfaA
IHE3034/pBR322
IHE3034Δ*vgrG2*/pBR322
IHE3034/pMMdaiF
IHE3034Δ*vgrG2*/pMMdaiF

**h**

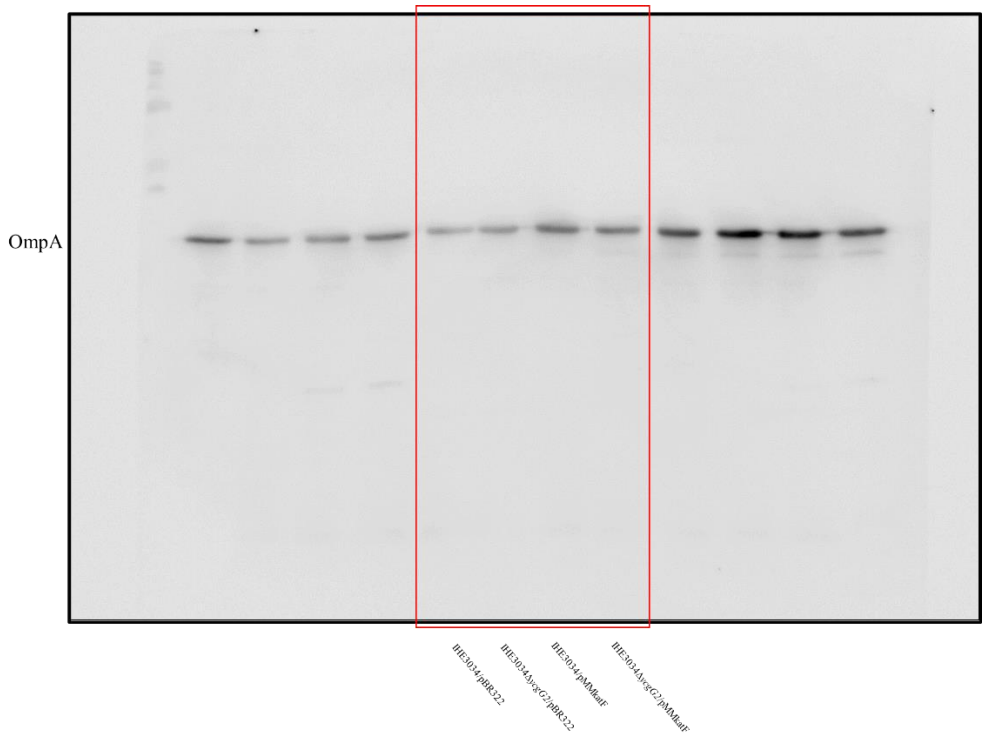

OmpA
IHE3034/pBR322
IHE3034Δ*vgrG2*/pBR322
IHE3034/pMMdaiF
IHE3034Δ*vgrG2*/pMMdaiF

Fig. S3.

**i**

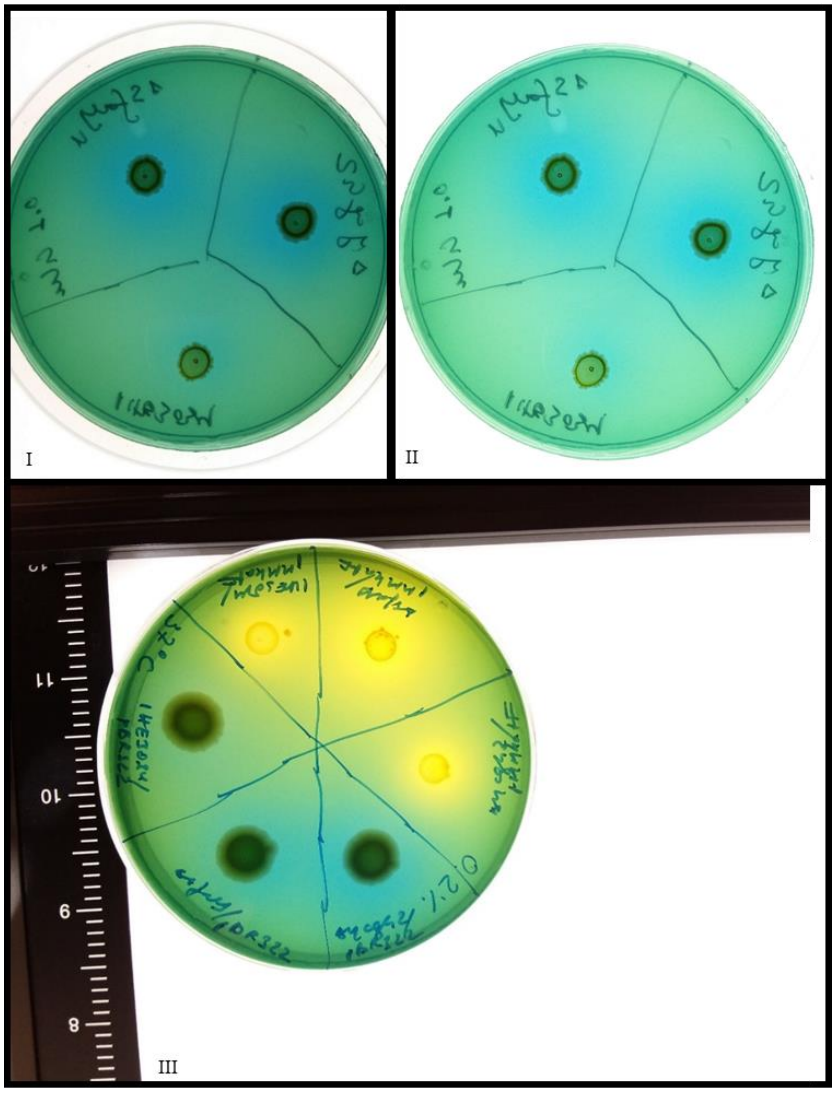



Fig. S3: Properties of the IHE3034 strain and its ycgG2deletion mutant.

No growth difference observed when IHE3034 bacteria and their corresponding Δ*ycgG2* mutant cells were incubated in Artificial Urine Medium (AUM) (a) and in LB supplemented with 0.5M NaCl (b), one representative replica of three independent experiments is shown.

(c) The full membrane of the immunoblot against YcgG2 present in **Fig.3a**.

(d) The full membrane of the immunoblot against OmpA present in **Fig.3a**.

(e) The full membrane of the immunoblot against SfaA present in **Fig. 3b**.

(f) The full membrane of the immunoblot against OmpA present in **Fig.3b.**

(g) The full membrane of the immunoblot against SfaA present in **Fig.3c.**

(h) The full membrane of the immunoblot against OmpA present in **Fig.3c**.

(i) Pictures of Simmon's plates with cropped labelling present in **Fig.3e** (see Box I) and in **Fig. 3f**.

**a**

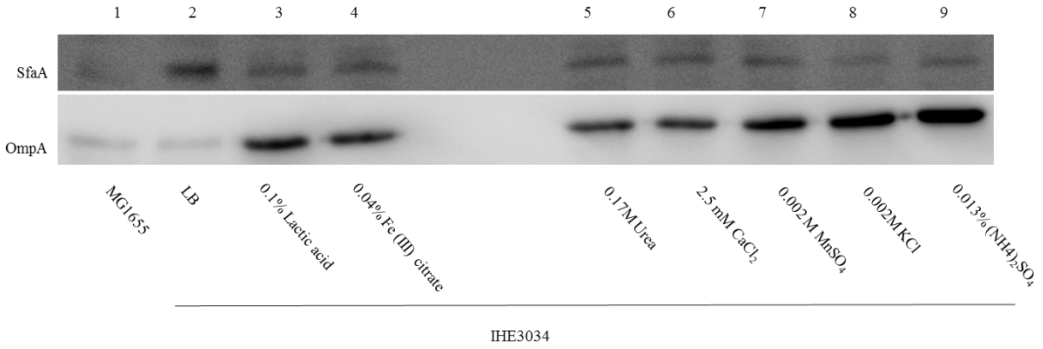
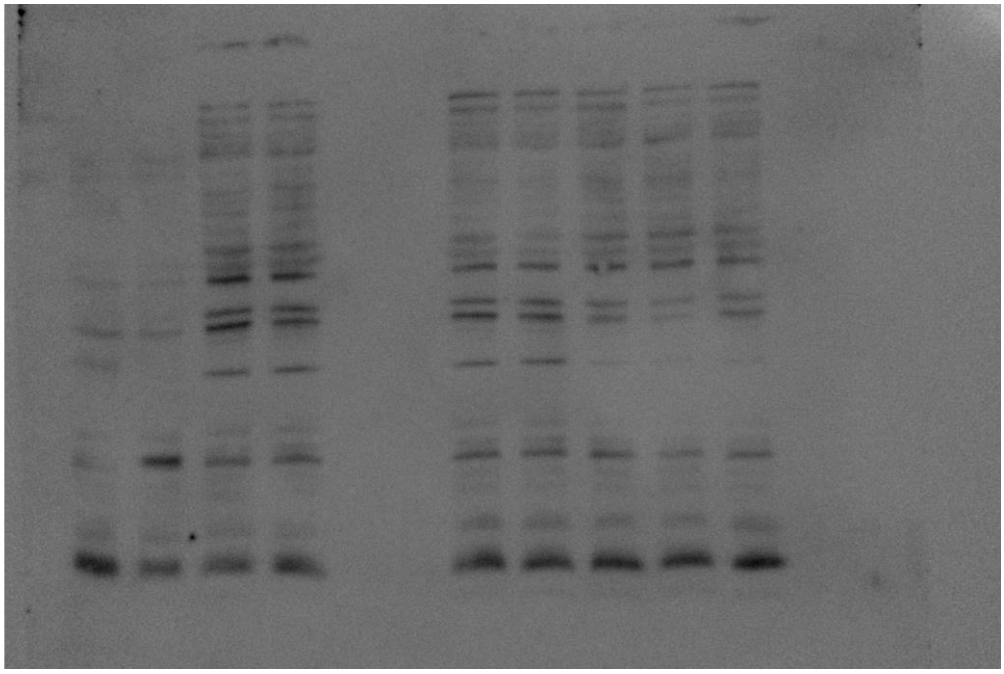

1
2
3
4
5
6
7
8
9
SfaA
OmpA
MG1655
LB
0.1% Lactic acid
0.04% Fe (III) citrate
0.17M Urea
2.5 mM $CaCl_2$
0.002M $MnSO_4$
0.002M KCl
0.013% $(NH4)_2SO_4$
IHE3034

Fig. S4.

**b**




**c**

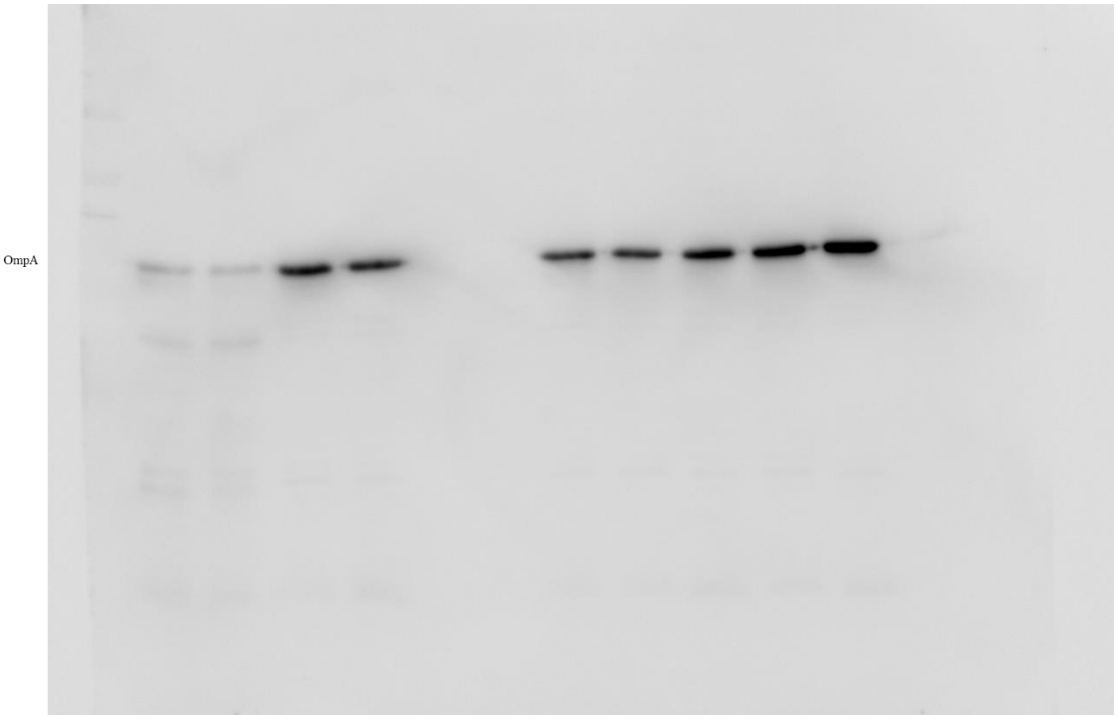



Fig. S4.

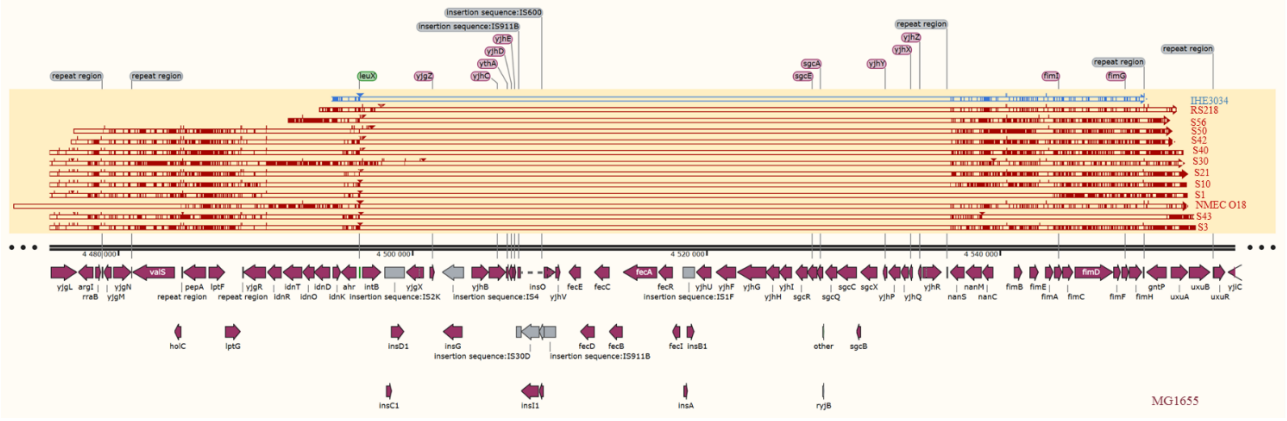
Fig. S4: The impact of the different components of the AUM on SfaA production of IHE3034 bacteria.

(a) SfaA production by IHE3034 bacteria incubated in LB (lane 2, positive control), 0.1% lactic acid (lane 3), 0.04% iron (III) citrate (lane 4), 0.17M urea (lane 5), 2.5 mM $CaCl_2$ (lane 6), 0.002 M $MnSO_4$ (lane 7), 0.002 M KCl (lane 8) and 0.013% ammonium sulfate (lane 9). MG1655 protein extract used as a negative control, OmpA levels served as a loading control.

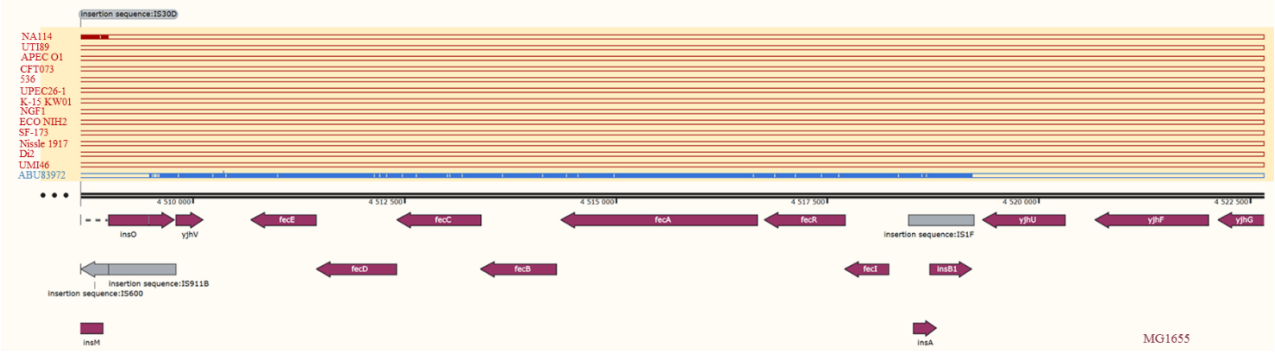
(b) The full membrane of the immunoblot against SfaA present in **Fig. S4a**.

(c) The full membrane of the immunoblot against OmpA present in **Fig. S4a.**

**a**


IHE3034
RS218
S56
S50
S42
S40
S30
S21
S10
S1
NMEC O18
S43
S3
MG1655

**b**


NA114
UTI89
APEC O1
CFT073
536
UPEC26-1
K-15 KW01
NGF1
ECO NIH2
SF-173
Nissle 1917
Dt2
UMI46
ABU83972
MG1655

**c**

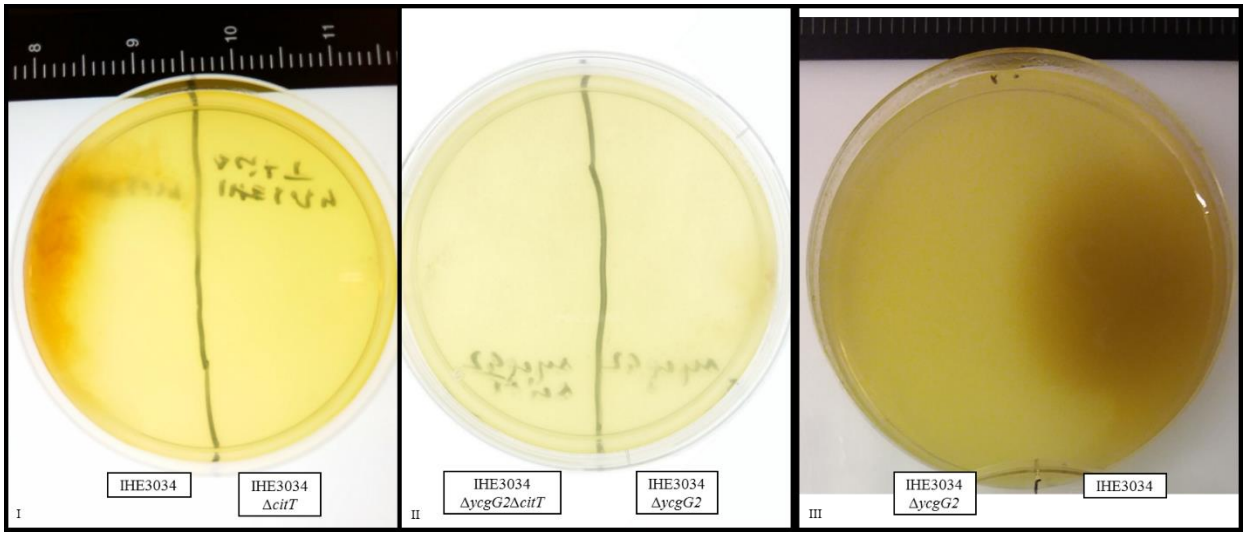

IHE3034
IHE3034 ΔcitT
I
IHE3034 ΔycgG2ΔcitT
IHE3034 ΔycgG2
II
IHE3034 ΔycgG2
IHE3034
III

Fig. S5.

Fig. S5: Graphical representation of the local alignment of the deleted ExPEC *fec* genetic region against the one of MG1655 genome.

(a) Local alignment of NMEC sequences (shown in red, the sequence of IHE3034 shown in blue) against the genome of MG1655 (detailed below).

(b) Local alignment of the uropathogenic and avian pathogenic *E. coli* sequences (shown in red) against the genome (shown below) of MG1655. The alignment of the sequence of ABU83972 showed presence of the *fec* gene cluster (shown in blue).

(c) The full pictures of the plates from ferric citrate *in-vitro* assay depicted in **Fig. 5d**.

298 **Table S1. Indel mutations in the genome of IHE3034F compared to the one of IHE3034**

| IHE3034 Locus/Gene | Position | IHE3034 | IHE3034F |
|---|---|---|---|
| ECOK1_0515/phage integrase gene | 558439 | GTGGGACATATT | G |
| ECOK1_0536/hypothetical gene | 574971 | T | TAA |
| ECOK1_0536/hypothetical gene | 574973 | TGG | T |
| ECOK1_0536/hypothetical gene | 575051 | T | TC |
| ECOK1_0900/*clpA* (pseudogene due to frameshift) | 931694 | CGTAAAGA | C |
| Intergenic region | 1161181 | C | CG |
| Intergenic region | 1161182 | C | CCG |
| ECOK1_2031/*uvrY* | 2054745 | G | GGT |
| Intergenic region | 2714858 | CGAGTCTTGAGTCTT | C |
| ECOK1_3036/hypothetical gene | 3107961 | C | CGTTACCA |
| ECOK1_3620/hypothetical gene | 3716051 | CA | C |
| ECOK1_4442/*btuB* (pseudogene due to frameshift) | 4567043 | A | AC |
| ECOK1_4650/*dcuA* | 4790789 | CG | C |
| ECOK1_4680/*queG* (pseudogene due to frameshift) | 4817885 | C | CA |
| ECOK1_4834/*hyi* (pseudogene due to frameshift) | 4976703 | C | CA |

299

300 **Table S2. SNPs mutations in the genome of IHE3034F compared to the one of IHE3034**

| IHE3034 Locus/Gene | Position | IHE3034 | IHE3034F |
|---|---|---|---|
| ECOK1_0421/*lon* | 463548 | T | G |
| Intergenic region | 558394 | C | A |
| ECOK1_0515/phage site-specific recombinase gene | 558421 | A | T |
| ECOK1_0515/phage site-specific recombinase gene | 558424 | C | T |
| ECOK1_0515/phage site-specific recombinase gene | 558427 | C | T |
| ECOK1_0515/phage site-specific recombinase gene | 558437 | C | G |
| ECOK1_0536/hypothetical gene | 574949 | T | C |
| ECOK1_0536/hypothetical gene | 574963 | C | T |
| ECOK1_0536/hypothetical gene | 574979 | T | C |
| ECOK1_0536/hypothetical gene | 574996 | T | C |
| ECOK1_0536/hypothetical gene | 575003 | G | C |
| ECOK1_0536/hypothetical gene | 575005 | A | C |
| ECOK1_0536/hypothetical gene | 575026 | T | C |
| ECOK1_0536/hypothetical gene | 575028 | T | C |
| ECOK1_0536/hypothetical gene | 575035 | T | C |
| ECOK1_0536/hypothetical gene | 575037 | G | A |
| ECOK1_0536/hypothetical gene | 575038 | G | A |
| ECOK1_0536/hypothetical gene | 575042 | T | C |
| ECOK1_0536/hypothetical gene | 575045 | G | A |
| ECOK1_0536/hypothetical gene | 575063 | C | T |
| ECOK1_0536/hypothetical gene | 575066 | G | A |

| | | | |
|---|---|---|---|
| ECOK1_0536/hypothetical gene | 575081 | G | A |
| ECOK1_0536/hypothetical gene | 575083 | C | T |
| ECOK1_0536/hypothetical gene | 575089 | A | T |
| Intergenic region | 605737 | A | C |
| ECOK1_0657/*gltJ* | 697000 | G | A |
| ECOK1_0989/*mukF* | 1022101 | T | C |
| ECOK1_1066/*putA* | 1108192 | C | A |
| ECOK1_1107/*iroN* | 1151518 | T | C |
| ECOK1_1107/*iroN* | 1151560 | T | C |
| ECOK1_1107/*iroN* | 1151581 | A | T |
| ECOK1_1107/*iroN* | 1151665 | G | A |
| ECOK1_1107/*iroN* | 1151767 | C | A |
| ECOK1_1107/*iroN* | 1152044 | A | G |
| ECOK1_1107/*iroN* | 1152514 | T | C |
| ECOK1_1108/*iroE* | 1154149 | T | C |
| ECOK1_1108/*iroE* | 1154179 | G | A |
| ECOK1_1108/*iroE* | 1154433 | A | G |
| ECOK1_1109/*iroD* | 1154671 | T | C |
| ECOK1_1109/*iroD* | 1154728 | C | T |
| ECOK1_1109/*iroD* | 1155093 | T | C |
| ECOK1_1109/*iroD* | 1155094 | G | A |
| ECOK1_1109/*iroD* | 1155388 | G | A |
| ECOK1_1109/*iroD* | 1155758 | G | A |
| ECOK1_1110/ABC transporter gene | 1155926 | C | A |
| ECOK1_1110/ABC transporter gene | 1155974 | T | C |
| ECOK1_1110/ABC transporter gene | 1156187 | A | G |
| Intergenic region | 1160966 | A | G |
| Intergenic region | 1160977 | G | A |
| Intergenic region | 1161036 | C | T |
| Intergenic region | 1161165 | C | T |
| Intergenic region | 1161189 | T | A |
| Intergenic region | 1161197 | C | T |
| ECOK1_1112/hypothetical gene | 1161357 | C | A |
| ECOK1_1112/hypothetical gene | 1161562 | C | A |
| ECOK1_1112/hypothetical gene | 1161573 | G | T |
| ECOK1_1112/hypothetical gene | 1161575 | G | A |
| ECOK1_1112/hypothetical gene | 1161576 | T | C |
| ECOK1_1301/*sitD* | 1326729 | A | G |
| ECOK1_1301/*sitD* | 1326803 | C | T |
| ECOK1_1301/*sitD* | 1326817 | T | C |
| ECOK1_1301/*sitD* | 1326872 | G | A |
| ECOK1_1302/*sitC* | 1328078 | A | G |
| ECOK1_1303/*sitB* | 1328118 | A | T |

| | | | |
|---|---|---|---|
| ECOK1_1303/*sitB* | 1328313 | T | C |
| ECOK1_1303/*sitB* | 1328373 | G | C |
| ECOK1_1303/*sitB* | 1328510 | T | C |
| ECOK1_1303/*sitB* | 1328547 | T | C |
| ECOK1_1303/*sitB* | 1328646 | A | C |
| ECOK1_1304/*sitA* | 1328984 | C | A |
| ECOK1_1304/*sitA* | 1329032 | G | A |
| ECOK1_1304/*sitA* | 1329041 | T | C |
| ECOK1_1304/*sitA* | 1329280 | T | C |
| ECOK1_1304/*sitA* | 1329341 | G | A |
| ECOK1_1304/*sitA* | 1329683 | C | A |
| ECOK1_1304/*sitA* | 1329704 | C | T |
| ECOK1_1553/auxiliary transport protein gene | 1564992 | G | C |
| ECOK1_2161/hypothetical gene | 2187637 | C | T |
| ECOK1_2162/tRNA-Asn gene | 2187821 | A | G |
| ECOK1_3005/hypothetical gene | 3083132 | A | G |
| ECOK1_3009/phage N-6-adenine-methyltransferase gene | 3085261 | C | T |
| ECOK1_3119/*rpoS*, pseudogene | 3182422 | G | C |
| ECOK1_3120/*rpoS* (*5*' end of the gene before the stop codon) | 3182582 | C | A |
| ECOK1_3464/hypothetical gene | 3559184 | A | G |
| ECOK1_3519/*sstT* | 3620440 | G | A |
| ECOK1_3819/*ompR* | 3901439 | T | A |
| ECOK1_4122/*uhpA* | 4229388 | C | G |

313 **Table S3. Bacterial strains used in this work**

| Strain | Description/Relevant characteristics | Reference/Source |
|---|---|---|
| *Escherichia coli* strains | | |
| MC4100 | F$^-$ , *(argF-lac)UI69, araOl39, rpsL 150, ptsF25, flbB5301, rbsR, deoC, relA* | [1] |
| RH90 | MC4100 *rpoS359::*Tn10 | [2] |
| BW25113 | F- **,** *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *λ*$^-$ , *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514* | [3,4] |
| JW0604-1 | BW25113Δ*citT750::kan* | [4] |
| MG1655 | *E. coli* K-12 wild type | [5] |
| IHE3034 | Clinical NMEC isolate, O18:K1:H7 | [6] |
| MAM20 | Δ*ycgG2::cat* mutant of IHE3034 | This work |
| AES1 | Δ*sfaX::aph* mutant of IHE3034 | [7] |
| AES153 | Δ*sfaY* mutant of IHE3034 | This work |
| NZ7 | AES153/pBR322 | This work |
| NZ11 | IHE3034/pBR322 | This work |
| NZ67 | IHE3034/pMMkatF | This work |
| NZ70 | AES153/pMMkatF | This work |
| NZ215 | IHE3034Δ*ycgG2/*pBR322 | This work |
| NZ216 | IHE3034Δ*ycgG2/*pMMkatF | This work |
| NZ217 | IHE3034Δ*citT::kan* | This work |
| NZ218 | IHE3034Δ*ycgG2*Δ*citT::kan* | This work |
| 536 | Clinical UTI pyelonephritis isolate, O6:K15:H31 | [8] |
| 536Δ102 | 536 (*leuX*$^-$) | [9] |
| 536-21 | 536 (PAI I$^-$, PAI II$^-$, *selC*$^-$, *leuX*$^-$) | [10] |

| | | |
|---|---|---|
| 536R3 | 536 (PAI I$^-$, PAI II$^-$, *selC*$^-$) | [11] |
| NZ225 | 536-21Δ*rpoS::Tn10* | This work |
| NZ226 | 536R3Δ*rpoS::Tn10* | This work |
| UTI89 | Clinical UTI cyctitis isolate, O18:K1:H7 | [12] |
| NZ219 | UTI89Δ*rpoS::Tn10* | This work |
| RS218 | Clinical NMEC isolate, O18:K1:H7 | [13] |
| DH5α | *ednA1 hsdR17 supE44 thi-1 recA1 gyrA relA1Δ(lacZ4A-argF)* | [14] |
| IHE1041 | Clinical UTI pyelonephritis isolate;<br>O1:K1:H7 | Prof. Timo Korhonen,<br>University of Helsinki, Finland |
| IHE1049 | Clinical UTI pyelonephritis isolate;<br>O1:K1:H7 | Prof. Timo Korhonen,<br>University of Helsinki, Finland |
| IHE1402 | Clinical UTI pyelonephritis isolate;<br>O6:K2:H1 | Prof. Timo Korhonen,<br>University of Helsinki, Finland |
| IHE1190 | Clinical UTI pyelonephritis isolate;<br>O18:K5:H7 | Prof. Timo Korhonen,<br>University of Helsinki, Finland |
| IHE1268 | Clinical UTI pyelonephritis isolate;<br>O18:K5:H7 | Prof. Timo Korhonen,<br>University of Helsinki, Finland |
| *Vibrio cholerae* strains | | |
| C6706 | El Tor, Inaba, Str$^R$ | [15] |
| C6706*luxO*$^c$ | C6706, LuxO (L104Q) constitutively active | [16] |
| NZ44 | C6706*luxO*$^c$/pBAD18 | This work |
| NZ92 | C6706*luxO*$^c$/pNZK16 | This work |
| NZ93 | C6706*luxO*$^c$/pNZK17 | This work |

314

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**Table S4. Plasmids used in this work**

| Plasmid | Description/Relevant characteristics | Reference/Source |
|---|---|---|
| pBAD18 | $Cb^R$, ori ColE1, inducible $P_{BAD}$ promoter | [17] |
| pBR322 | $Cb^R$, $Tc^R$, *rep* ori (pMB1), cloning vector | [18] |
| pMMkatF | $Cb^R$, 4.2-kb subclone of $rpoS_{EC}$ in pAT153 | [19] |
| pNZK16 | $Cb^R$, pBAD18, *ycgG* from MG1655 | This work |
| pNZK17 | $Cb^R$, pBAD18, *ycgG2* from IHE3034 | This work |
| pSIM6 | $Cb^R$, ori SC101, temperature sensitive, carries the α-red recombinase genes, temperature inducible | [20] |
| pKD3 | $Cm^R$, ori R6Kγ, template for generation of cat fragment flanked with FRT sites. | [3] |
| pKO3 | $Cm^R$, ori M13, *sacB*, $repA^{ts}$, suicide vector | [21] |

334 **Table S5. Primers used in this work**

| **Primer:** | **Sequence (5'->3'):** |
|---|---|
| *ycgG2*delFw | TTTATTCTGTTAATCGCCTGCGCCGCTGCCTTCCTGCTTGTGTGTAGGCTGGAGCTGCTTC |
| *ycgG2*delRv | TGTCTATAATTACACCGCACCATGGCCTTGCTGATCAATTTTCACATATGAATATCCTCCTTAG |
| 1312Fw | AGAGCTCATTCTGCCTACATTGTGCTAAAAAATTAA |
| *ycgG2*dwdelRv | CCGGATCCTGCCAGCAACCCATTCAGCCGCATACTGGCAGTGAGAAAC |
| EAL6up | GCGGATCCGGCGGTAATGATATTAC |
| EALdelendup | CTCTATATTGAGACTCTGCAGCAGGGATAATCCTTTTTCACAGAC |
| SfaII-3 | CGGTCGACGGATCAGCATCACTAGG |
| EALdelstartdo | CTGCTGCAGAGTCTCAATATAGAGTGACATTACTCCTCCGG |
| Fw*sfaH*dw | GTGACTTTTAGCTACAACTAGAATGCAG |
| dd*sfaX*Rv | ATGCGGCCGCTGATAATCAATATCATTTAGCAAAAGAAAAAGCAA |
| *ycgG*K-12Fw | TGAGCTCCAAGCAAATGCGCAATACAC |
| *ycgG*K-12Rv | GGGAAGCTTTCACTCAACCACAACCTTCAC |
| *ycgG*K1FW | TGAGCTCTGGGATTTTGATATTTATTCTGTTAATCGCCTGC |
| *ycgG*K1RV | GGGAAGCTTTCACTCAACCACAATCTTCACCTTCG |
| pBAD18FW | ATGCCATAGCATTTTTATCC |
| pBAD18RV | GATTTAATCTGTATCAGG |
| T7PmRBS*ycgG*K-12Fw | GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAA GGATATACCAATGCGCAATACACTCATACCCATC |
| ComplRV*ycgG* | AAGCTTTCACTCAACCACAACCTTCACCTTCGGTTTAGAAAGGATA |
| T7PmRBS*ycgG*2K1Fw | GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAA GGATATACCATTGATATTTATTCTGTTAATCGCCTGC |
| up*citT*Fw | AAGCACTTGATAAATTTGGAAATATTAATTTTCGGAGA |
| dw*citT*Rv | GGGTGTGGAACTCATACATACACTGAA |
| 3120FW | ATGAGTCAGAATACGCTGAAAGTTCA |

| | |
|---|---|
| *rpoS*Rv | TTATTCGCGGAACAGCGCTTCGATATTCA |

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