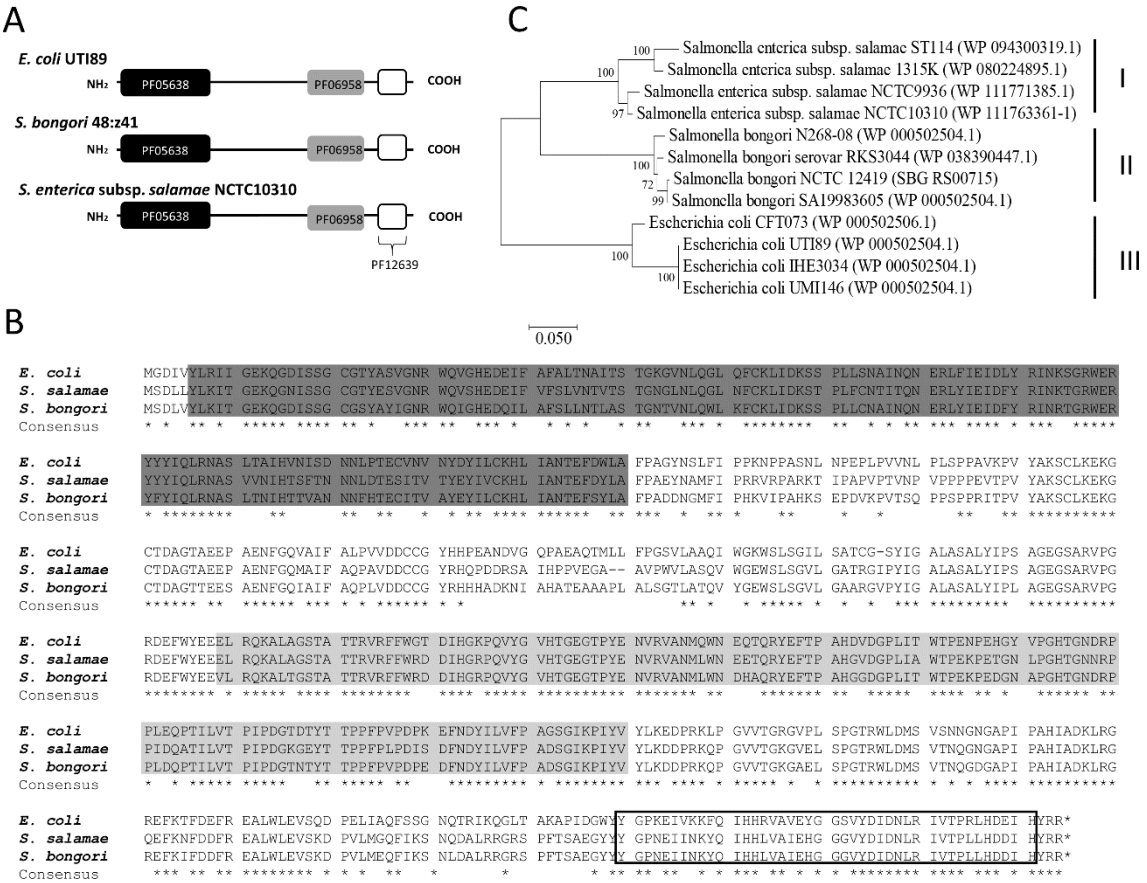


**Figure S1.** Usp homologs encoded by PAI*usp*. (A) Schematic representation of Usp proteins encoded by PAI*usp* from *E. coli* UTI89 (Accession no. WP_000502504.1), *S. bongori* serovar 48:z4:-- str. RKS3044 (Accession no. WP_038390447.1) and *S. enterica* subsp. *salamae* NCTC10310 (Accession no. WP_111763361.1). Black, light grey and white boxes represent conserved domains PF05638 (Hcp domain), PFO6958 (S-type pyocin domain) and PF12639 (colicin nuclease domain), respectivly. (B) Protein sequence comparison between Usp homologs of *E. coli* UTI89, *S. bongori* serovar 48:z4:-- str. RKS3044 and *S. enterica* subsp. *salamae* NCTC10310. Usp protein sequences were aligned using the multiple sequence alignment tool ClustalW. Identical and conserved residues are indicated below the alignment by an asterix. Dark grey, light grey and white boxes represent Hcp, S-type and colicin nuclease domain, respectively. (C) The neighbor-joining phylogenetic tree (bootstrap n = 1000; Poisson correction; Complete gap deletion; Midpoint rooted) showing the phylogenetic relationship between the Usp proteins from various species. Clades are indicated by large Roman numerals.

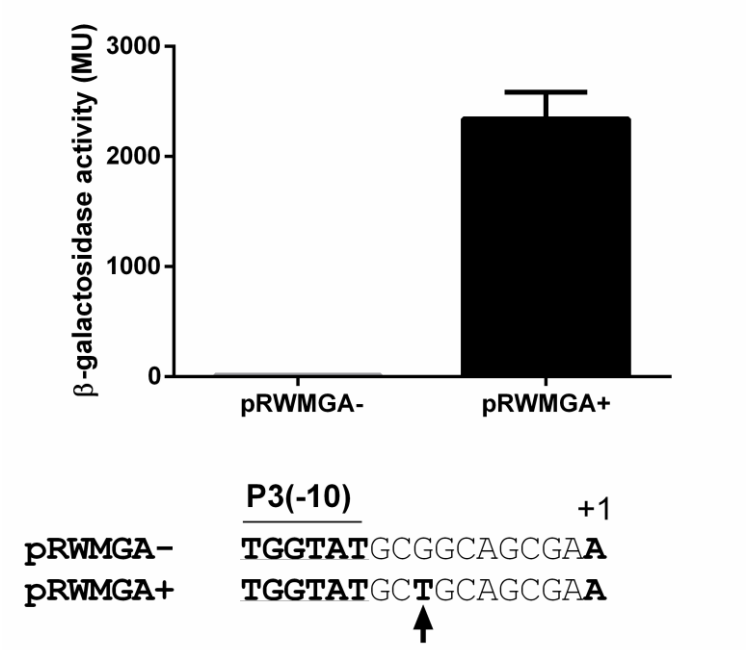


**Figure S2.** Isolation of *E. coli* strain conveying high P3 promoter activity on MacConkey agar plates. *E. coli* containing pRWAro- plasmid (*aroP* P3 promoter from *E. coli* K-12 MG1655 which does not harbor PAI*usp*, fused with *lacZ* gene) was plated on MacConkey agar. Remarkably, within 2-3 days of growth we observed some red colonies from which plasmid DNA was isolated and the promoter region sequenced promoter. Surprisingly, sequencing revealed a single modification in the discriminator region; -8G to T substitution, relative to the transcription start point (the new plasmid was termed pRWAro+). Subsequently, we compared non-mutated, wild type (pRMGA-) and modified (pRMGA+) promoter P3 activity on the basis of β-galactosidase activity. Units for β-galactosidase assay are those defined by Miller, 1974. Error bars represent standard deviations of the means of two independent experiments. Position of the spontaneous mutation is indicated with an arrow.

**Table S1** PAI*usp* containing strains identified by PSI-BLAST and tBLASTn analysis, excluding *E. coli* strains.

| Organisms containing PAIusp | Strain | Genome RefSeq ID | Usp protein accession number or locus tag |
|---|---|---|---|
| *Salmonella bongori* | NCTC 12419 | NC_015761.1 | SBG_RS00715 |
| *Salmonella bongori* | N268-08 | NC_021870.1 | AGR57335.1 |
| *Salmonella bongori* serovar 48:z41:-- | RKS3044 | NZ_CP006692.1 | WP_038390447.1 |
| *Salmonella bongori* serovar 66:z41:- | SA19983605 | NZ_CP022120.1 | LFZ56_RS17695 |
| *Salmonella bongori* serovar 44:z39:- | BCW_1554 | NZ_MXOF01000001.1 | WP_079777515.1 |
| *Salmonella enterica* subsp. *salamae* serovar 57:z29:z42 | ST114 | NZ_CP022467.1 | WP_094300319.1 |
| *Salmonella enterica* subsp. *salamae* | NCTC9930 | NZ_LS483456.1 | DQN98_RS21100 |
| *Salmonella enterica* subsp. *salamae* | RKS2993 | NZ_JXTT01000055 | WP_046094099.1 |
| *Salmonella enterica* subsp. *salamae* serovar Greenside | NCTC9936 | NZ_LS483475.1 | WP_111771385.1 |
| *Salmonella enterica* subsp. *salamae* | NCTC10310 | NZ_LS483477.1 | WP_111763361.1 |
| *Salmonella enterica* subsp. *salamae* serovar 55:k:z39 | 1315K | NZ_CP022139.1 | WP_080224895.1 |
| *Salmonella enterica* subsp. *salamae* serovar 58:d:z6 | 327-85 | NZ_MXLI01000001.1 | WP_079805736.1 |
| *Salmonella enterica* subsp. *salamae* serovar 50:b:z6 | BCW_1520 | NZ_MXPI01000002.1 | WP_079818387.1 |
| *Salmonella enterica* subsp. *salamae* serovar 48:d:z6 | BCW_1519 | NZ_MXPJ01000002.1 | WP_079814527.1 |
| *Salmonella* enterica subsp. *salamae* | NCTC10436 | NZ_LS483428.1 | WP_111753446.1 |
| *Salmonella enterica* subsp. *salamae* | NCTC9948 | LS483495.1 | SQJ44863.1 |
| *Salmonella enterica* subsp. *salamae* | NCTC9930 | LS483456.1 | SQH95318.1 |

| Strains or plasmids | Relevant charasteristic(s)[a] | Reference |
|---|---|---|
| Strains | | |
| *E. coli* DH5α | Cloning host; F– Φ80*lac*ZΔM15 Δ(*lac*ZYA-argF) U169 *rec*A1 *end*A1 *hsd*R17 (rK–, mK+) *pho*A *sup*E44 λ– *thi*-1 *gyr*A96 *rel*A1 | Sambrook *et al.* (1989) |
| *E. coli* BL21 (DE3) | F- *omp*T *hsd* $S_B$($r_B$-$m_B$-) *dcm gal*λ (DE3) | Promega |
| *E. coli* JW1889 | *F-Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *λ-*, *ΔaraF751::kan*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514* | Baba *et al.* (2006) |
| *E. coli* JW1316 | *F-Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *λ-*, *ΔtyrR760::kan*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514* | Baba *et al.* (2006) |
| *E. coli* TA211 | wild-type strain (UPEC isolate) | IMI |
| *S.bongori* NCTC 12419 | wild-type strain | ATTC |
| Plasmids | | |
| pRW50 | $Tc^R$; Low-copy-number promoterless *lac*Z trancriptional fusion vector | Lodge *et al.* (1992) |
| pRWBP | $Tc^R$; 403 bp *Eco*RI-*Hind*III fragment from *S. bongori* NCTC 12419 *aro*P-*usp* intergenic region: *usp-lac*Z transcription fusion | This study |
| pRWBPM10 | $Tc^R$; pRWBP derivate; P3 (-35) mutation: CACTCT-->CA**AG**CT | This study |
| pRWBPM35 | $Tc^R$; pRWBP derivate; P3 (-10) mutation: TGGTAT-->T**TAATG** | This study |
| pRCPL | $Tc^R$; 527 bp EcoRI-HindIII fragment from *E. coli* TA211 *aroP-usp* intergenic region: *usp-lacZ* trancription fusion | This study |
| pRWC10 | $Tc^R$; pRCPL derivate; P3 (-10) mutation: TGGTAT-->T**TAATG** | This study |
| pRWC35 | $Tc^R$; pRCPL derivate; P3 (-10) mutation: TGGTAT-->T**TAATG** | This study |
| pJCAT | $Ap^R$; High-copy-number promoterless *cat* trancription fusion vector (pJET1.2 cloning vector (Thermo Fisher Scientific) derivate) | This study |
| pJBLC | $Ap^R$; 403 bp *Pst*I-*Bst*EII fragment from *S. bongori aroP-usp* intergenic region: *usp-cat* transcription fusion | This study |
| pJBSC | $Ap^R$; pJBLC derivate; 247 bp fragment withouth both TyrR binging boxes | This study |
| pJBTC | $Ap^R$; pJBLC derivate; 315 bp fragment withouth weak TyrR binding box | This study |
| pJBTD2 | $Ap^R$; 111 bp PstI-BstEII fragment from S. bongori aroP-usp intergenic region only with strong TyrR box and P3 promoter | This study |
| pJBTD3 | $Ap^R$; pJBTD2 derivate: 111 bp fragment extended for 64 bp | This study |
| pJCLCH | $Ap^R$; High-copy number plasmid with N-terminally $His_6$-tagged Cat under *E.coli* TA211 *usp* promoter | This study |
| pET8TH | $Ap^R$; pET8c -derived expression vector with $P_{lacUV5}$ promoter: expression and production of recombinant N-terminally $His_6$-tagged TyrR | This study |

[a] - abbreviations: Tc, tetracycline; Ap, ampicillin; R, resistance; ATCC, American Type Culture Collection; IMI

**Table 2.** Usp levels from *E. coli* and *S. bongori usp* promoters in w.t. *E. coli* and isogenic *hns* defective strain.

| *E. coli* | | |
|---|---|---|
| 25°C | w.t. | 2.0 |
| | *hns*- | 35.4 |
| 37°C | w.t | 9.4 |
| | *hns*- | 26.4 |
| 42°C | w.t. | 9.4 |
| | *hns*- | 34 |

| *S. bongori* | | |
|---|---|---|
| 25°C | w.t. | 0.24 |
| | *hns*- | 1.5 |
| 37°C | w.t | 2.0 |
| | *hns*- | 14 |
| 42°C | w.t. | 1.9 |
| | *hns*- | 24 |

Top, Usp from *E. coli* promoter; $10^8$ cells (w.t. and *hns*- defective) grown at various temperatures. Bottom, Usp from *S. bongori* promoter; $10^8$ cells (w.t. and *hns*- defective) grown at various temperatures. .