Episomal F TraR activates the σ^E-dependent stress pathway to anticipate conjugational stress
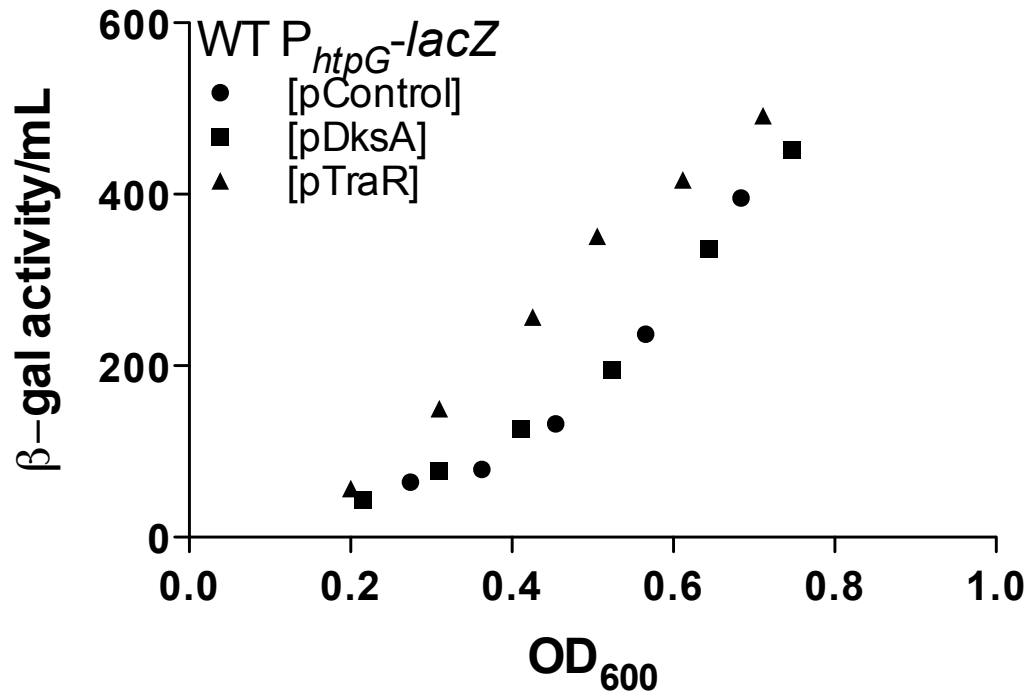
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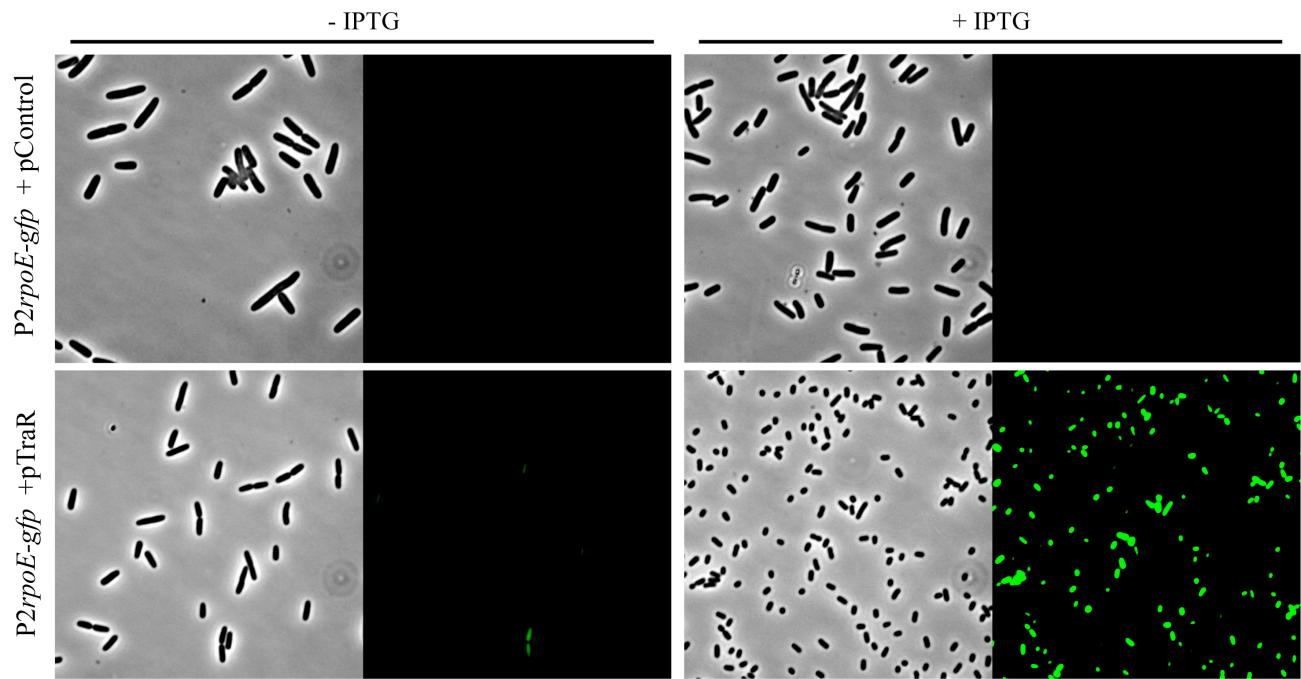
Supplemental – Strain Table

Name	Genotype or Relevant Genotype	Reference or Source
Plasmids		
pR1	Amp^R Kn^R Cm^R	O. Garcia (Pasteur)
pR1*drd19*	Amp^r Km^r Cm^r Sm^r IncFII *finO*	O. Garcia (Pasteur)
pControl	pBA169 Amp^R	(1)
pDksA	pBA169/*dksA* Amp^R	(2)
pTraR	pBA169/*traR* Amp^R	(2)
pOX38	IncFI, *tra$^+$ finO$^-$*, RepFIA$^+$, f1 *Hin*dIII fragment of F	(3)
F'	pOX38 Kn^R	(2)
F'Δ*traR*	pOX38 Δ*traR*::FRT Kn^R	(2)
pUA E16	P2$_{rpoE}$-*gfp-kan*	Carol Gross
pCP20	FLP recombinase vector	(4)
pKD46	Vector used for linear gene replacement	(4)
pSEB015	Plasmid used for in vitro transcription Amp^R	(5)
Parental strains		
MG1655	F$^-$ λ$^-$ *ilvG$^-$ rfb-50 rph-1*, sequenced wild-type K12	(6)
CF9240	MG1655 Δ*dksA*::Tet^R	Michael Cashel
CAG39758	MG1655 Δ*lacIZ* λ P3$_{rpoH}$-*lacZ*	(7)
MC1061	*araD139* Δ*(araA-leu)7697* Δ*lacX74 galK16 galE15(galS)* λ$^-$ *e14$^-$ mcrA0 relA1 rpsL150(strR) spoT1 mcrB1 hsdR2*	
CAG33315	MC1061 λP3$_{rpoH}$-*lacZ* Δ*degS*	(8)
CH1385	MG1655 Δ*rseA*::Tet^R	(9)
CH1399	39758 x P1 CH1385	This study
CAG55816	MG1655 pUA E16	Carol Gross
Experimental Strains		
CH3060	MG1655 Strep^R [pR1]	This study
CH3061	MG1655 Strep^R [pR1*drd19*]	This study
CH3095	CF9240 [pR1]	This study
CH3096	CF9240 [pR1*drd19*]	This study
CH1329	39758 [pTraR]	This study
CH1330	39758 [pDksA]	This study
CH1331	39758 [pControl]	This study
CH1037	MG1655 [pTraR]	(Blankschien *et al*., 2009)
CH1039	MG1655 [pControl]	(Blankschien *et al*., 2009)
CH1474	CAG33315 [pTraR]	This study
CH1475	CAG33315 [pDksA]	This study
CH1476	CAG33315 [pControl]	This study
CH1410	CH1399 [pTraR]	This study
CH1411	CH1399 [pDksA]	This study
CH1412	CH1399 [pControl]	This study
CH4203	CAG55816 [F']	This study
CH4204	CAG55816 [F'Δ*traR*]	This study
CH5684	CAG55816 [pControl]	This study
CH5686	CAG55816 [pTraR]	This study
CH1018	MG1655 [F']	(Blankschien *et al*., 2009)
CH2728	MG1655 [F'Δ*traR*]	(Blankschien *et al*., 2009)

F' 1 G T G A G T G A T G A A G C C G A T G A A G C A T A T T C A G T G A C A G A A C A A C T G A C C A T
        | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | # | | | | | | | | | | | | | | | | | |
pR1 1 G T G A G T G A T G A A G C C G A T G A A G C A T A T T C A G T A A C A G A A C A A C T G A C C A T

F' 51 G A C A G G A A T A A A C C G G A T A C G C C A G A A A A T A A A T G C T C A T G G T A T T C C T G
         | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
pR1 51 G A C A G G A A T A A A C C G G A T A C G C C A G A A A A T A A A T G C T C A T G G T A T T C C T G

F' 101 T T T A T C T C T G T G A A G C A T G C G G A A A T C C T A T T C C G G A A G C C C G G C G G A A A
          | | | | | | | | | | | | | | | | | | | | | | # | | | | | | | | | | | | | | | | | | | | | | | | | | | |
pR1 101 T T T A T C T C T G T G A A G C A T G C G G T A A T C C T A T T C C G G A A G C C C G G C G G A A A

F' 151 A T A T T T C C C G G T G T G A C G T T G T G C G T T G A A T G T C A G G C G T A T C A G G A A A G
          | | | | | | | | | | | | | | | | | | | | | | # | | | # | | | | | | | | | | | | | | | | | | | | | | | |
pR1 151 A T A T T T C C C G G T G T G A C G T T A T G T G T T G A A T G T C A G G C G T A T C A G G A A A G

F' 201 A C A G A G A A A A C A T T A T G C A T A A
          # | | | | | | | | | | | | | | | | | | | |
pR1 201 G C A G A G A A A A C A T T A T G C A T A A

Supplemental Figure 1

Sequence alignment of *traR* between F' and pR1 plasmids. These nucleotide changes do not lead to any amino acid substitutions.

Supplemental Figure 2

The σ^{32}-dependent promoter *hptG* is modestly and transiently activated by induction of TraR.

Beta-galactosidase activity of the P*htpG-lacZ* promoter fusion was measured in LB at 32°C with or without

TraR induction (0.1mM IPTG). The rate of β-gal synthesis between OD600 of 0.2 and 0.4 are the

following: pControl= 377.5 (R2-0.91), pDksA = 422.1 (R2-0.99), pTraR= 886.0 (R2-0.99). The rate of

β-gal synthesis between OD600 of 0.4 and 0.8 is 1071.0 for pControl (R^2- 0.99), 977.8 for pDksa

(R^2- 0.990) and 707.8 for pTraR (R^2- 0.976). Rates of beta-galactosidase activity in exponential phase,

from OD600 0.2 to 0.8, are 875.1 (pControl), 804.2 (pDksA), 806.4 (pTraR). Data shown are

representative of three independent experiments.

Supplemental Figure 3

Artificial induction of TraR alone is sufficient to increase σ^E activity. Microscopy images from cultures grown at 32°C in LB and sampled at log phase (OD_{600} 0.4 − 0.5) with or without IPTG, left and right respectively. Strains contain a chromosomal P2$_{rpoE}$-*gfp* fusion with either pControl (bottom) or pTraR (top). Images shown are phase contrast (left) and GFP fluorescence (right) and were taken at identical light and fluorescence exposures. Data shown are representative of three independent experiments.

Supplemental References

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