SUPPLEMENTARY INFORMATION

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Supplementary Methods

Concentrating indole with C18 columns

Indole production in *E. coli* occurs mostly during the transition from exponential to stationary phase[1]. To measure the low concentration of indole accurately during lag and exponential phase, a pre-concentration step was added before the Kovacs assay, using C18 solid phase extraction (SPE) cartridges (SampliQ C18, Agilent, CA, USA). C18 SPE cartridges concentrate low quantities of non-polar analytes, and their use for concentrating various indole analogues has already been established[2].

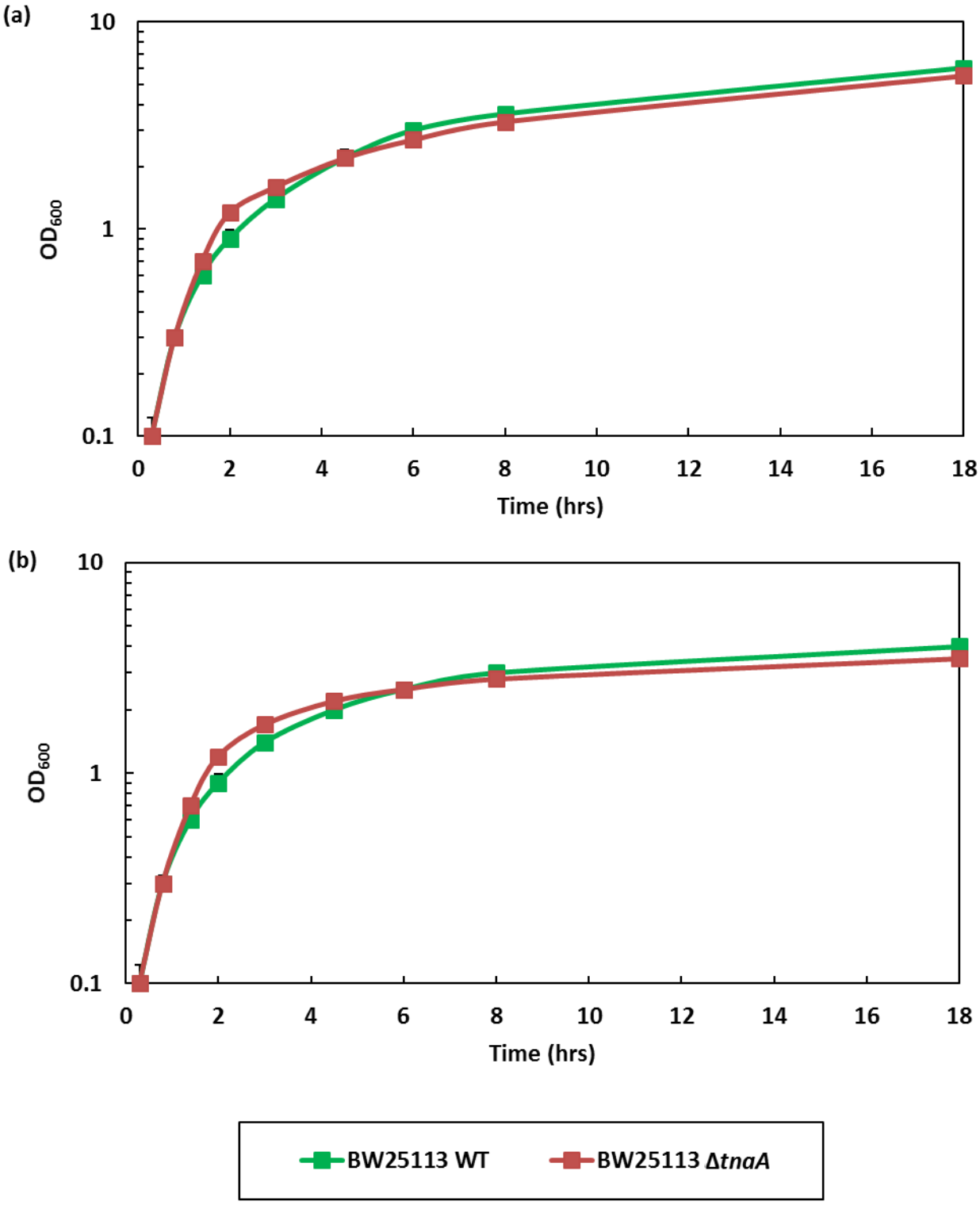
The procedure was performed according to the manufacturer's instructions with minor modifications. Before use, each cartridge (500 mg octadecylsilane – 6 ml) was equilibrated by flowing-through 10 ml 1-pentanol, followed by 10 ml deionised water. A sample (50 ml) from a lag-phase or exponential-phase bacterial culture was taken, and cells harvested by centrifugation at 2755 x g for 10 min (Eppendorf 5810 R centrifuge). The supernatant was flowed through an equilibrated cartridge and the cartidge was then washed with 10 ml deionised water. Indole was eluted from the cartridge with 5 ml 1-pentanol, resulting in an eluate containing indole concentrated ten-fold.

Kovacs assay

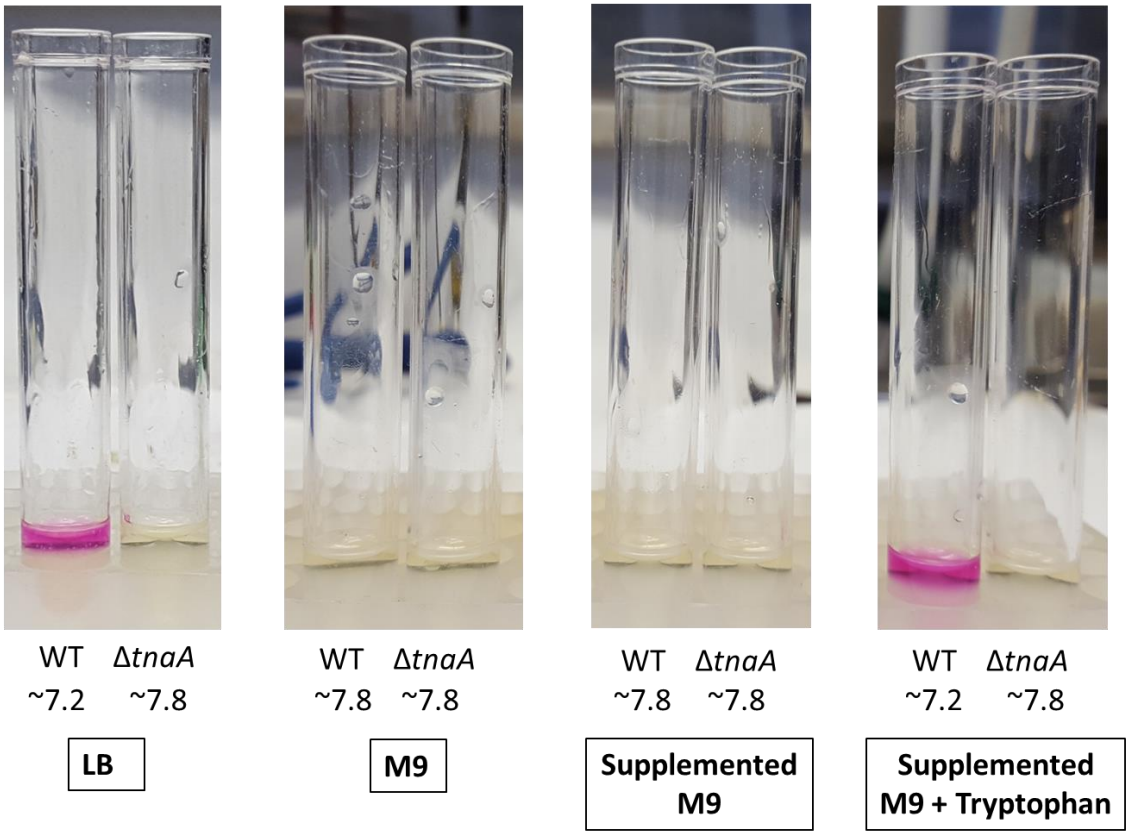
The Kovacs assay is an established technique providing consistent results from different laboratories[3,4]. The assay was performed as previously described[1] to determine the presence of indole in culture supernatants. Briefly, a sample (1 ml) from a stationary-phase culture was removed, and cells harvested by centrifugation at 11337 x g for 3 min (Eppendorf Minispin microfuge). The supernatant was removed and assayed: 300 µl of Kovacs reagent (10 g of *p*-dimethylamino-benzaldehyde dissolved in a mixture of 50 ml of HCl and 150 ml of amyl alcohol) was added to the supernatant and incubated for 2 minutes.

The presence of indole was indicated by the formation of red colour following the addition of Kovacs reagent.

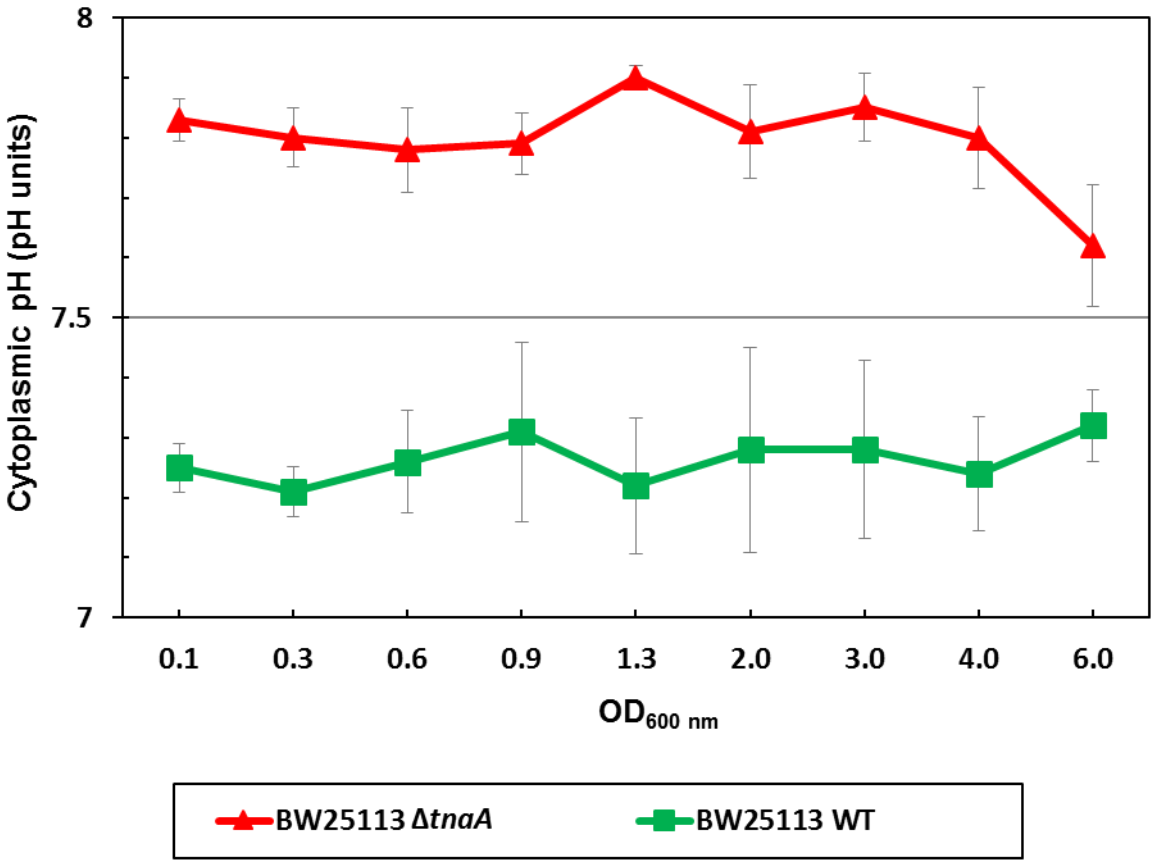
The Kovacs assay was also used to determine the concentration of indole in lag and exponential phase culture supernatants, after a pre-concentraion step with C18 columns. The assay was performed in a 96-well plate as previously described[5]. Briefly, 100 µl of x10 indole concentrated samples (in 1-pentanol) were incubated with 150 µl of Kovacs reagent for 5 min at room temperature. The reaction produced a soluble red product, which was measured spectrophotometrically at 530 nm (SpectraMax 190 Microplate reader; Molecular Devices, CA, USA). At least six known indole concentrations from 0 to 300 µM (in 1-pentanol) were assayed in triplicate and the mean results were used to construct a standard curve. Indole concentrations in unknown samples (also tested in triplicate) were calculated by comparison.



Supplementary Fig. S1: Growth curves of *E. coli* BW25113 WT and Δ*tnaA* growing in (a) LB and (b) M9/glucose (0.4%). Overnight cultures were diluted to OD_{600} = 0.05 in fresh LB or M9 and cultures were grown at 37 °C and sampled at intervals between OD_{600} = 0.1 – 0.6 for LB and OD_{600} = 0.1 – 0.4 for M9. The final sample was taken after overnight incubation (~ 18 hrs). The transition between exponential to stationary phase was observed around the same time (~ 2 hrs after sub-culture) in both media.



Supplementary Fig. S2: The absence of indole production correlates with the higher cytoplasmic pH. The cytoplasmic pH of the wild-type *E. coli* is ~ 7.2 when indole is produced and ~ 7.8 when indole is absent. The mutant strain lacks the ability to convert tryptophan to indole under any conditions so the cytoplasmic pH is always ~ 7.8. The presence of indole is indicated by the formation of red colour following the addition of Kovacs reagent. Samples were taken from stationary-phase cultures of BW25113 WT and BW25113 Δ*tnaA* grown in four different media: LB, M9 (containing glucose as a carbon source), supplemented M9 (glucose, VitB1, trace elements & casamino acids) and supplemented M9 + tryptophan. The average cytoplasmic pH (Fig. 1) is added for comparison.



Supplementary Fig. S3: The cytoplasmic pH of *E. coli* BW25113 WT and Δ*tnaA* growing in LB, measured by flow cytometry . Cultures of BW25113 and BW25113 Δ*tnaA* in LB were sampled throughout exponential and stationary phase, and samples were analysed by flow cytometry. The cytoplasmic pH of each sample was determined by the average fluorescence intensity of pHluorin normalised to mCherry and compared to a standard curve. pH values are in agreement with those obtained by fluorescence spectroscopy (Fig. 1). Data are presented as means ± SD, and the final point in each line is an overnight sample (~ 18 hrs incubation).

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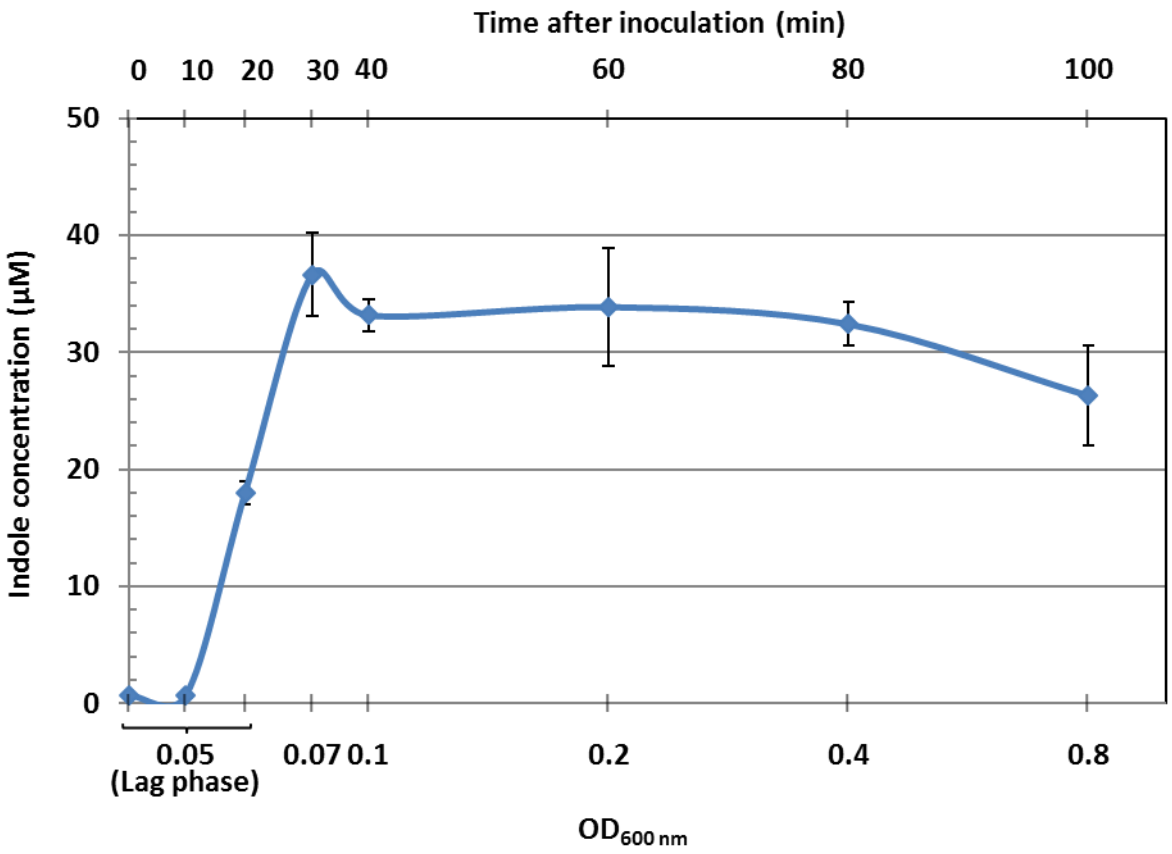

Supplementary Fig. S4: Indole concentration in the LB supernatant of *E. coli* BW25113 WT during lag and exponential phase. An overnight culture of BW25113 WT in LB was subcultured into fresh LB (OD_{600} = 0.05). Samples were taken throughout the lag phase, which lasted ~ 20 min, and the exponential phase. Indole was measured by Kovacs assay after passing each sample through a C18 solid phase extraction column, which concentrated indole 10-fold. 30 µM (± 10 µM) indole was made during the lag phase and this concentration remained constant throughout exponential phase.

Supplementary Table 1: Primers used to generate the pSCM001 plasmid using Gibson Assembly

Primer Name	Sequence (5'-3')
pBAD TOPO_FWD	ACATGGCATGGATGAACTATACAAATGAAAACGGTCTCCAGCTTGGCT
pBAD TOPO_REV	TCGCCATGTTGTCTTCTTCGCCTTTTGAAACCATGGTATGTATATCTCCTTCTTAAAGTTAAACA
mCherry_FWD	AAGAAGGAGATATACATACCATGGTTTCAAAAGGCGAAGAAGACAACATGGCGATTATCAAGG
mCherry_REV	ATagagccgccagagccgccGTACAGTTCATCCATACCGCCGGTAGAGT
pHluorin_FWD	ACggcggctctggcggctctATGAGTAAAGGAGAAGAACTTTTCA
pHluorin_REV	ACAGCCAAGCTGGAGACCGTTTTCATTTGTATAGTTCATCCATGCCA

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Small caps: (Gly-Gly-Ser) x2 Bridge

Supplementary References

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2. Yong, J.W.H., Ge, L., Wong, W.S., Ma, Z. & Tan, S.N. Analyses of Indole Compounds in Sugar Cane (*Saccharum officinarum* L.) Juice by High Performance Liquid Chromatography and Liquid Chromatography-Mass Spectrometry after Solid-Phase Extraction. *Separations* **4**, doi:10.3390/separations4010007 (2017).
3. Chant, E.L. & Summers, D.K. Indole signalling contributes to the stable maintenance of *Escherichia coli* multicopy plasmids. *Mol. Microbiol* **63**, 35–43 (2007).
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5. Darkoh, C., Chappell, C., Gonzales, C. & Okhuysen, P. A Rapid and Specific Method for the Detection of Indole in Complex Biological Samples. *Appl. Environ. Microbiol* **81**, 8093-8097 (2015).