Escherichia coli biofilm response to salicylic acid

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SUPPLEMENTAL MATERIAL

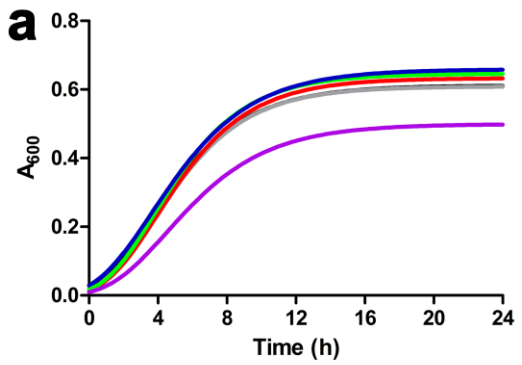
Synthesis of 4-acetamidosalicylic acid

4-acetamidosalicylic acid (4-AcASA) was synthesized as reported in the following procedure. A suspension of 4-ASA (1 g, 6.53 mmol) in ethanol (10 mL) and acetic anhydride (0.62 mL, 6.58 mmol) was stirred at 40 °C for 30 min and then allowed to cool at room temperature. The mixture was then concentrated *in vacuo* and extracted with ethyl acetate (3 x 2 mL). The organic phase was dried over Na_2SO_4, filtered and then concentrated under reduced pressure. The crude product was purified by flash column chromatography using dichloromethane/methanol (9:1) as eluent to afford the final product 4-AcASA. Yield: 48 %. Brown solid. TLC: DCM/MeOH (9:1) Rf = 0.52. M.p.: 230-231 °C (decomp). ^{1}H NMR (DMSO-d6): 6.40 (dd, 1H, ArH, J=1.2, J=8.1), 6.87 (d, 1H, ArH, J=8.1), 7.02 (t, 1H, ArH, J=8.1), 7.16 (s, 1H, OH), 9.30 (s, 1H, NH), 9.76 (s, 1H, COOH). ^{13}C NMR (CD_3OD): 22.7, 107.2, 111.0, 129.3, 139.8, 157.7, 170.4.

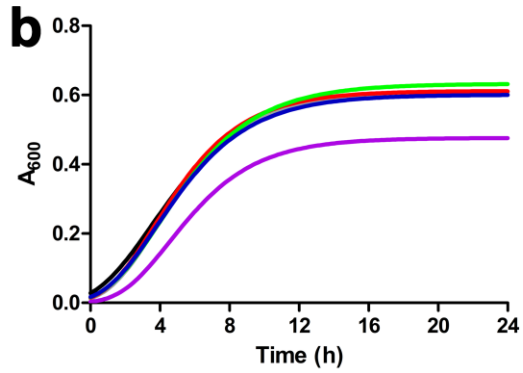
Planktonic grow of 4-ASA and 4-AcASA

Cmpd	Concentration (μM)	A_{600} ml^{-1}
4-ASA	**0**	0.031 ± 0.003^{a}
	0.183	0.030 ± 0.004^{a}
	1.83	0.032 ± 0.006^{a}
	18.3	0.029 ± 0.002^{a}
	183	0.027 ± 0.004^{a}
	1,830	0.029 ± 0.003^{a}
	1,830 (glucose)	0.121 ± 0.007^{b}
	3,000 (glucose)	0.172 ± 0.011^{c}
4-AcASA	**0**	0.031 ± 0.003^{a}
	0.183	0.029 ± 0.006^{a}
	1.83	0.032 ± 0.006^{a}
	18.3	0.028 ± 0.003^{a}
	183	0.025 ± 0.004^{a}
	1,830	0.029 ± 0.004^{a}
	1,830 (glucose)	0.121 ± 0.007^{b}
	3,000 (glucose)	0.172 ± 0.011^{c}

Table S1. Planktonic growth in the presence of 4-ASA and 4-AcASA as the sole carbon and energy source. Data represent the mean ± standard deviation of three independent measurements. Different superscript letters indicate statistically significant differences (Tukey's HSD, $p \leq 0.001$) between the means of three independent replicates. The positive controls were set up with the mineral medium supplemented with glucose at both 1,830 μM and 3,000 μM.



Concentration (μM)	λ (min)	μ_m (A_{600} min^{-1}) $\times 10^{-3}$	R^2
0	91.28±8.12[a]	1.23±0.12[a]	0.990±0.024
0.183	80.06±12.65[a]	1.23±0.04[a]	0.997±0.019
1.83	86.79±10.60[a]	1.26±0.06[a]	0.998±0.027
18.3	84.43±2.83[a]	1.29±0.05[a]	0.990±0.025
183	88.18±4.32[a]	1.28±0.05[a]	0.992±0.021
1,830	80.79±2.59[a]	0.96±0.05[b]	0.986±0.032



Concentration (μM)	λ (min)	μ_m (A_{600} min^{-1}) $\times 10^{-3}$	R^2
0	91.28±8.12[a]	1.23±0.12[a]	0.990±0.024
0.183	90.18±3.93[a]	1.28±0.12[a]	0.996±0.031
1.83	81.02±1.95[a]	1.26±0.08[a]	0.997±0.028
18.3	86.51±3.40[a]	1.24±0.11[a]	0.997±0.023
183	78.36±1.90[a]	1.21±0.02[ab]	0.996±0.025
1,830	128.15±13.06[b]	1.07±0.03[b]	0.998±0.023

Figure S1. Planktonic growth without and with 4-ASA and 4-AcASA. A_{600}-based growth curve of *E. coli* without and with 4-ASA (a) and 4-AcASA (b) at different concentrations (left) and growth parameters lag time length (λ), maximum specific growth rate (μ_m) and the Goodness of Fit (R^2) obtained by the Gompertz model (right). Data represent the mean ± standard deviation of three independent measurements. Different superscript letters indicate statistically significant differences (Tukey's HSD, $p \leq 0.001$) between the means of three independent replicates.