Salicylate Increases Fitness Cost Associated with MarA-Mediated Antibiotic Resistance

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Supporting Material

Figure S1. Green fluorescence level of WT with and without genomically integrated sfGFP determined by flow cytometry. WT strain with and without sfGFP. Each dot represents a green fluorescence reading of one cell in a sample of 5000 cells by flow cytometry. Black and green boxes show thresholds for distinguishing between fluorescent and non-fluorescent strains.

Figure S2. Minimum inhibitory concentration of carbenicillin in MarA⁻, AcrB⁻, WT, and MarA⁺ strains. Error bars show standard deviation from six biological replicates. *P<0.05; **P<0.01; ***P<0.001, Student’s t-test.
Figure S3. Lag time ($\lambda$) and carrying capacity ($A$) extracted from Gompertz model fits for each strain. Data points show mean values and standard deviation from six biological replicates. Differences between the strains are not statistically significant, $P > 0.05$ by a Student’s t test.

Figure S4. Competition assay between WT strains with and without genomically integrated \textit{sfgfp}. Fraction of cells of each strain over time after competition between the WT and WT (sfGFP) with initially equal proportions of the competing strains in a well-mixed liquid culture. Relative proportions were obtained using counts of each fluorescent cell from flow cytometry. Error bars show standard deviation from three biological replicates.
Figure S5. Fluorescence distributions from (a) marA reporter and (b) acrAB reporter in the WT strain with and without salicylate exposure.

Figure S6. Dose-response curve between carbenicillin and salicylate for the WT strain. Optical density of WT strain growing in cultures with carbenicillin and salicylate. Data points show mean values and standard deviation from three biological replicates.
Figure S7. Growth cost of strains as a function of salicylate concentration. Growth cost is defined as the reduction of growth rate of cells treated with salicylate relative to untreated cells. Dashed lines are fits to a Hill function \( h(x) = \frac{x^n}{K^n + x^n} \), where \( K \) equals to the salicylate concentration which inhibits cell growth by 50\%, as described in Ref (1). \( K=5.05 \) (3.45, 5.83) mM, \( n=2.04 \) (1.37, 4.26) for the AcrB\(^{-}\) strain; \( K=3.40 \) (1.49, 4.07) mM, \( n=1.18 \) (0.87, 2.71) for the MarA\(^{-}\) strain; \( K=2.92 \) (1.15, 3.88) mM, \( n=0.85 \) (0.55, 1.94) for the WT strain; \( K=2.44 \) (1.05, 2.67) mM, \( n=0.95 \) (0.85, 2.21) for the MarA\(^{+}\) strain. 95\% confidence intervals from nonlinear least square fitting are listed in parentheses. Data points and error bars show mean and standard deviation of experimental data from at least four biological replicates.
Supporting References