

Role of the Rcs Phosphorelay in Intrinsic Resistance to Penicillin, Phosphomycin, and Cefsulodin in *Escherichia coli* K12

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SUPPLEMENTAL MATERIAL

| Strain | WT new | | | | | | | | | | | |
|-------------------------|-----------------------|-------------|-------------|------|------|------|------|------|------|------|------|------|
| | Antibiotic Penicillin | | | | | | | | | | | |
| $\mu\text{g}/\text{mL}$ | 100 | 50 | 25 | 12.5 | 6.3 | 3.1 | 1.6 | 0.8 | 0.4 | 0.2 | 0.1 | 0.0 |
| 0.00 | 0.00 | 0.00 | 0.13 | 0.32 | 0.45 | 0.42 | 0.40 | 0.35 | 0.36 | 0.36 | 0.38 | 0.37 |
| 0.00 | 0.01 | 0.20 | 0.29 | 0.44 | 0.32 | 0.39 | 0.33 | 0.35 | 0.32 | 0.41 | 0.41 | 0.40 |
| 0.00 | 0.00 | 0.19 | 0.43 | 0.32 | 0.35 | 0.32 | 0.44 | 0.29 | 0.35 | 0.34 | 0.34 | 0.42 |
| 0.00 | -0.01 | 0.36 | 0.38 | 0.45 | 0.47 | 0.54 | 0.55 | 0.43 | 0.47 | 0.53 | 0.53 | 0.64 |
| -0.01 | -0.01 | 0.36 | 0.33 | 0.38 | 0.49 | 0.50 | 0.60 | 0.48 | 0.47 | 0.50 | 0.50 | 0.59 |
| -0.01 | 0.00 | 0.30 | 0.34 | 0.38 | 0.49 | 0.43 | 0.47 | 0.45 | 0.45 | 0.48 | 0.48 | 0.56 |
| -0.01 | -0.01 | -0.01 | 0.43 | 0.48 | 0.50 | 0.47 | 0.50 | 0.48 | 0.46 | 0.45 | 0.45 | 0.45 |
| 0.00 | 0.00 | 0.22 | 0.29 | 0.38 | 0.30 | 0.40 | 0.39 | 0.46 | 0.52 | 0.47 | 0.47 | 0.44 |
| 0.02 | 0.02 | 0.19 | 0.28 | 0.42 | 0.38 | 0.39 | 0.37 | 0.51 | 0.39 | 0.37 | 0.37 | 0.41 |

| Strain | ΔrcsB new | | | | | | | | | | | |
|-------------------------|-------------------------|-------------|-------------|-------------|------|------|------|------|------|------|------|------|
| | Antibiotic Penicillin | | | | | | | | | | | |
| $\mu\text{g}/\text{mL}$ | 100 | 50 | 25 | 12.5 | 6.3 | 3.1 | 1.6 | 0.8 | 0.4 | 0.2 | 0.1 | 0.0 |
| 0.00 | 0.00 | 0.00 | -0.01 | 0.37 | 0.33 | 0.41 | 0.33 | 0.38 | 0.36 | 0.35 | 0.38 | 0.35 |
| -0.01 | 0.00 | 0.00 | 0.00 | 0.31 | 0.48 | 0.35 | 0.43 | 0.34 | 0.33 | 0.34 | 0.37 | 0.38 |
| 0.00 | 0.01 | 0.00 | 0.00 | 0.30 | 0.37 | 0.31 | 0.41 | 0.39 | 0.35 | 0.33 | 0.36 | 0.40 |
| 0.00 | 0.00 | 0.22 | 0.27 | 0.34 | 0.38 | 0.42 | 0.46 | 0.45 | 0.43 | 0.39 | 0.39 | 0.53 |
| 0.00 | 0.00 | 0.29 | 0.30 | 0.37 | 0.36 | 0.36 | 0.40 | 0.40 | 0.42 | 0.38 | 0.38 | 0.52 |
| 0.00 | 0.01 | 0.32 | 0.34 | 0.37 | 0.35 | 0.33 | 0.36 | 0.34 | 0.32 | 0.44 | 0.44 | 0.48 |
| -0.02 | -0.03 | -0.03 | 0.39 | 0.27 | 0.27 | 0.26 | 0.27 | 0.50 | 0.30 | 0.30 | 0.30 | 0.37 |
| -0.02 | -0.02 | -0.01 | 0.37 | 0.26 | 0.27 | 0.19 | 0.25 | 0.31 | 0.31 | 0.31 | 0.31 | 0.42 |
| -0.01 | -0.01 | 0.23 | 0.28 | 0.26 | 0.28 | 0.23 | 0.34 | 0.31 | 0.37 | 0.37 | 0.37 | 0.36 |

FIG. S1 Cumulative OD₆₀₀ values of WT new and ΔrcsB new strains incubated with a concentration gradient of penicillin. Each box represents a well and the numbers represent the OD₆₀₀ value obtained from a Nanodrop 200C spectrophotometer. Shades of green indicates relative OD₆₀₀ levels, where darker shades indicate higher levels. Yellow indicates no bacterial growth. Bolded OD₆₀₀ values indicates our interpretation of the highest concentration of penicillin where bacteria grew. Therefore, the corresponding MIC for our WT new strains on penicillin is interpreted to be 50 $\mu\text{g}/\text{mL}$, with 8 of 9 replicates agreeing. The corresponding MIC for our ΔrcsB NEW strain is around 25-50 $\mu\text{g}/\text{mL}$ where 5 of 9 replicates show a MIC of 25 $\mu\text{g}/\text{mL}$, and 4 of 9 replicates show a MIC of 50 $\mu\text{g}/\text{mL}$.