

**Supplementary Information For:**

**Generation of a *Gluconobacter oxydans* knockout collection for improved extraction of rare earth elements**

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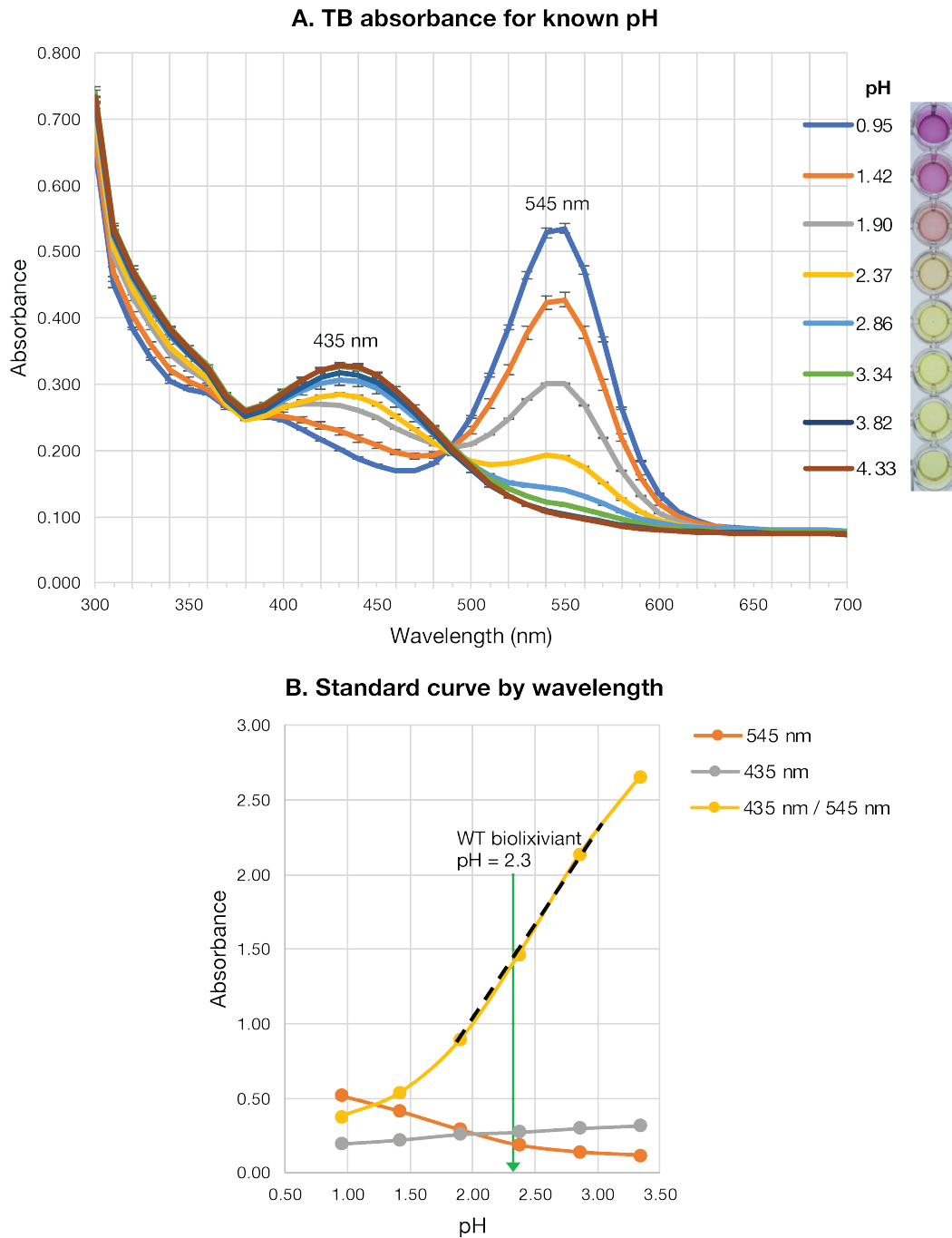
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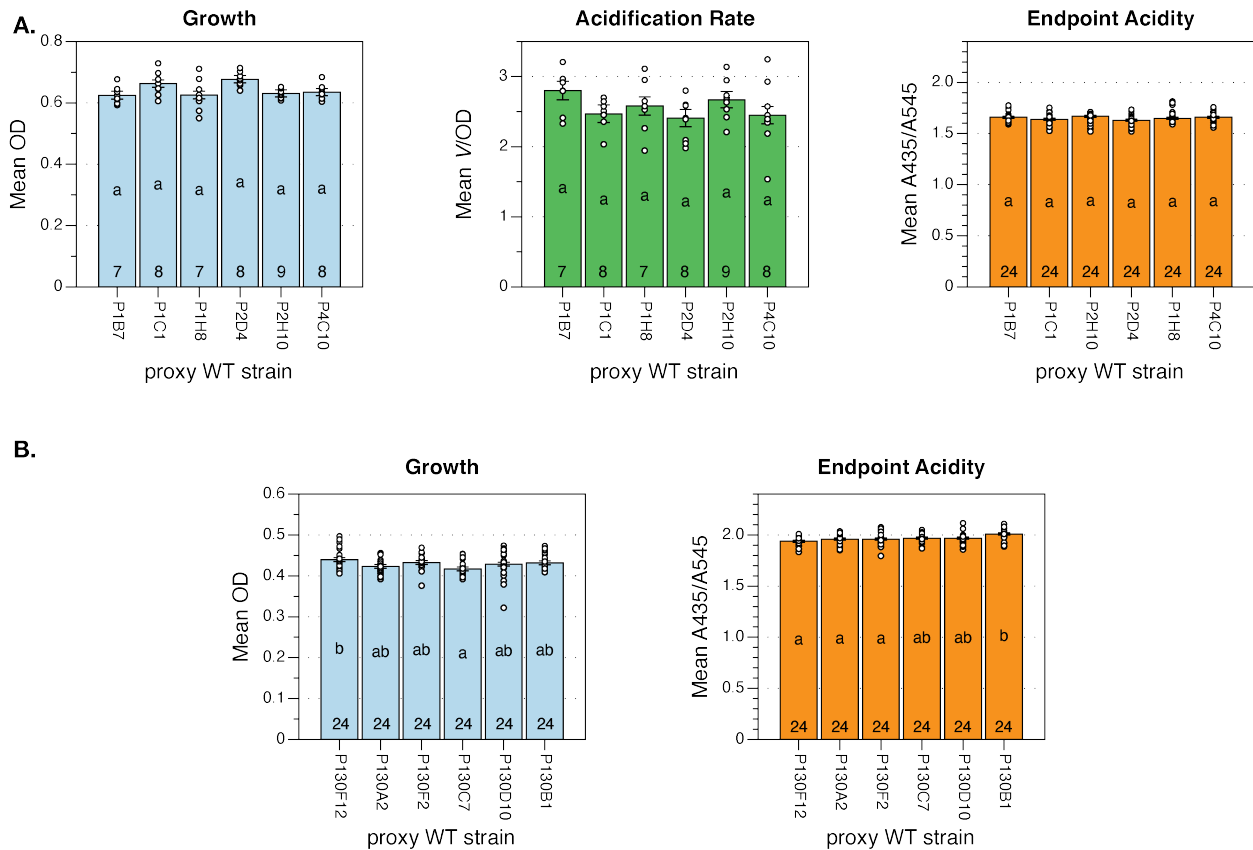
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**Supplementary Figure 1.** Calibration data for Thymol Blue assay for media acidification. **(A)** Thymol Blue (TB) absorbance spectra for solutions of known pH. Error bars are standard deviation and  $n$  is 3 biological replicates. **(B)** Calibration curve to connect TB absorbance and pH. Black dashed line shows linearity of 435 nm / 545 nm between just below pH of 2.0 to a pH of 3.0. Green arrow indicates pH of the previously reported wild type *G. oxydans*-produced bioRxiviant.



**Supplementary Figure 2.** Comparison of individual proxy WT disruption strains type A (**A**) and type B (**B**) growth (blue bars), initial acidification rate (green bars), and endpoint acidity (orange bars). Multiple comparisons were made for each set of proxy WT strains using the emmeans package in R with a Tukey  $p$ -value adjustment. Letters denote significance groups. Individual data points are shown as black circles. Error bars are standard error. The number of biological replicates for each strain measured for a particular phenotype is shown at the bottom of the corresponding bar.