Structural, functional, and substrate specificity analysis of YbtT, a type II thioesterase in the yersiniabactin biosynthetic pathway of pathogenic *Enterobacteriaceae*

RUNNING TITLE: YbtT Structure and Function

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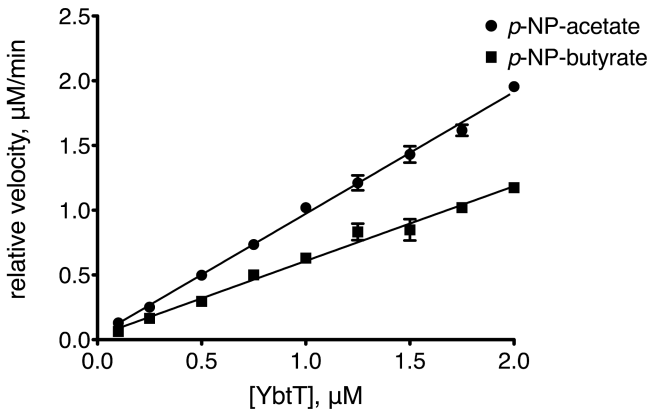
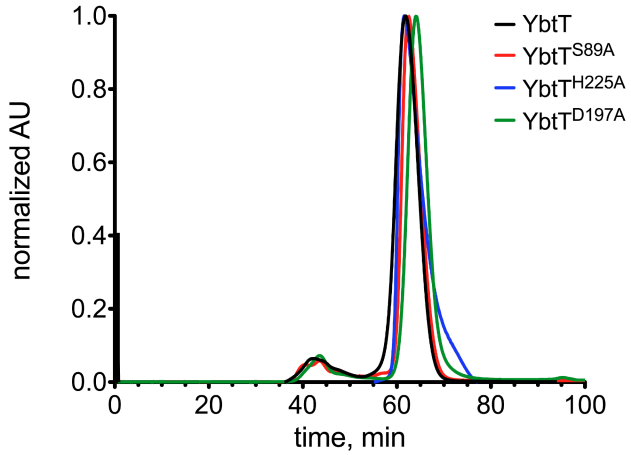
Supporting Information

Supplemental Figures S1-S3



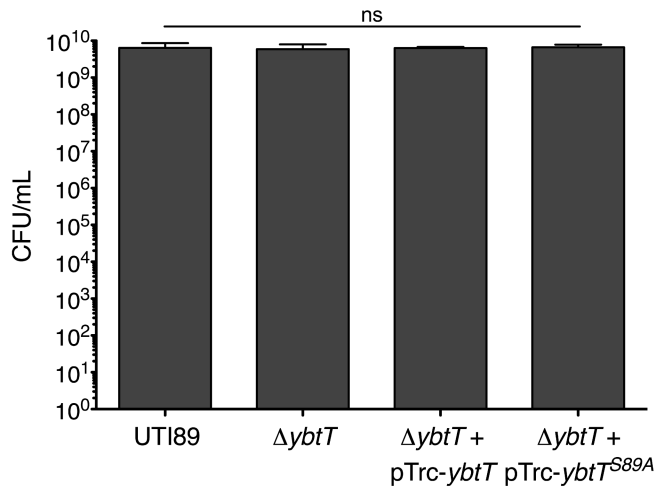
Supplemental Figure S1. YbtT activity on *p*-nitrophenyl ester substrates is linear with respect to enzyme concentration

Hydrolysis of *p*-nitrophenyl-acetate and –butyrate was monitored at concentrations of YbtT from 0.1-2 µM. The concentration of substrate was held constant in all reactions at 1 mM. Time courses were collected for 10 min at each concentration of enzyme and linear regressions were performed to determine the slopes (relative velocity). Relative velocity was plotted as a function of YbtT concentration and a linear regression was performed (solid lines). For both substrates, the relative velocity was proportional to the concentration of YbtT. Mean and standard errors plotted, n = 2. *p*-nitrophenyl-acetate R^2 = 0.9974, *p*-nitrophenyl-butyrate R^2 = 0.9896.



Supplemental Figure S2. YbtT, YbtT-S89A, YbtT-H225A, and YbtT-D197A exhibit the same elution profiles on a gel filtration column

YbtT, YbtT-S89A, YbtT-H225A, and YbtT-D197A were purified by Ni affinity chromatography and then further purified by gel filtration on a 16/600 75 pg column with 50 mM potassium phosphate pH 7.4+100 mM KCl+2 mM TCEP, except for YbtT-D197A which was run with 300 mM KCl in the buffer. The proteins were detected by absorbance at 280 nm. Each trace was normalized to its maximum absorbance.



Supplemental Figure S3. Deletion of the *ybtT* gene and genetic complementation do not affect bacterial growth

UTI89, its isogenic mutant Δ*ybtT*, and Δ*ybtT* strains complemented with either wildtype *ybtT* or *ybtT-S89A* cloned into the plasmid pTrcHis2 were grown under iron-restricted conditions for 18 hours and bacterial growth was assessed by colony forming units (CFU). Mean of n = 3 with standard deviation plotted, one-way ANOVA compared with multiple comparison post-test to Δ*ybtT*, ns $p > 0.05$.