SUPPLEMENTAL INFORMATION

Insights into the function of the *N*-acetyltransferase SatA that detoxifies streptothricin in *Bacillus subtilis* and *Bacillus anthracis*

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Running title: Functional analysis of SatA

SUPPLEMENTAL FIGURES

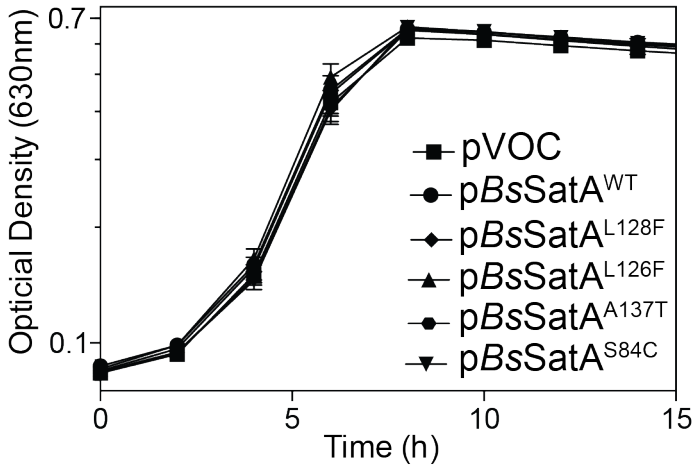

Figure S1. **Variant *B. subtilis satA* alleles have no growth defect in *S. enterica*.** Strains carrying wild-type (*satA*$^+$, circles), variant *satA* alleles, or empty vector (squares) were grown in glycerol (22 mM) minimal medium (1). All strains carried Δ*metE2702 ara-9* chromosomal mutations and the indicated plasmid: JE22263 (/pCV1), JE22334 (/pBsSATA1), JE23884 (/pBsSATA10), JE23887 (/pBsSATA11), JE23888 (/pBsSATA12), JE23889 (/pBsSATA13). Error bars represent one standard deviation and are present, although in many cases they are too small to be visible.

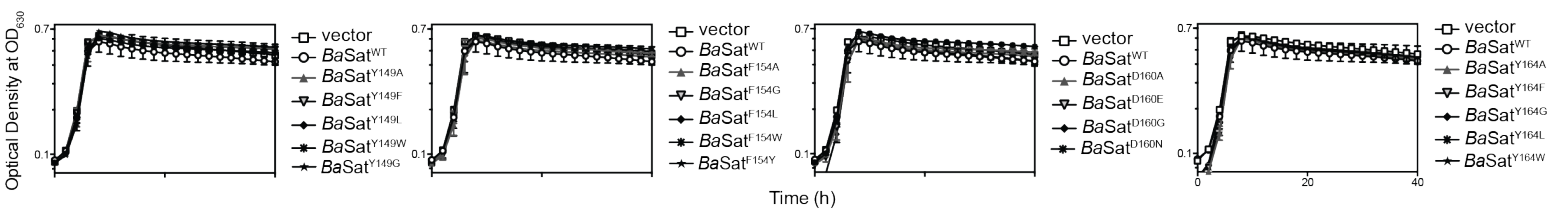

Figure S2. **Mutant *B. anthracis satA* alleles have no growth defect in *S. enterica*.** Strains carrying wild-type (*satA*$^+$, circles), variant *satA* alleles, or empty vector (squares) were grown in glycerol (22 mM) minimal medium (1) with 250 μM L-(+)-arabinose. Error bars represent one standard deviation and are present, although in many cases they are too small to be visible.

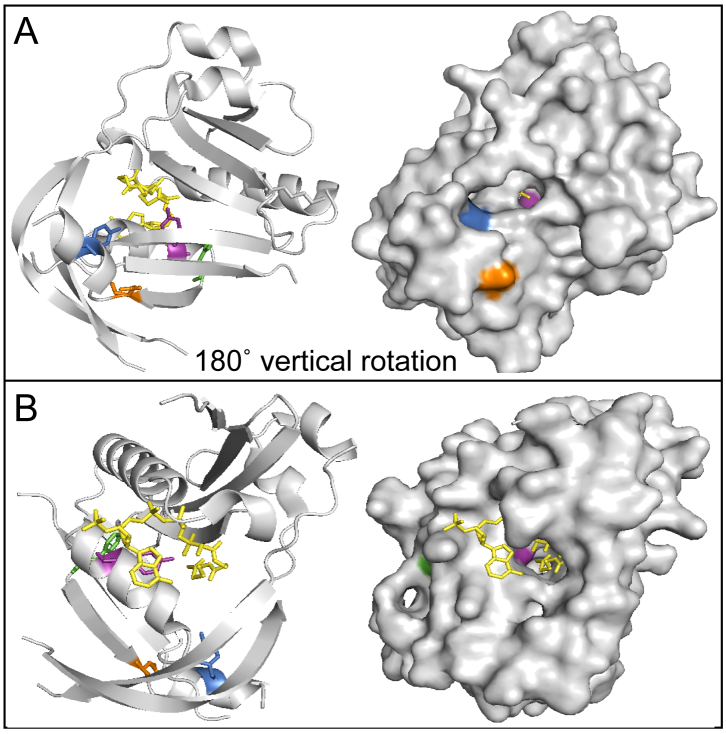

Figure S3. Surface model of *Ba*SatA (PDB 3PP9). Ribbon (left) or surface (right) model of *Ba*SatA. Panels B is a 180˚ vertical rotation of panel A, intended to display the full surface of the enzyme. Conserved aromatic residues are colored: Y149 (purple), F154 (green), D160 (orange), and Y164 (blue), while AcCoA is shown in yellow.

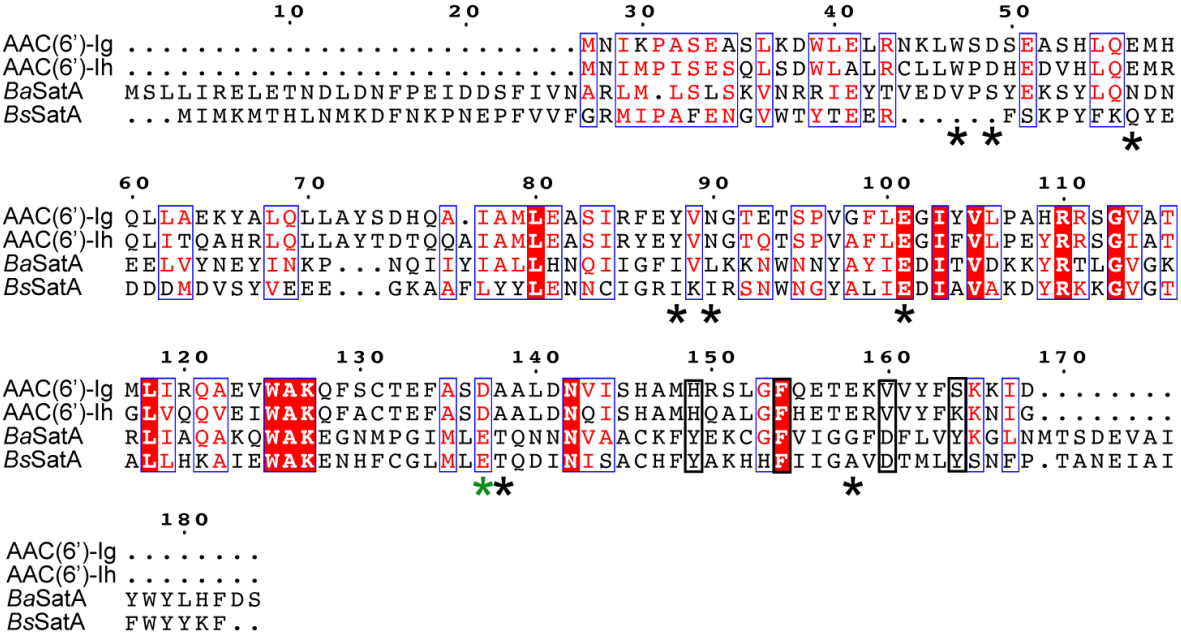

Figure S4. Alignment of AAC(6') and SatA proteins. Protein sequences were aligned using Geneious software (2) (https://www.geneious.com) and the figure was generated using ESPript (3). Conserved residues are highlighted red while similar residues are boxed. Numbers refer to the residue number of *B. anthracis* SatA. Residues substituted in the *Ba*SatA (Y149, F154, D160, and Y164) are outlined in black. Residues of AAC(6')-Ig known to bind tobramycin are indicated with asterisks below the residue. The green asterisk corresponds to the putative active site glutamate for SatA and a reported binding site of tobramycin for AAC(6')-Ig.

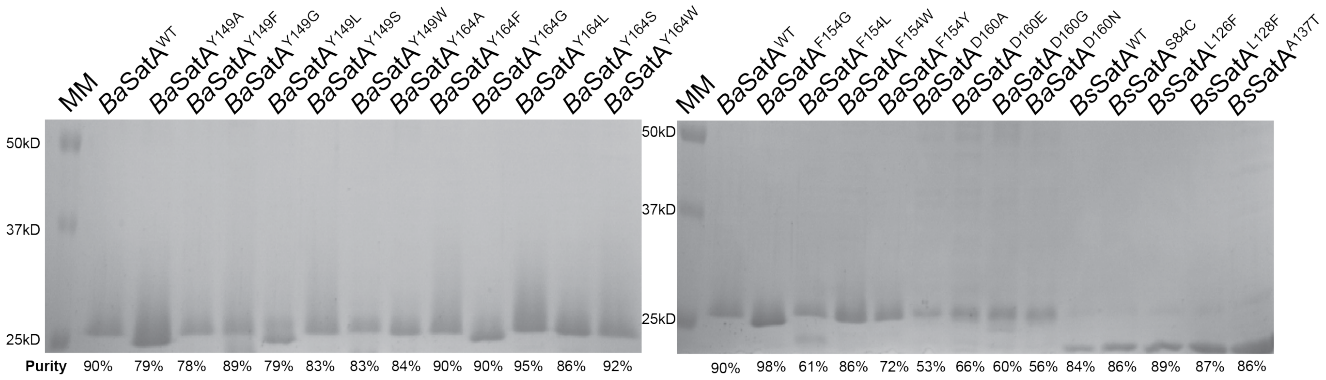

Figure S5. Assessment of *Bs*SatA and *Ba*SatA variants purity. Samples of each variant were run on SDS-PAGE, followed by staining with Coomassie Brilliant Blue R. Protein purity was calculated by running various dilutions of proteins on a separate 12% SDS-PAGE gel and bands were quantified using Total Lab software (4). MM stands for molecular mass marker.

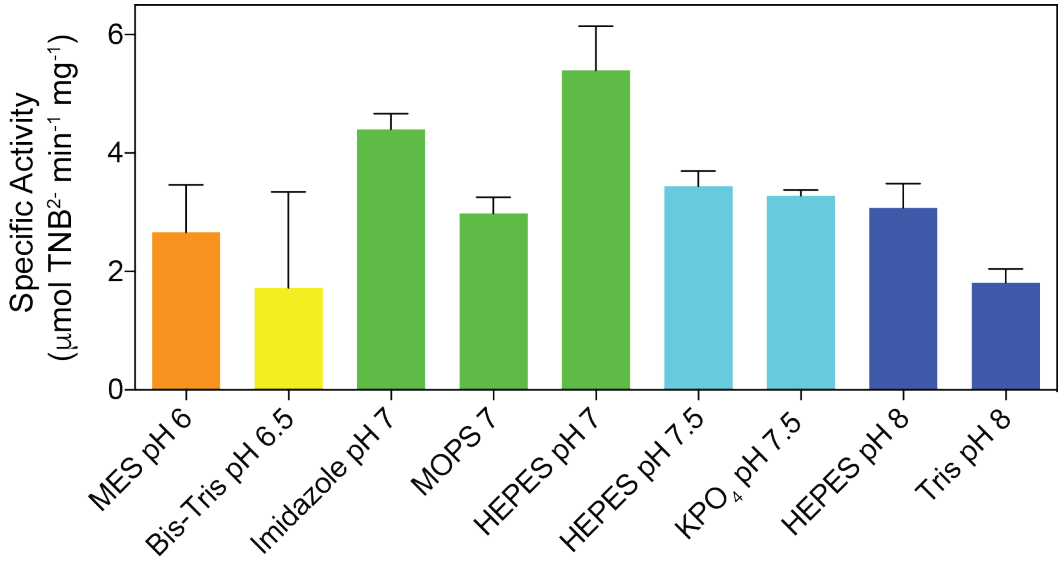

Figure S6. *Ba*SatA *in vitro* activity is highest in HEPES buffer. Various buffers (50 mM) were used to determine the specific activity of *Ba*SatA under saturating conditions (10 μM streptothricin and 500 μM AcCoA) using a continuous DTNB spectrophotometric assay (5) as described in the *Material and Methods*. Abbreviations: MES (2-(*N*-morpholino)ethanesulfonic acid), Bis-Tris (Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane), MOPS (3-(*N*-morpholino)propanesulfonic acid), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), and Tris (*tris*(hydroxymethyl)aminomethane).

REFERENCES

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