SUPPLEMENTAL INFORMATION

In *Bacillus* subtilis, the SatA (formerly YyaR) acetyltransferase detoxifies streptothricin via lysine acetylation

Rachel M. Burckhardt and Jorge C. Escalante-Semerena*
Department of Microbiology, University of Georgia, Athens

*Corresponding author: Department of Microbiology, University of Georgia, 212C Biological Sciences Building, 120 Cedar Street, Athens, GA 30602, USA; T: +1(706)-542-2651; F: +1(706)-542-2815; Email: jcescala@uga.edu, URL: www.escalab.com

Running title: *B. subtlis* SatA acetylates streptothricin

Figure S1. Minimal Inhibitory concentration of streptothricin. Cultures of a *B. subtilis satA*$^+$ strain (JE9142) were grown in minimal glycerol medium at 37 ºC, with various concentrations of streptothricin added. After 24 h, the final optical density at 600 nm was recorded, and compared to that of the culture grown in the same medium devoid of streptothricin.

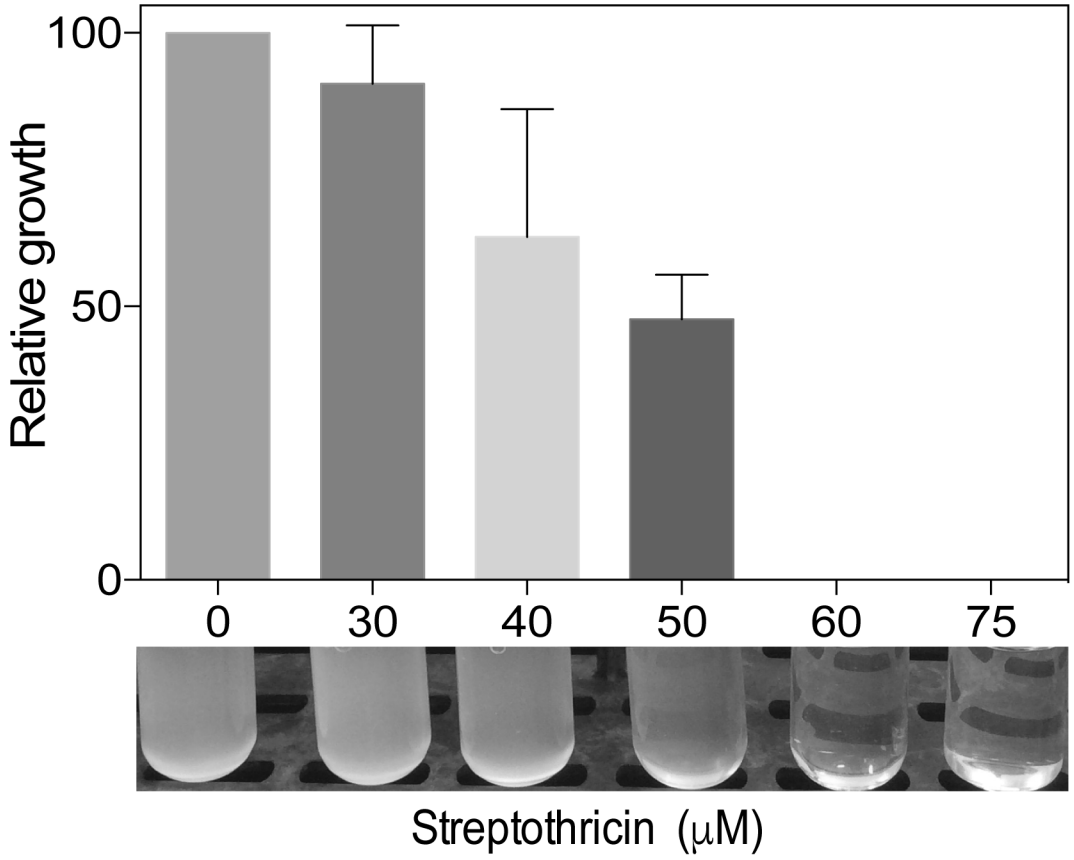


Figure S2. Alignment of various streptothricin acetyltransferase proteins. Streptothricin acetyltransferase protein sequences were aligned using Geneious software program available online at https://www.geneious.com (1); the figure was generated using ESPript (2). Structural components of the *Bacillus anthracis* str. Ames (PBD 3PP9) SatA homologue are shown above the indicated protein sequence; β refers to a β strand, TT refers to a turn, η refers to a 3_{10} helix, and α refers to an α helix.

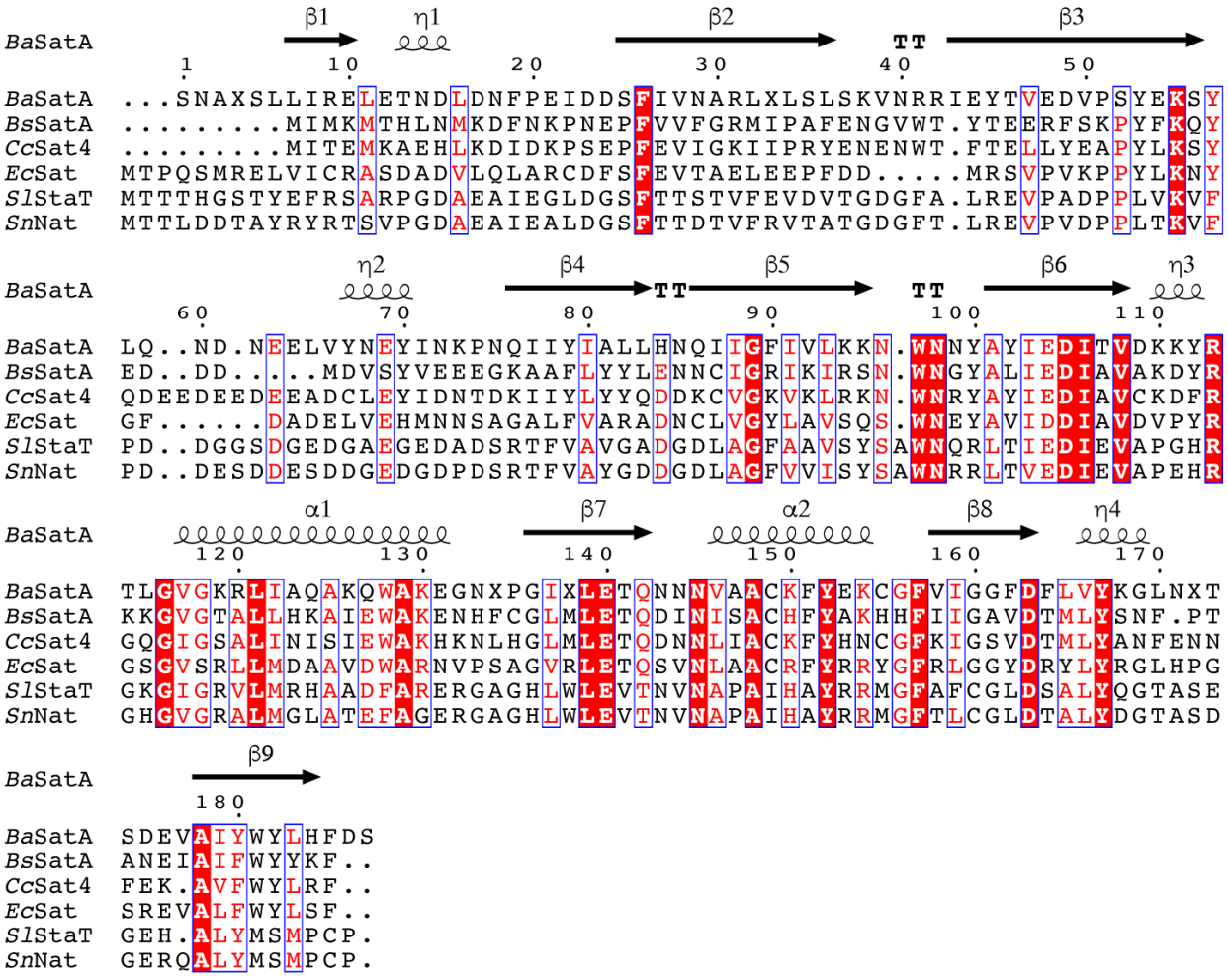


Figure S3. Assessment of H_6-*Bs*SatA purity. The purity of **H_6-*Bs*SatA** was determined by SDS-PAGE (3), followed by Coomassie Brilliant Blue R (ACROS Organics) staining (4).

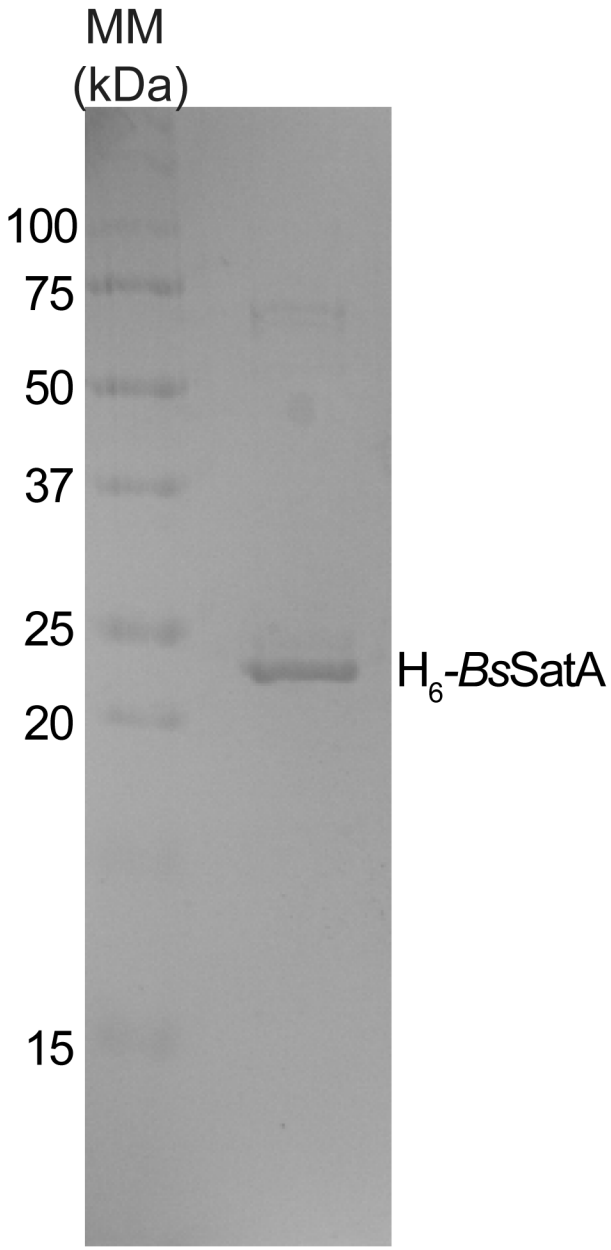
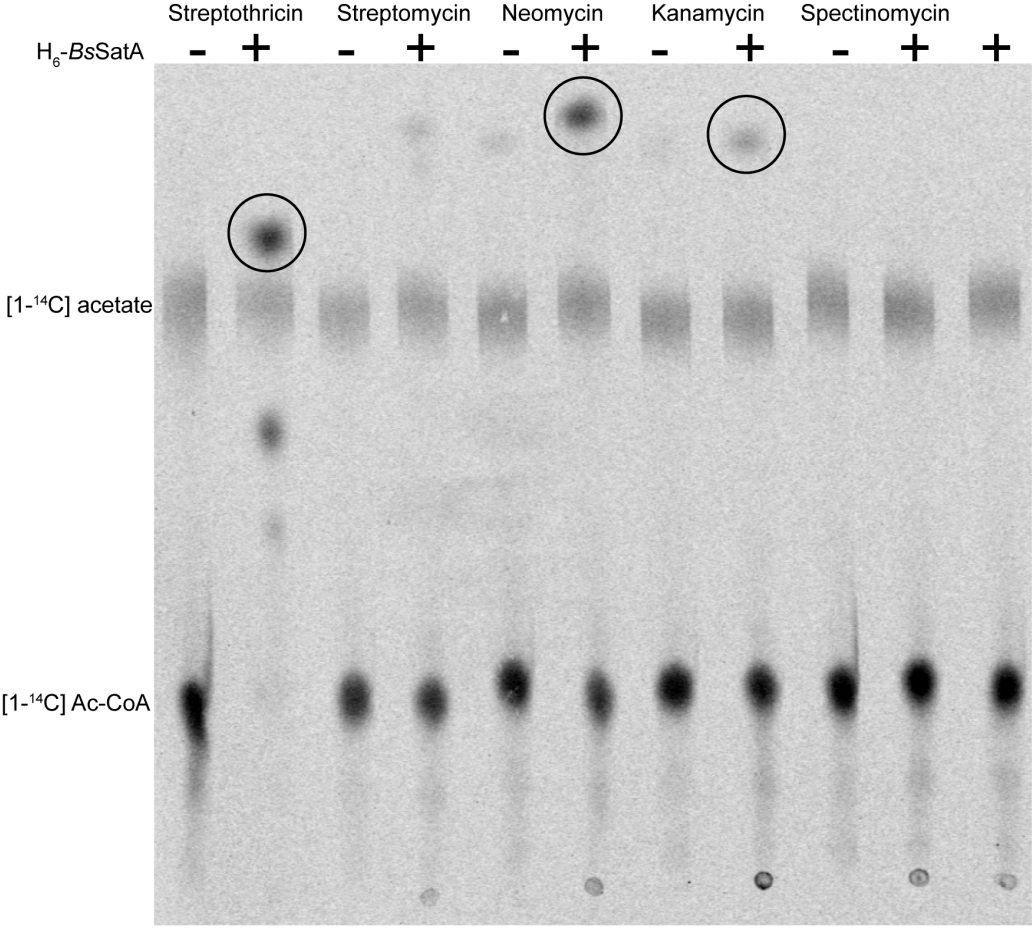


Figure S4. H_6-*Bs*SatA acetylates aminoglycoside antibiotics. Reactions of [1-^{14}C]acetyl-CoA, antibiotic, and H_6-*Bs*SatA were incubated at 37 ºC for 1 h, and spotted onto a thin layer chromatography (TLC) silica plate. The plate incubated for 1 to 2 h, and was developed for a phosphor image. The movement of the radiolabeled acetyl group onto the antibiotic can be seen when H_6-*Bs*SatA is present for streptothricin (positive control), neomycin, and kanamycin. No transfer of label is seen in the substrate only or enzyme only controls. TLC reactions were performed and imaged as described in the *Material and Methods*.



References

1. **Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A.** 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28:**1647-1649.
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3. **Laemmli UK.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680-685.
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