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 °C, 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.



Streptothricin (µM)

Figure S2. Alignment of various streptothricin acetyltransferase proteins. Streptothricin acetyltransferase protein sequences were aligned using Geneious software program available online at <u>https://www.geneious.com</u> (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 3₁₀ helix, and α refers to an α helix.

		β1	η1	β2		β3
BaSatA	1	10	20	30	4 0	50
BaSatA BsSatA CcSat4 EcSat SlStaT SnNat	MTPQSMREI MTTTHGSTY MTTLDDTAY	LIRELET MIMKMTE MITEMKA LVICRASI KEFRSARE KRYRTSVE	NDLDNFPEID HLNMKDFNKPN AEHLKDIDKPS ADVLQLARCD GDAEAIEGLD GDAEAIEALD	DSFIVNARLXLS EPFVVFGRMIPA EPFEVIGKIIPR FSFEVTAELEEP GSFTTSTVFEVD GSFTTDTVFRVT	LSKVNRRIE FENGVWT.Y YENENWT.F FDDM VTGDGFA.L ATGDGFT.L	Y TVE D V P SY E K S Y TEERFSK PYF K Q Y TELLYEAPYLKSY R SVPVK P PYLKN Y REVPADPPLVKVF R EVPVDPPLTKVF
<i>Ba</i> SatA	60	η2 2000 70	β4 8 0 8 0	→ TT β5	→ TT	β6 η3 200 110
BaSatA BsSatA CcSat4 EcSat SlStaT SnNat	LQND.N EDDD QDEEDEEDE GFI PDDGGS PDDESD	ELVYNEY MDVSY EEADCLEY ADELVEE GEDGAEG ESDDGEL	INKPNQIIY VEEEGKAAFL IDNTDKIIYL MNNSAGALFV GDADSRTFVA	ALLHNQIIGFIV YYLENNCIGRIK YYQDDKCVGKVK ARADNCLVGYLA VGADGDLAGFAA YGDDGDLAGFVV	LKKN.WNNY IRSN.WNGY LRKN.WNRY VSQS.WNEY VSYSAWNQR ISYSAWNRR	A Y I E D I T VDKKY R AL I E D I A VAKDY R A Y I E D I A VCKDF R A V I D D I A VCKDF R L T I E D I E VAPGHR L T VED I E VAPEHR
<i>Ba</i> SatA	<u>00000</u> 120	α1 20000000 13	$\frac{\beta^2}{14}$	$ \xrightarrow{7} \qquad \alpha 2 \\ 0 \\ 0 \\ 1 \\ 5 \\ 0 \\ 1 \\ 5 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0$	<u>βε</u> 160	$\stackrel{\eta 4}{\longrightarrow} \qquad \stackrel{\eta 4}{\underbrace{\circ \circ \circ \circ}_{170}}$
BaSatA BsSatA CcSat4 EcSat SlStaT SnNat	TLGVGKRLI KKGVGTALI GQGIGSALI GSGVSRLLM GKGIGRVLM GHGVGRALM	LAQAKQWA LHKAIEWA LNISIEWA 4DAAVDWA 4RHAADFA 4GLATEFA	KEGNXPGIXL KENHFCGLML KHKNLHGLML RNVPSAGVRL RERGAGHLWL GERGAGHLWL	ETQNNNVAACKF ETQDINISACHF ETQDNNLIACKF ETQSVNLAACRF EVTNVNAPAIHA EVTNVNAPAIHA	YEKCGFVIG YAKHHFIIG YHNCGFKIG YRRYGFRLG YRRMGFAFC YRRMGFTLC	GFDFLVYKGLNXT AVDTMLYSNF.PT SVDTMLYANFENN GYDRYLYRGLHPG GLDSALYQGTASE GLDTALYDGTASD
<i>Ba</i> SatA	β9 180	→				
BaSatA BsSatA CcSat4 EcSat SlStaT SnNat	SDEV <mark>AIY</mark> WY ANEIAIFWY FEK.AVFWY SREVALFWY GEH.ALYMS GERQALYMS	LHFDS YKF LRF LSF MPCP. MPCP.				





Figure S4. H₆-*Bs*SatA acetylates aminoglycoside antibiotics. Reactions of $[1-^{14}C]$ acetyl-CoA, antibiotic, and H₆-*Bs*SatA were incubated at 37 °C 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 H₆-*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

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