



Figures and figure supplements

A switch in surface polymer biogenesis triggers growth-phase-dependent and antibiotic-induced bacteriolysis

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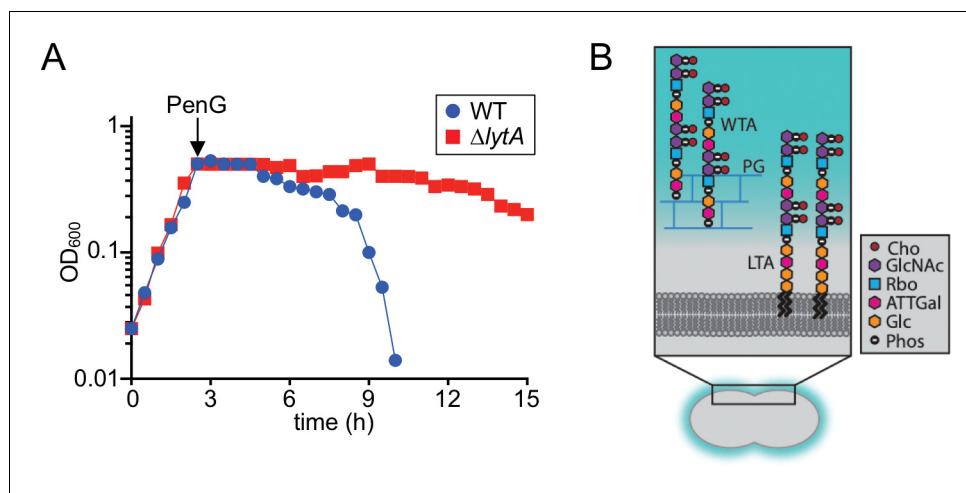


Figure 1. Beta-lactam induced lysis of *Sp* and overview of its cell envelope. **(A)** The indicated strains were grown in THY at 37 °C in 5% CO₂. At an OD₆₀₀ of ~0.5, they were challenged with penicillin G (PenG) (0.5 µg/ml final). Growth was monitored every 30 min for 15 hr. **(B)** Schematic diagram of the cell envelope of *Sp*. The cell wall peptidoglycan (PG) (blue) contains Wall Teichoic Acid (WTA) polymers and the lipid bilayer contains Lipoteichoic Acid (LTA). The constituents of the repeating unit in LTAs and WTAs are indicated; Cho, choline; GlcNAc, N-acetylglucosamine; Rbo, ribitol; ATTGal, 2-acetamido-4-amino-2,4,6-trideoxygalactose; Glc, glucose; Phos, phosphate. The following figure supplement is available for **Figure 1**.

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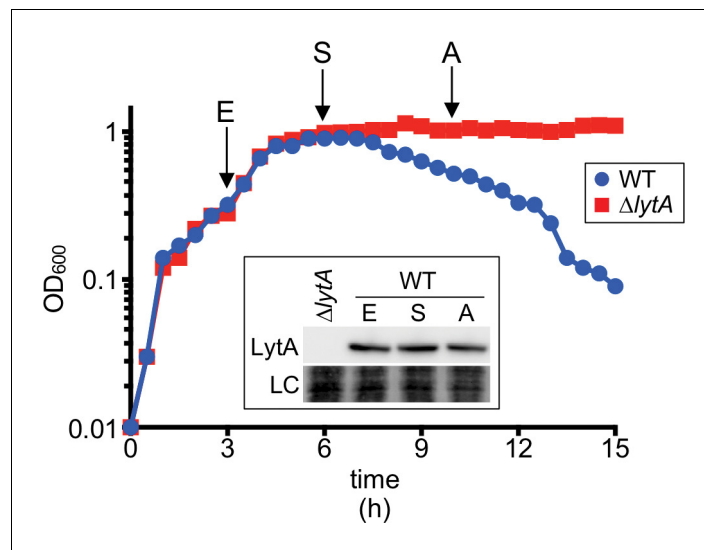


Figure 1—figure supplement 1. LytA levels remain constant before and at the onset of growth-phase-dependent autolysis. Growth curves of the indicated strains at 37°C in 5% CO₂. Measurements of culture OD₆₀₀ were recorded approximately every 30 min for 15 hr. Samples were collected at the indicated time points (E, exponential; S, stationary; A, autolysis), normalized to an OD₆₀₀ of 0.5 and analyzed by SDS-PAGE and immunoblotting using an anti-LytA antibody (LytA). LC (loading control) indicates a Ponceau S stain of the nitrocellulose membrane used for immunoblot analysis.

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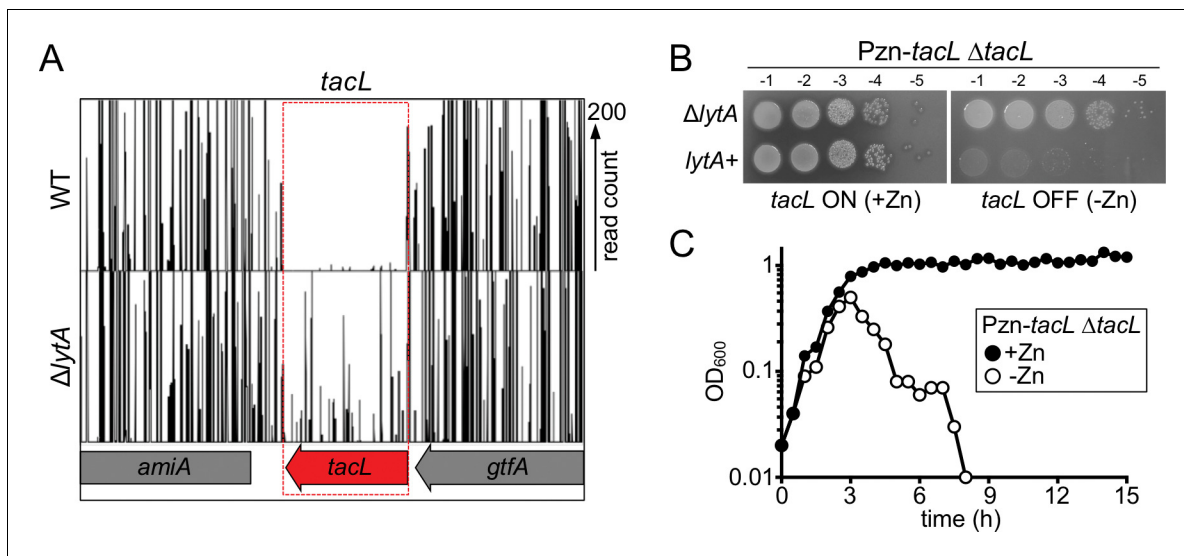


Figure 2. The essential gene *tacL* can be inactivated in cells lacking *lytA*. (A) Transposon insertion profiles from the Tn-Seq screen. Mariner transposon libraries were generated in wild-type (WT) and $\Delta lytA$ mutant strains and insertion sites were mapped to the *Sp* genome using Illumina sequencing. The height of each line reflects the number of sequencing reads at each position. Note that transposon insertions in *tacL* were much more readily isolated in cells lacking *lytA*. (B) Spot dilutions of the indicated strains in the presence and absence of inducer (Zn). The indicated strains were grown to exponential phase, normalized for OD₆₀₀ and serially diluted. Aliquots (5 μ l) of each dilution were spotted onto TSAII 5% SB plates in the presence or absence of 100 μ M ZnCl₂. Plates were incubated at 37 °C in 5% CO₂ and imaged. (C) Depletion of *tacL* results in growth arrest and lysis in exponential phase while its overexpression results in protection against growth-phase-dependent autolysis. Strains containing a zinc-inducible *tacL* allele (*Pzn-tacL*) were grown in THY to mid-exponential phase. Cultures were diluted into fresh THY to an OD₆₀₀ of 0.025 in the presence or absence of 100 μ M ZnCl₂ and grown at 37 °C in 5% CO₂. Growth was monitored by taking OD₆₀₀ measurements approximately every 30 min for 15 hr.

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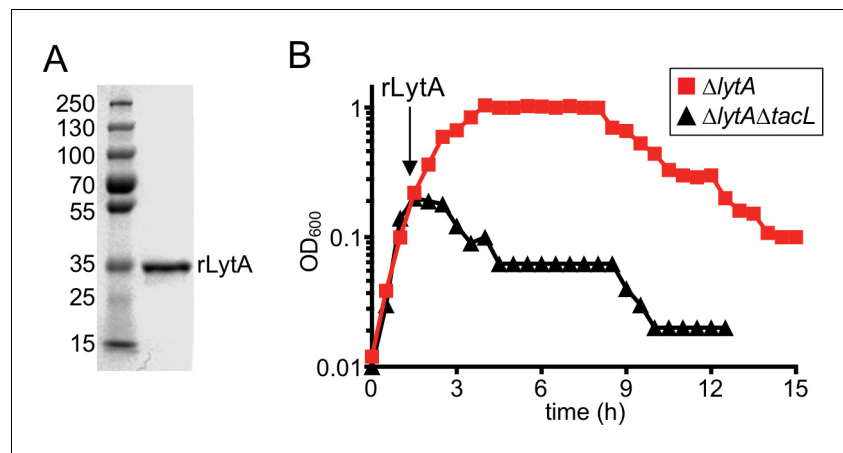


Figure 3. Cells lacking TacL are hypersensitive to exogenous LytA. (A) Coomassie-stained gel of recombinant LytA (rLytA) purified from *E. coli*. Molecular weight markers (in kDa) are shown. (B) Growth curves of the indicated strains before and after the addition of 1 $\mu\text{g/ml}$ rLytA. Cells lacking *lytA* (ΔlytA) or *tacL* and *lytA* ($\Delta\text{lytA} \Delta\text{tacL}$) were challenged with rLytA at an OD_{600} of ~ 0.2 . In the absence of TacL, cells rapidly lyse after rLytA addition. By contrast and as reported previously, the ΔlytA (TacL^+) strain only lyses in stationary phase in a manner similar to LytA^+ cells (Fernebro et al., 2004; Mellroth et al., 2012).

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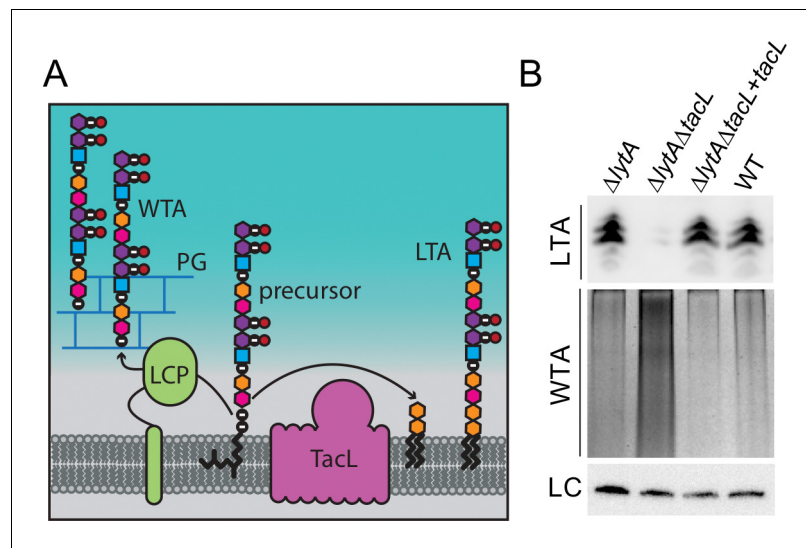


Figure 4. Cells lacking TacL contain altered levels of teichoic acids. (A) Schematic model depicting the final steps in the synthesis of WTA and LTA in *Sp*. WTAs and LTAs are thought to be synthesized from a common precursor polymer that is linked to an undecaprenyl phosphate lipid carrier (Denapante et al., 2012). The polymer is transferred to the PG by LCP proteins to form WTA (Denapante et al., 2012). TacL is hypothesized to transfer the precursor to a glycolipid anchor to generate LTA (Heß et al., 2017). (B) Analysis of LTA and WTA levels during exponential growth in wild-type (WT) or cells lacking LytA (Δ lytA), LytA and TacL (Δ lytA Δ tacL) or the double mutant harboring a Zn-inducible tacL allele (+tacL) grown in the presence of 100 μ M ZnCl₂. Top: Immunoblot analysis of membrane-associated LTAs separated by 16% Tris-tricine SDS-PAGE and probed with a monoclonal antibody specific for phosphocholine. Middle: Analysis of WTAs released from purified cell wall sacculi and separated by SDS-PAGE followed by alcian blue-silver staining. Bottom: LC, loading control.

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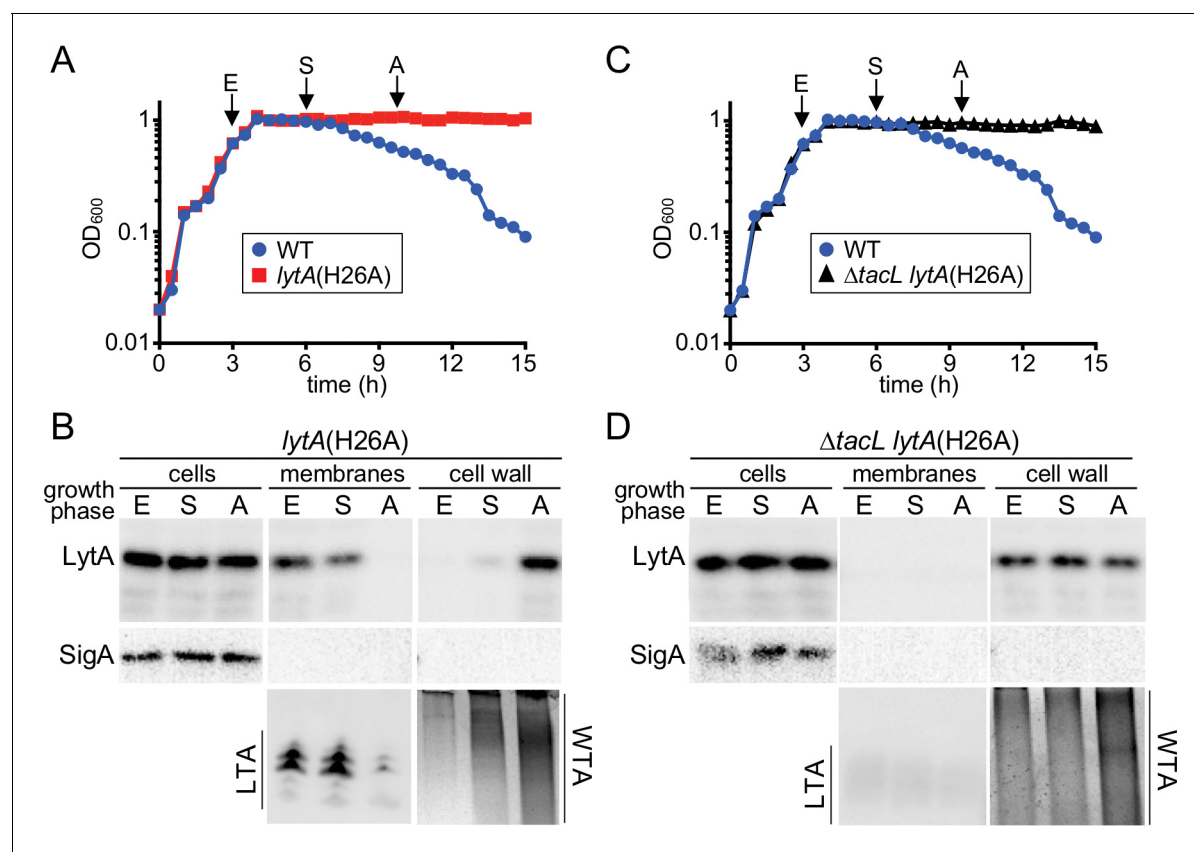


Figure 5. A switch from LTA to WTA synthesis and LytA localization occurs at the onset of autolysis. (**A and C**) Growth curves of the indicated strains cultured at 37 °C in 5% CO₂. At the indicated time points (E, exponential; S, stationary; A, autolysis), samples from the *lytA(H26A)* mutants were collected, normalized to an OD₆₀₀ of 0.5 and processed as described in Materials and methods. The WT growth curve was used as a reference for the timing of autolysis. (**B and D**) Samples from (**A**) and (**C**) were analyzed to detect LytA(H26A) in whole cell lysates (cells), associated with protoplast membranes, or cell wall sacculi. The cytoplasmic protein SigA was used as a control for protoplast integrity. The immunoblots were from the same membrane and exposure but were cropped to re-order the lanes for clarity. LTAs in the membrane fraction and WTA in the cell wall fraction were monitored by immunoblot and alcian blue-silver staining, respectively.

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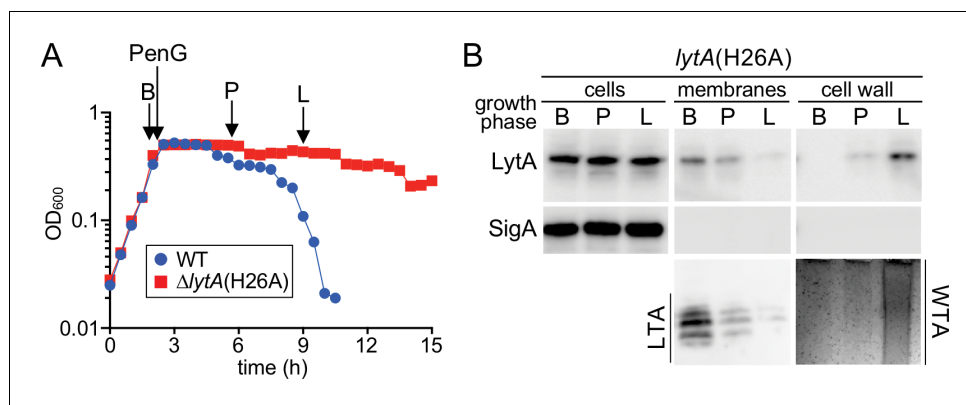


Figure 6. A switch from LTA to WTA synthesis and LytA localization occurs after exposure to penicillin. **(A)** Growth curves of the indicated strains before and after challenge with penicillin G (PenG) (0.5 μ g/ml final). At the indicated time points (B, before PenG addition; A, after addition; L, lysis), samples from the *lytA*(H26A) strain were collected, normalized to an OD₆₀₀ of 0.5 and processed as described in Materials and methods. The growth curve of WT treated with PenG was used as reference for the timing of lysis **(B)** Samples from **(A)** were analyzed to detect LytA (H26A) in whole cell lysates (cells), associated with protoplast membranes, or with cell wall sacculi. The cytoplasmic protein SigA was used as a control for protoplast integrity. The immunoblots were from the same membrane and exposure but were cropped to re-order the lanes for clarity. LTAs in the membrane fraction and WTA in the cell wall fraction were monitored by immunoblot and alcian blue-silver staining, respectively.

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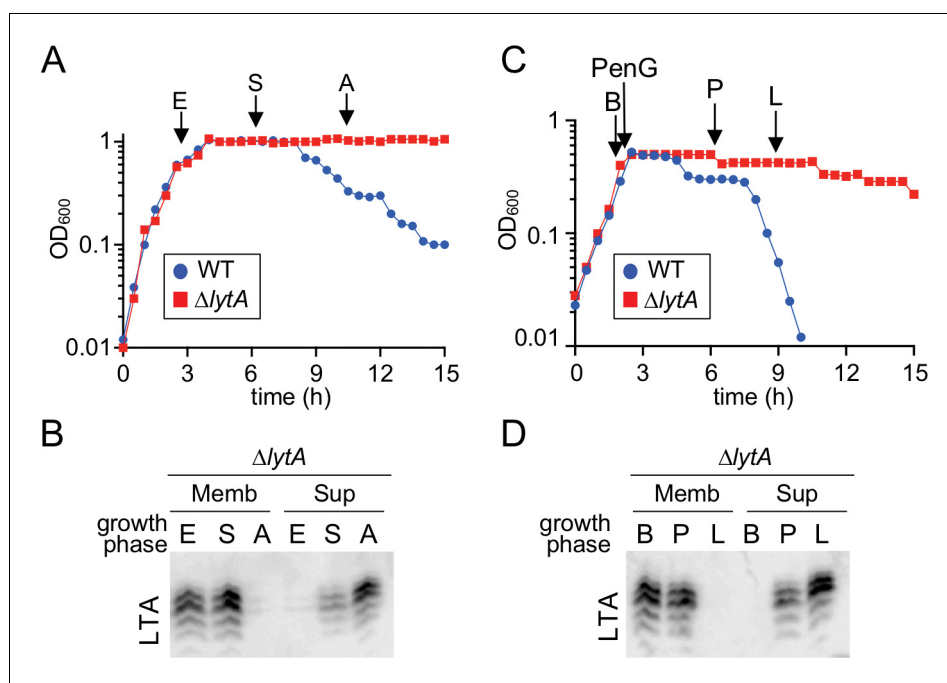


Figure 7. Teichoic acids are released into the culture medium during autolysis. (A) Growth curve of the indicated strains cultured at 37 °C in 5% CO₂. At the indicated time points (E, exponential; S, stationary; A, autolysis), samples from the $\Delta lytA$ mutant were collected, normalized to an OD₆₀₀ of 0.5 and processed as described in Materials and methods. The WT growth curve was used as a reference for the timing of autolysis. (B) Samples from (A) were analyzed to detect LTA in membranes (Memb) or culture medium (Sup, supernatant) by immunoblot. (C) Growth curve of the indicated strains cultured at 37 °C in 5% CO₂. At an OD₆₀₀ of ~0.5 the cultures were challenged with penicillin G (PenG) (0.5 µg/ml final). At the indicated time points (B, before PenG addition; A, after addition; L, lysis), samples from the $\Delta lytA$ mutant were collected and normalized to an OD₆₀₀ of 0.5. (D) Samples from (C) were analyzed to detect LTA in membranes (Memb) or supernatant (Sup) by immunoblot.

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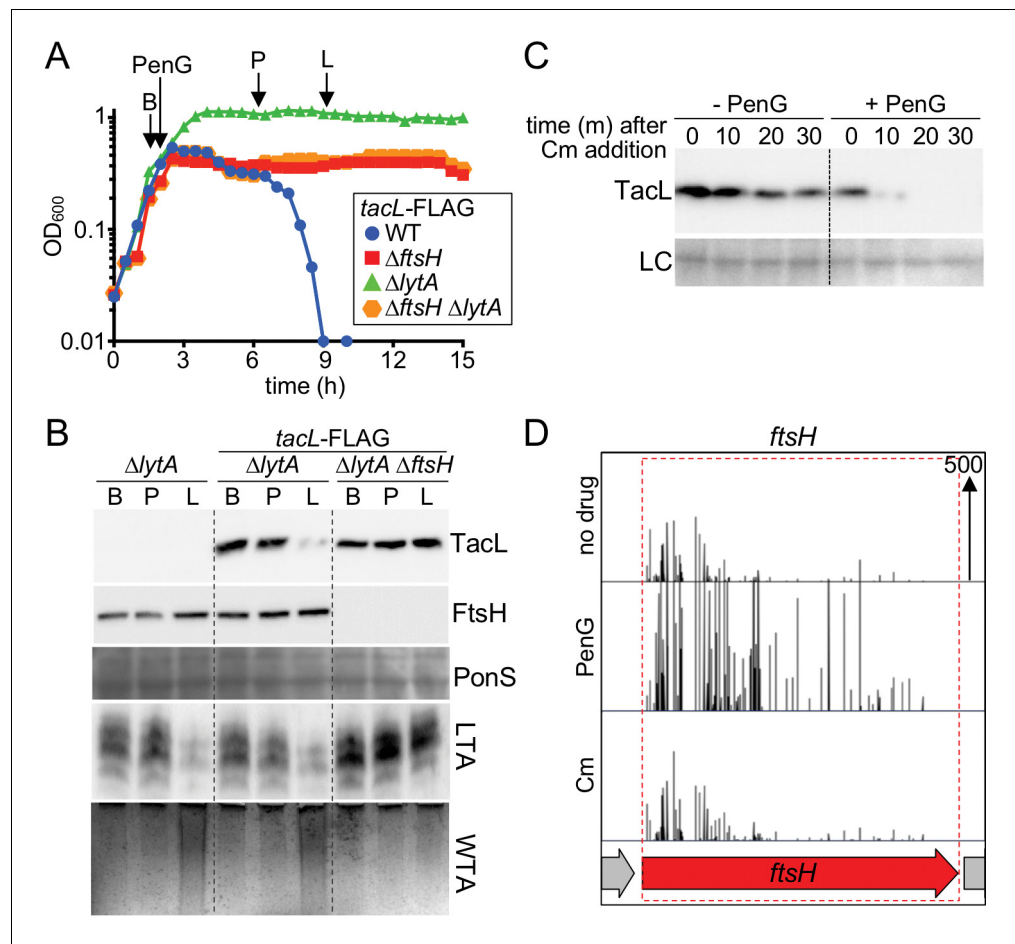


Figure 8. Penicillin treatment causes a reduction in TacL protein levels that depends on FtsH. **(A)** Growth curves of the indicated strains harboring a functional TacL-FLAG fusion as the sole source of TacL. At an OD₆₀₀ of ~0.5 the cultures were challenged with penicillin G (PenG) (0.5 µg/ml final). At the indicated time points (B, before penG addition; A, after addition; L, lysis), samples from the $\Delta lytA$ and $\Delta ftsH \Delta lytA$ strains were collected and normalized to an OD₆₀₀ of 0.5. Samples from a TacL⁺ $\Delta lytA$ strain were collected at the same time points. The growth curve of this strain was omitted from the figure for clarity. The growth curve of the TacL-FLAG LytA⁺ strain (WT) treated with PenG was used as reference for the timing of lysis. **(B)** Samples from **(A)** were processed as described in Materials and methods to detect TacL-FLAG and FtsH from whole cell lysates, LTA from membrane preparations, and WTAs released from purified cell wall sacculi. A region of the nitrocellulose membrane used for immunoblot analysis was stained with Ponceau S (PonS) to control for loading. **(C)** TacL-FLAG stability before and after PenG exposure. Wild-type *Sp* harboring TacL-FLAG was grown as in **(A)**. Prior to PenG exposure (-PenG) or 2 hr after addition (+PenG), cultures were treated with chloramphenicol (Cm) (50 µg/ml final) to block translation. Samples were collected immediately before and 10, 20, 30 min after Cm addition and analyzed by SDS-PAGE and anti-FLAG immunoblotting to detect TacL. A region of the nitrocellulose membrane used for immunoblot analysis was stained with Ponceau S (PonS) to control for loading. **(D)** Transposon insertion profiles from a mariner transposon library generated in wild-type *Sp* and grown in the presence or absence of sub-inhibitory doses of penicillin G (PenG) or chloramphenicol (Cm). Transposon insertions in *ftsH* were significantly enriched ($p < 0.0001$) in the presence of PenG compared to no drug or the Cm-treated control. The following figure supplements are available for **Figure 8**.

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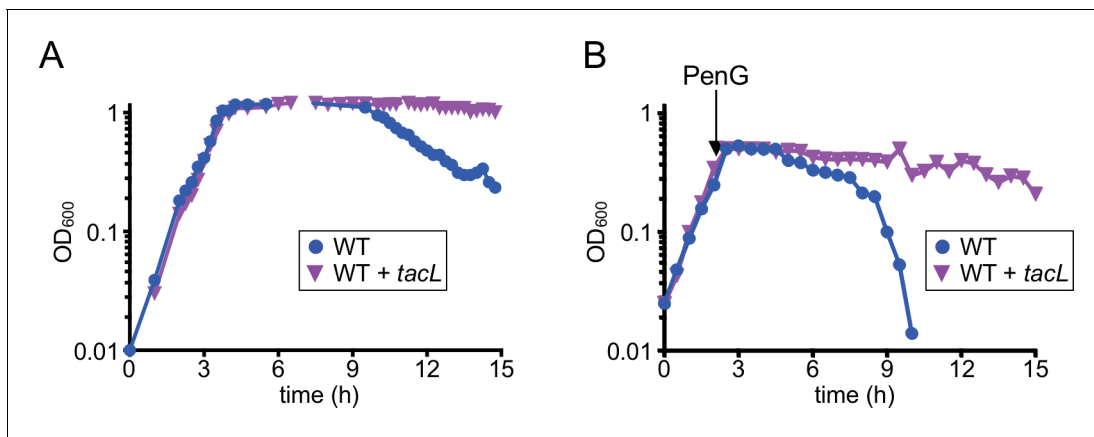


Figure 8—figure supplement 1. *tacL* overexpression prevents growth-phase-dependent and antibiotic-induced autolysis. (A) Growth curves of wild-type (WT) *Sp* and a strain harboring a zinc-inducible *tacL* allele (WT + *tacL*). Exponentially growing cultures were diluted into fresh THY at an OD₆₀₀ of 0.025 in the presence of 100 μM ZnCl₂ and incubated at 37°C in 5% CO₂. (B) The same strains as in (A) were grown in THY in the presence of 100 μM ZnCl₂ to an OD₆₀₀ of 0.5 and then challenged with penicillin G (0.5 μg/ml final). Growth was monitored approximately every 30 min for 15 hr. Note that *tacL* overexpression blocks cell lysis in both conditions.

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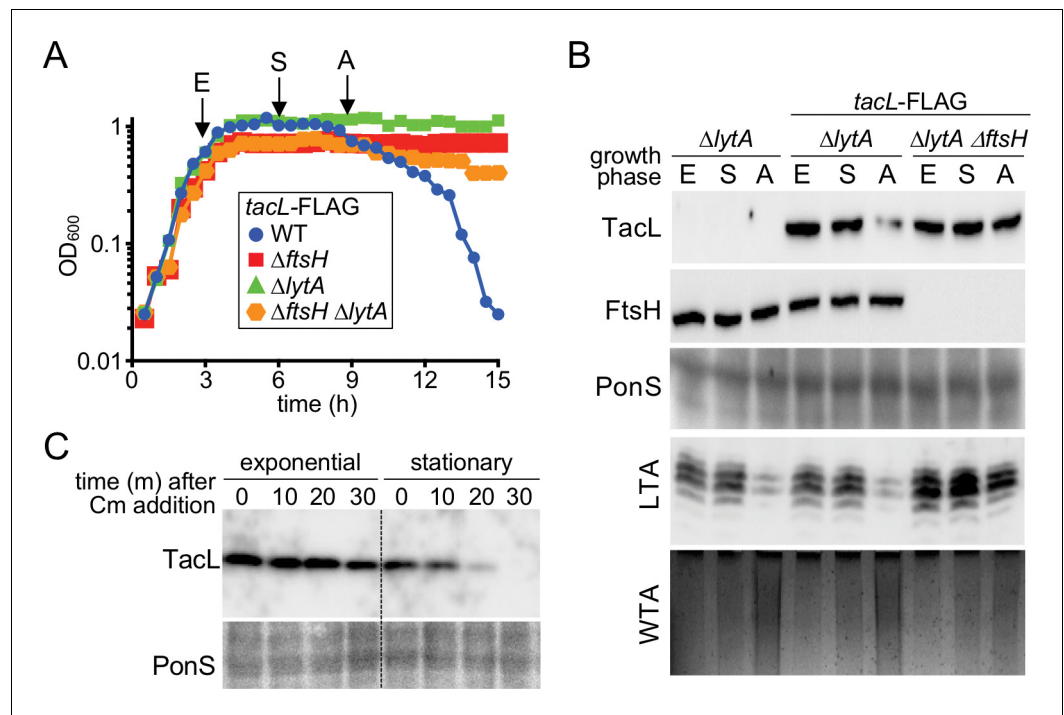


Figure 8—figure supplement 2. Reduction in TacL protein levels correlates with the growth phase-dependent switch from LTA to WTA synthesis and autolysis. **(A)** Growth curves of the indicated strains harboring a functional TacL-FLAG fusion as the sole source of TacL. At the indicated time points (E, exponential phase; S, stationary phase; A, autolysis), samples from the $\Delta lytA$ and $\Delta ftsH \Delta lytA$ strains were collected and normalized to an OD₆₀₀ of 0.5. Samples from a TacL⁺ $\Delta lytA$ strain were collected at the same time points. The growth curve of this strain was omitted from the figure for clarity. The growth curve of the TacL-FLAG LytA⁺ strain (WT) was used as reference for the timing of autolysis. **(B)** Samples from **(A)** were processed as described in Materials and methods to detect TacL-FLAG and FtsH from whole cell lysates, LTA from membrane preparations, and WTAs released from purified cell wall sacculi. A region of the nitrocellulose membrane used for immunoblot analysis was stained with Ponceau S (PonS) to control for loading. **(C)** TacL-FLAG stability in exponential and stationary phases. Wild-type *Sp* harboring TacL-FLAG was grown as in **(A)**. In exponential phase (E) or 2 hr after entry into stationary phase (S), cultures were treated with chloramphenicol (Cm) (50 μ g/ml final) to block translation. Samples were collected immediately before and 10, 20, 30 min after Cm addition and analyzed by SDS-PAGE and anti-FLAG immunoblotting to detect TacL. A region of the nitrocellulose membrane used for immunoblot analysis was stained with Ponceau S (PonS) to control for loading.

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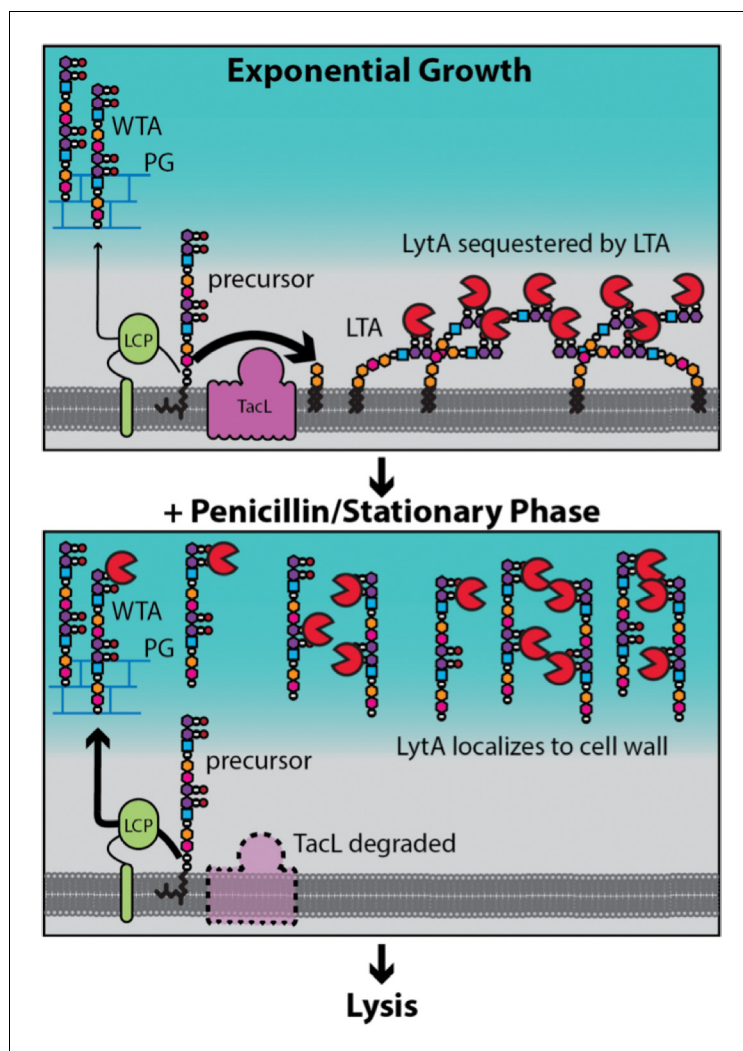


Figure 9. Model for LytA regulation. During exponential growth, TacL-dependent LTA synthesis dominates over LCP-dependent production of WTA. LytA bound to the phosphocholine moieties (purple/black balls) on LTA is sequestered away from WTA preventing LytA from targeting the cell wall. Upon entry into stationary phase or exposure to cell wall targeting antibiotics (penicillin), TacL is degraded in an FtsH-dependent manner leading to a reduction in LTA synthesis and an increase in WTA levels. Association of LytA with newly synthesized WTA leads to cell wall cleavage and lysis.

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