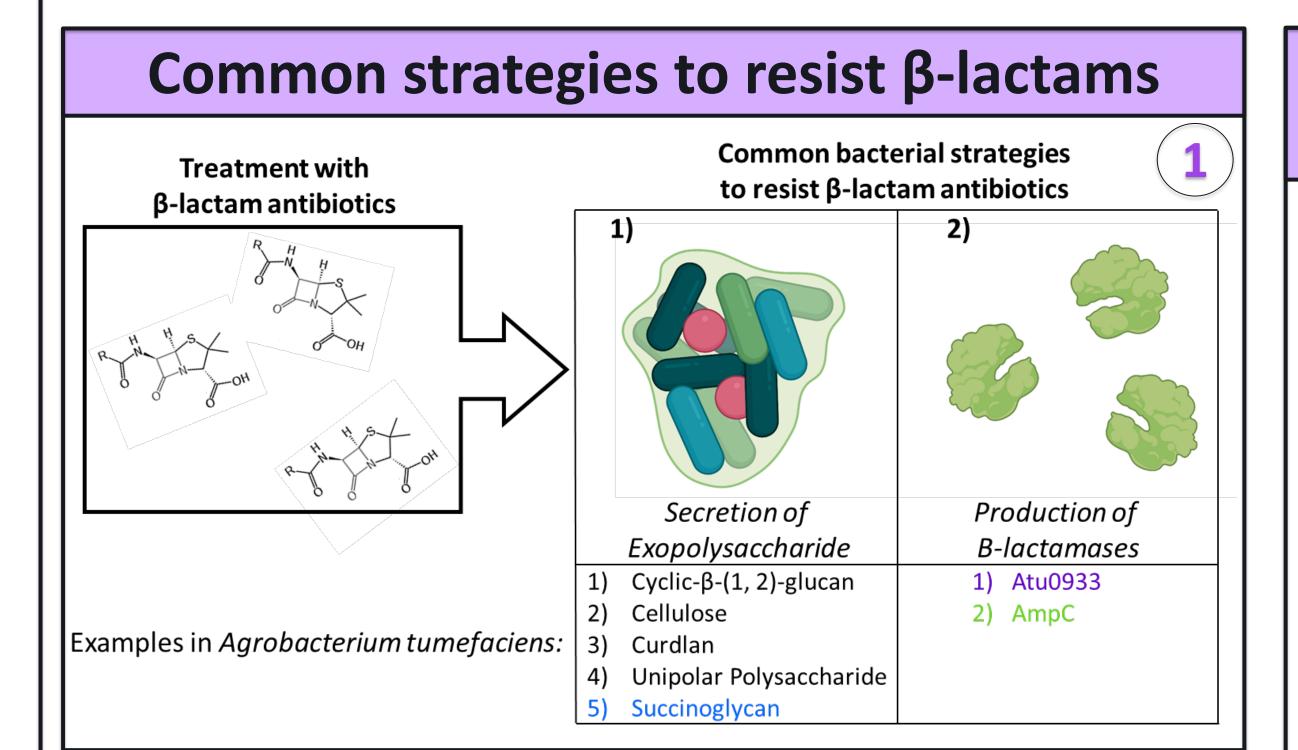


Succinoglycan and β-lactamase Production Confers Resistance to Cell-Wall Targeting Antibiotics in *Agrobacterium tumefaciens*



Amara Mason, Jacob Bouchier and Pamela J.B. Brown Division of Biological Sciences, University of Missouri, Columbia MO

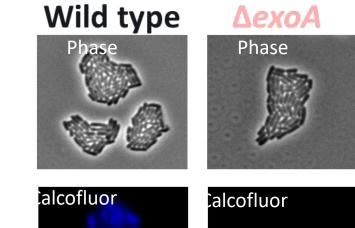


Outer Membrane Chys Ch

The succinoglycan biosynthesis pathway is activated by the ChvG-ChvI two-component system. ExoR is know to be degraded in the presence of acid, leading to phosphorylation of ChvI and succinoglycan biosynthesis.

Succinoglycan biosynthesis requires ExoA, a glycosyltransferase.

Succinoglycan production and export is dependent on ExoA



to β-lactam resistance.

Calcofluor white binds succinoglycan allowing for visualization of succinoglycan using fluorescence microscopy. Here we show that ExoA is required for succinoglycan production.

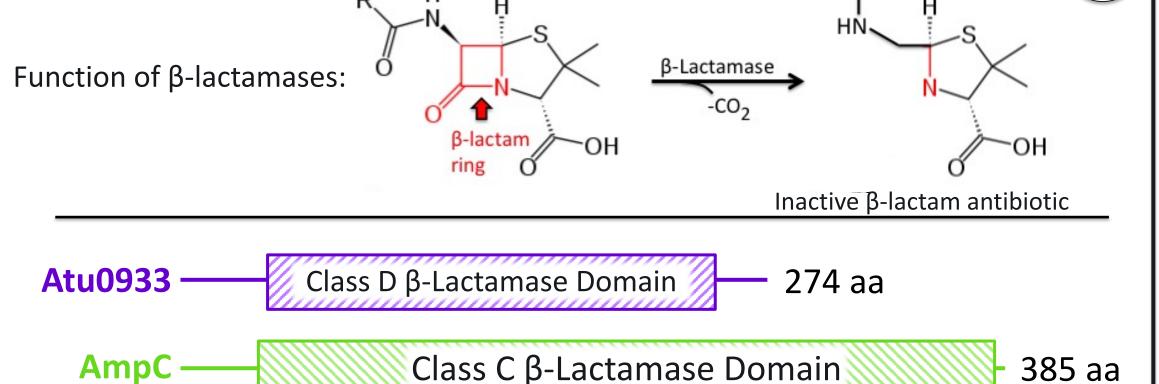
Research Question: How does Agrobacterium tumefaciens tolerate beta-lactam antibiotics? Using an antibiotic disc diffusion assay we find that ΔexoA, ΔchvG, and ΔchvI, show increased sensitivity to β-lactam antibiotics. We also see increased size in zones of inhibition when adding sulbactam, a β-lactamase inhibitor. From these data we can conclude 2 things: 1) Succinoglycan contributes to β-lactam resistance. 2) There are β-lactamases contributing

Objective and Hypothesis

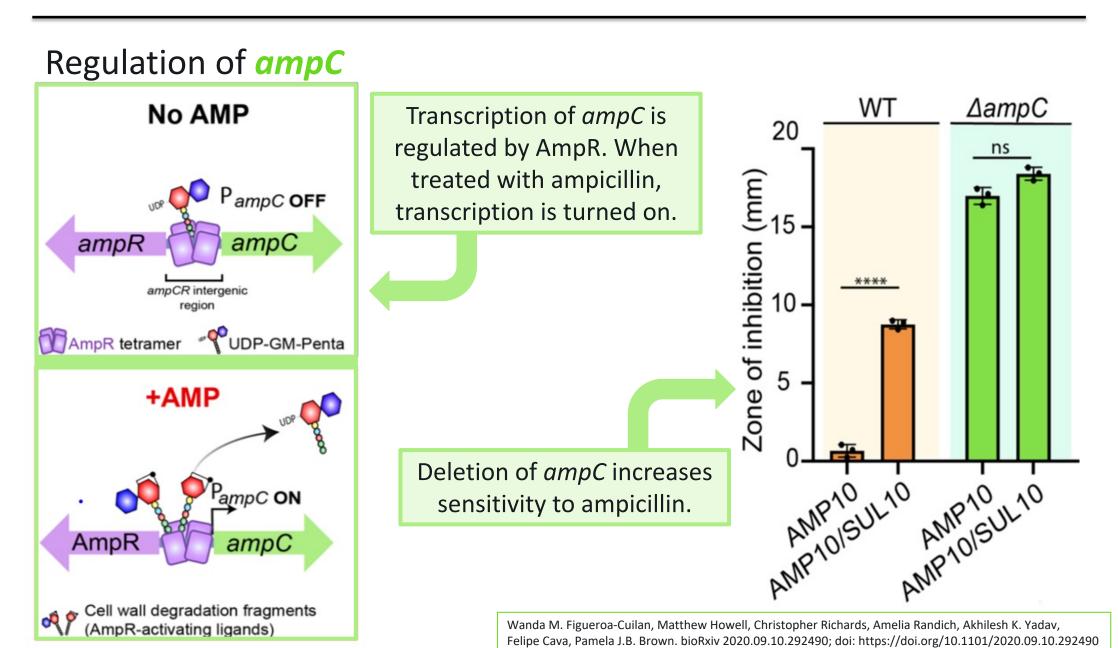
Hypothesis: *A. tumefaciens* secretes β-lactamases which contribute to survival during cell wall-targeting antibiotic stress.

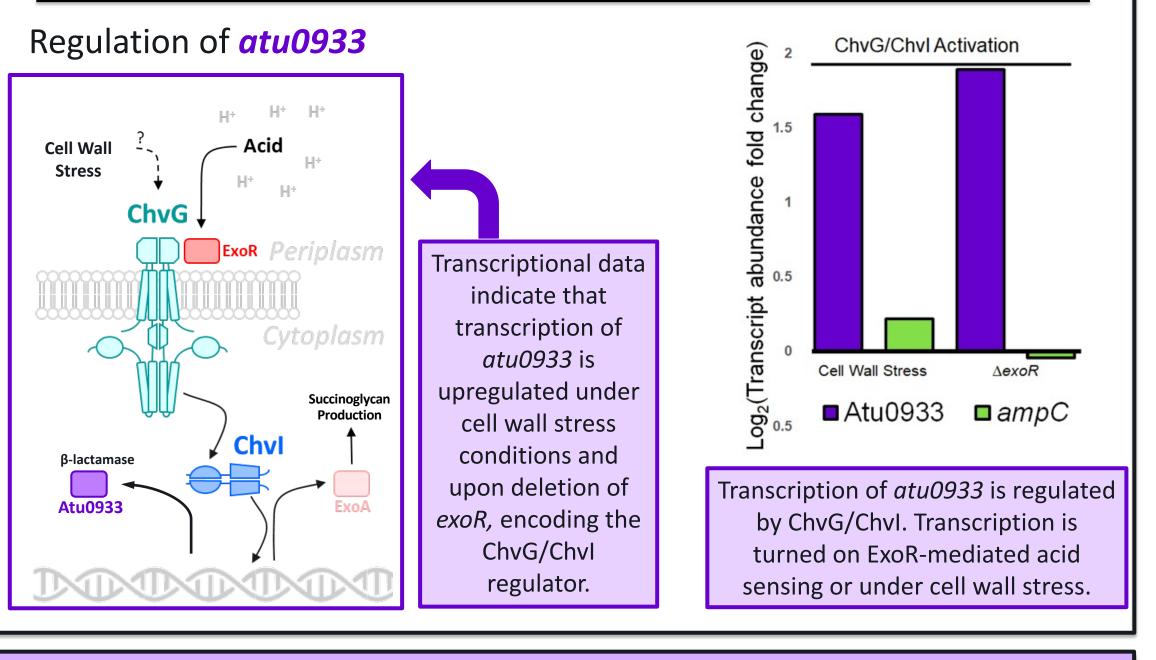
The genome of *A. tumefaciens* encodes two β-lactamases that are differentially regulated

Active β-lactam antibiotic

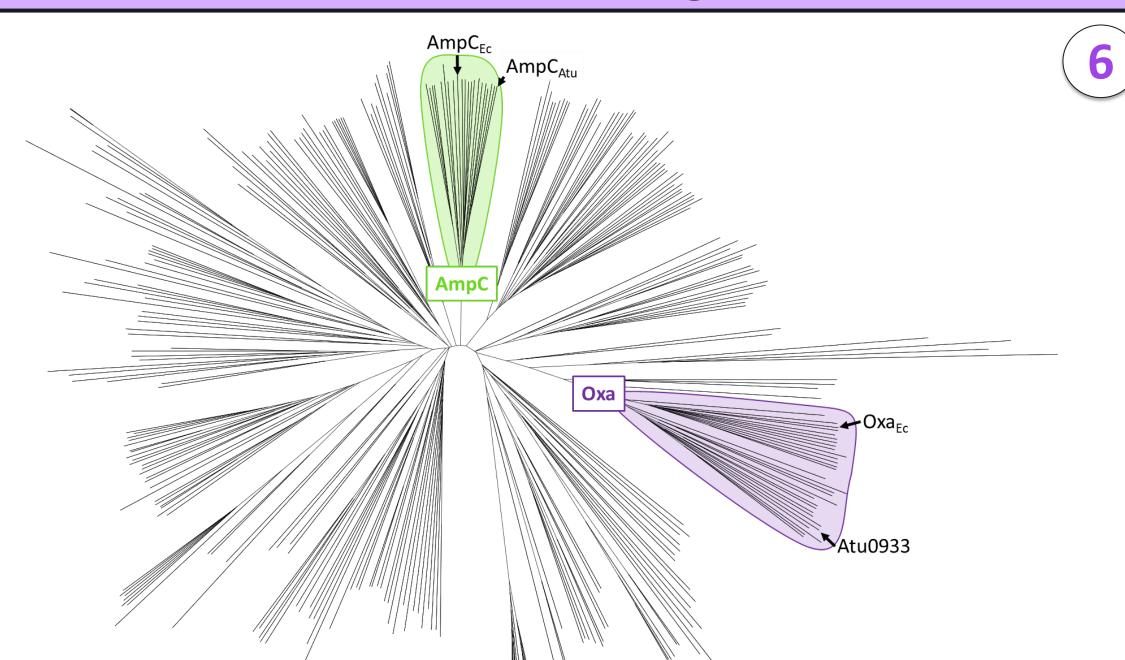


Bioinformatic analysis suggests that Atu0933 is a class D β -lactamase, an understudied group of β -lactamases. AmpC belongs to a well-studied group of β -lactamases that can be inhibited by sulbactam. Our lab has shown that AmpC is likely the culprit for the differences in zones of inhibition upon addition of sulbactam.

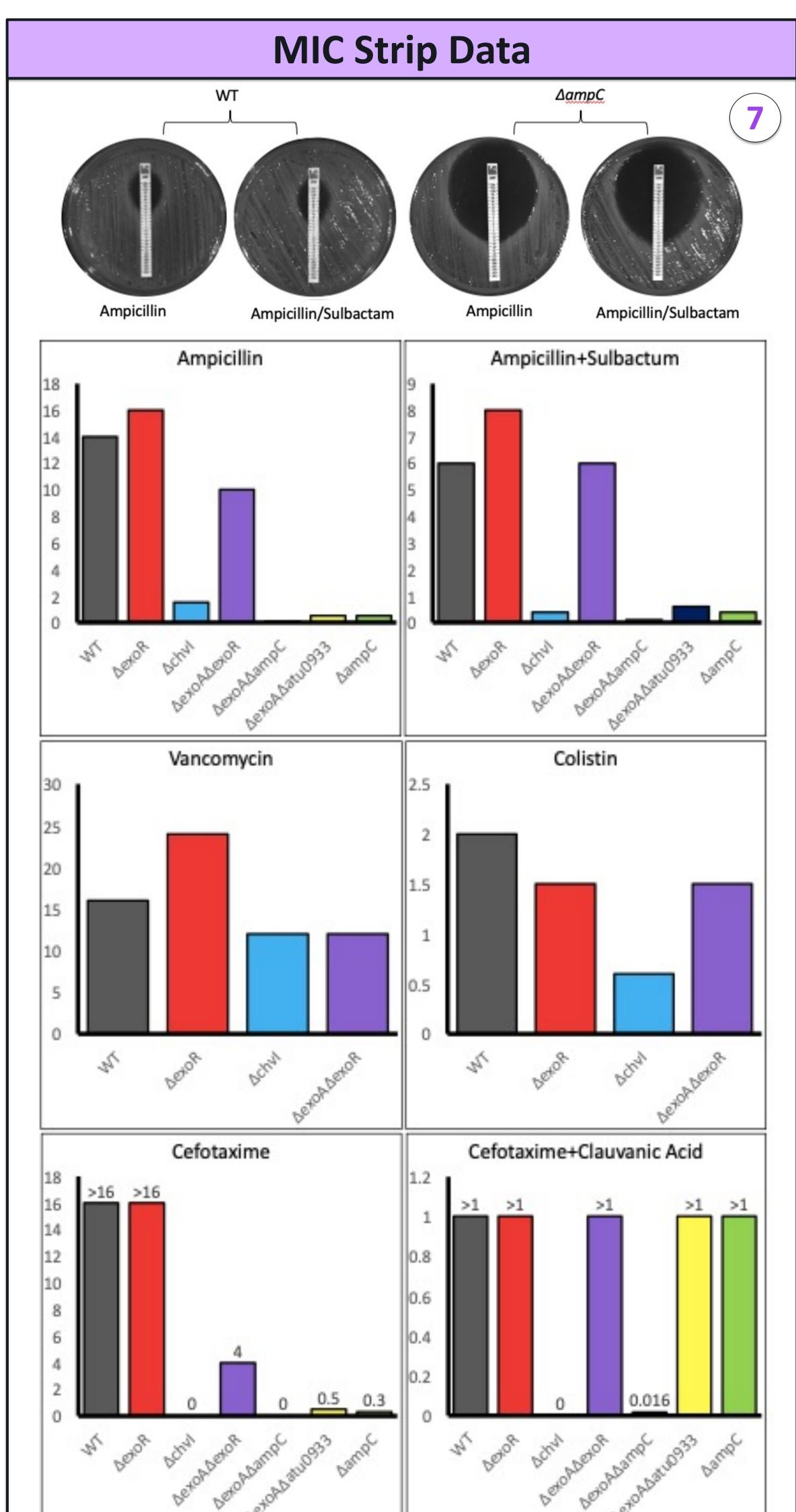




Phylogenetic analysis indicates Atu0933 may be an Oxa ortholog



Oxa orthologs typically have specificity for penicillins and cephalosporins, but not carbapenems nor monobactams. Some Oxa orthologs can also be inhibited by clavulanic acid, while others cannot be.



Summary of Results

- Δ*exoR* constitutively produces succinoglycan, providing resistance to each of antibiotic stress
- Δ*chvI* has extreme sensitivity to ampicillin and cefotaxime suggesting that the ChvG-ChvI pathway is required for some level of resistance
- The loss of succinoglycan through the deletion of ΔexoA, resulted in increased sensitivity to ampicillin, but not to the level of the chvl deletion, suggesting that chvl regulates additional resistance mechanisms

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