

## Succinoglycan and $\beta$ -lactamase Production Confers Resistance to Cell Wall Antibiotics in *Agrobacterium tumefaciens*

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Peptidoglycan (PG) is an essential feature of the cell wall in almost all bacteria. When PG is compromised, bacteria succumb to osmotic pressures and lyse. For this reason, PG-targeting  $\beta$ -lactam antibiotics including penicillins, carbapenems, monobactams, and cephalosporins are widely used to combat bacterial disease both clinically and agriculturally. However, many bacteria have evolved strategies to survive  $\beta$ -lactam treatment such as production of exopolysaccharides and  $\beta$ -lactamases. Exopolysaccharides form protective barriers against antibiotics, while  $\beta$ -lactamases inactivate  $\beta$ -lactam antibiotics. The plant pathogen *Agrobacterium tumefaciens* secretes multiple exopolysaccharides and encodes two  $\beta$ -lactamases. Here we link a well-known pathogenesis pathway to exopolysaccharide succinoglycan secretion and  $\beta$ -lactamase production. Using antibiotic disk diffusion assays we found that deletion of *chvI*, which regulates succinoglycan production, results in heightened sensitivity to  $\beta$ -lactam antibiotics. We also found that deletion of *exoA*, a gene associated with production of succinoglycan, failed to increase sensitivity to  $\beta$ -lactam antibiotics to the level of the *chvI* deletion. Next, we investigated the  $\beta$ -lactamase AmpC and found that deletion of *ampC* in the *exoA* deletion background only partially increased sensitivity to  $\beta$ -lactam antibiotics. This suggests ChvI regulates additional resistance mechanisms. We propose that additional resistance occurs through the production of the other  $\beta$ -lactamase, *atu0933*, which is known to be regulated by ChvI. Together, our findings suggest that *A. tumefaciens* has evolved to withstand natural  $\beta$ -lactam antibiotics from competing soil bacteria and fungi. This work is likely to inform on future crop disease management, particularly in areas devastated by the prevalence of *Agrobacterium* species.