

Regulation of disease susceptibility in *Physcomitrella*

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We have investigated the susceptibility of *Physcomitrella* to microbial pathogens, with the goal of establishing a pathosystem in which both the plant host and the pathogen are amenable to direct genetic manipulation through homologous recombination and other methods. *Physcomitrella* was susceptible to a variety of bacterial pathogens including *Pseudomonas aeruginosa*, *Lysobacter enzymogenes* and *Xanthomonas* spp. The bacteria multiplied *in planta* and caused host cell death. We focused on *L. enzymogenes* because this pathogen caused extensive and rapid programmed cell death (PCD) in *Physcomitrella*.

First, we used transposon insertion and deletion mutants in the bacterial genome to test whether the outcome of bacterial infection is under genetic control of the pathogen. We found that the ability of *L. enzymogenes* to infect *Physcomitrella* depended on classical bacterial virulence mechanisms including: (i) a type-three secretion system (TTSS) which is predicted to deliver virulence effector molecules into host cells and (ii) the production of lytic enzymes, such as chitinase, glucanase and proteases. Both of these virulence mechanisms are important for pathogens of higher plants and animals.

Second, we created gene knockouts in *Physcomitrella* to test whether the outcome of infection is under genetic control of the host plant. We found that the ability of *L. enzymogenes* to infect *Physcomitrella* depended on the presence of plant genes that regulate PCD. These genes comprise both positive and negative effectors of PCD that appear to act in (or on) a core PCD pathway. Their deletion in knockout lines affected the ability of *Physcomitrella* to undergo PCD in response to a broad range of biotic and abiotic stress signals. Knockout lines for negative regulators of PCD were severely compromised in their ability to be infected by *L. enzymogenes*, while knockout lines for positive regulators of PCD were essentially resistant to infection. Similar effects were observed when other pathogens were used as the inoculum.

These studies establish *Physcomitrella* as a tractable and relevant plant system for studying PCD and its role in infection and provide the foundation for characterizing other pathogen and plant genes that contribute to the progression of infection.