Cell biology

Bacteria deliver water channels to infect plants

Gwyn A. Beattie

A wide range of harmful bacteria introduce proteins into plant cells. Some of these proteins move to the cell membrane and serve as channels for water and nutrients, creating favourable conditions for bacterial growth beside plant cells. See p.586

For many pathogenic bacteria, a key step in becoming harmful is acquiring the ability to inject proteins directly into the cells of the host they infect. These 'effector' proteins often interact with host targets to reduce the host's defence response. For pathogens that infect plants, these effectors might also help the bacteria to overcome water and nutrient shortages in the plants' intercellular spaces, called the apoplast. On page 586, Nomura *et al.*[1] solve a long-standing mystery about the biochemical role of a major family of effector proteins. The authors find that these proteins induce the release of water into the apoplast, giving rise to a phenomenon called water soaking.

Nomura *et al.* show that bacterial effectors belonging to the AvrE/DspE family fold into a structure resembling a type of protein that forms a channel in the outer membrane of bacteria, called a bacterial porin. When inserted into the cell membranes of plant cells, the bacterial porin-like effector proteins create channels that are permeable to water and solutes such as nutrients (Fig. 1). This bacterial manipulation of the plant creates a water- and nutrient-rich environment for bacterial growth in the apoplast. The authors also found that blocking these channels inhibits bacterial infection of plants.

Many bacterial pathogens that target plants rely on effector proteins, although the effector repertoires of each pathogen vary from one protein to dozens. The AvrE/DspE family of effectors is thought to have been an early innovation during the evolution of bacterial pathogens. This is because this family is found in most effector-producing bacterial pathogens and the genes encoding these effectors are located beside those encoding the effector-injection machinery. Yet despite this evolutionary conservation and wide distribution, the biochemical roles of this effector family in host cells have remained mysterious, until now.

The difficulty in gaining a mechanistic understanding of these effectors lies in their extremely large size (around 2,000 amino-acid residues) and their toxicity when expressed in yeast and cultured plant cells. They show little similarity to proteins with known functions, although a role in the cell membrane was suggested when an effector was shown to move to the cell membrane after being injected into a plant cell[2]. Although they are large proteins with many possible domains, none of the domains has a clear function, and most are present in some but not all members of this family of proteins.

"The study demonstrates an exciting application of these findings in managing bacterial plant diseases."

Many clues have arisen regarding the roles of this family of proteins since its discovery almost 35 years ago[3]. Although the proteins are associated with the suppression of plant-defence responses, similar to the action of effectors from other protein families, AvrE/DspE-family effectors are essential to many pathogens' ability to cause disease. This family is also uniquely associated with macroscopic water soaking of infected plant tissue, suggesting a role in modulating the hydration of the apoplast.

One possible mechanism underlying water soaking was thought to be the leakage of water from plant cells undergoing a process of death called necrosis, but this idea was discounted on the basis of a detailed timeline of events during infection[4]. Another mechanism might be a reduced loss of water from plant pores, called stomata, that connect the apoplast to the outside air. Indeed, studies have associated an AvrE/DspE-family effector with stomatal closing[5,6], but the report by Nomura *et al.* shows that there is more to the story.

A key clue to solving this effector-function mystery came from using artificial intelligence in the form of the protein-folding-prediction program[7] AlphaFold2, which indicated that AvrE/DspE-family effector proteins are structurally related to bacterial porins. The authors tested whether this unexpected prediction was correct by performing targeted studies to assess whether the proteins had porin-like, or channel, functions. Nomura and colleagues

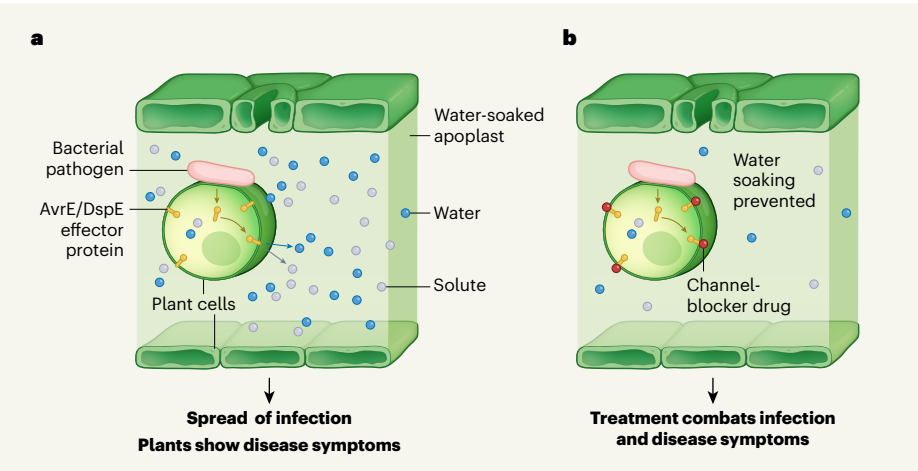

Figure 1 | Harmful bacteria can introduce water- and solute-permeable channels into the cell membranes of plants. a, Bacterial pathogens that infect plants can inject proteins, called effectors, into the host's cells. Nomura *et al.*[1] report that effector proteins in the AvrE/DspE family fold into structures that resemble bacterial proteins (called porins), which form channels in the outer membrane of bacterial cells. After injection into plant cells, these proteins insert into the plant cell membrane and function as water- and solute-permeable channels. These channels enable the movement of water and nutrients into the spaces between the plant cells, termed the apoplast, which results in an aqueous, nutrient-rich environment. This hydration state, called water soaking, is crucial for the growth of invading plant pathogens and the development of disease symptoms in infected plants. **b**, Treatment with a compound that blocks these channels prevents the movement of water and solutes into the apoplast, inhibits the growth of harmful bacteria and reduces or prevents the development of disease symptoms.

used a combination of approaches centring on a model system for plant-membrane studies comprising the developing eggs (oocytes) of the African clawed frog *Xenopus laevis*, and lipid vesicles (termed liposomes) generated *in vitro*.

The authors demonstrated that effector expression in *X. laevis* oocytes promoted ion currents across the cell membrane, as well as swelling and bursting of the cells dependent on solute concentration (osmolarity) and passive uptake of a fluorescent dye, all of which were consistent with a channel function. Moreover, although the presence of the effector protein in liposomes containing a fluorescent dye resulted in release of the dye, liposomes containing a fluorescent protein that was larger than the predicted size of the channel did not release the fluorescent protein.

Collectively, the authors' results indicate that AvrE/DspE-family effectors function as water- and solute-permeable channels in the cell membranes of eukaryotes (organisms that have a nucleus). These are among the first known effectors of plant-targeting bacteria to exhibit a direct cellular function in plant cells, rather than operating through host proteins. The findings add to a growing body of evidence that manipulation of apoplastic water is a primary control point for pathogens.

The study also demonstrates an exciting application of these findings in managing bacterial plant diseases. The authors predicted the channel diameter and identified chemicals that were then engineered to block the channels. These synthetic compounds not only inhibited the channel activities of the effectors in *X. laevis* oocytes, but also reduced or eliminated plant infections by two bacterial pathogens. AvrE- and DspE-mediated infections by *Pseudomonas syringae* pv. *tomato* on the model thale cress plant *Arabidopsis thaliana* and *Erwinia amylovora* (fire blight) on pear fruits were inhibited, respectively. These findings provide a new and potentially effective management strategy for bacterial pathogens. Moreover, by targeting an activity required for plant infection but not for bacterial growth, as such, channel blockers of this type should avoid favouring the emergence of drug-resistant pathogens, as occurs with antimicrobial treatments.

The authors have identified a key pathogen factor that helps to combat the limited water availability in the apoplast and therefore promote bacterial growth. A major unresolved question is how these proteins actually manage to modulate water and solute movement to generate a suitable aqueous environment in the apoplast. Answering this question will require a better understanding of the dynamics of water and solute gradients across the cell membrane of plants. The discovery of similar effectors made by eukaryotic pathogens called oomycetes[8] raises the question of whether other plant-targeting pathogens also use such channels during infection.

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1. Nomura, K. *et al*. *Nature* **621**, 586–591 (2023).
2. Xin, X.-F. *et al*. *Plant Physiol*. **169**, 793–802 (2015).
3. Kobayashi, D. Y., Tamaki, S. J. & Keen, N. T. *Proc. Natl Acad. Sci. USA* **86**, 157–161 (1989).
4. Gentzel, I. *et al*. *Cell Host Microbe* **30**, 502–517 (2022).
5. Hu, Y. *et al*. *Cell Host Microbe* **30**, 518–529 (2022).
6. Roussin-Léveillée, C. *et al*. *Cell Host Microbe* **30**, 489–501 (2022).
7. Jumper, J. *et al*. *Nature* **596**, 583–589 (2021).
8. Deb, D., Mackey, D., Opiyo, S. O. & McDowell, J. M. *PLoS ONE* **13**, e0195559 (2018).

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Polymer chemistry

Waste product from wood finally used to make glue

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Scientists have had limited success in converting lignin, a structural component of plants, into high-value products. The discovery that lignin can be used as a wood glue could be a game-changer for biorefineries. **See p.511**

On page 511, Yang *et al*.[1] describe a remarkable strategy for making a wood adhesive from lignin – the extraordinary polymer that strengthens and binds together plant cells. Lignin waste streams (known as technical lignins), currently generated from wood pulp and paper production, are notoriously ineffective for making adhesives of the required performance and at the scale needed for manufacturing wood composites. Now, for the first time, a lignin waste stream can be used as a high-value feedstock for manufacturing wood adhesives and other products. Furthermore, the findings could pave the way for a new generation of integrated plant-processing biorefineries – facilities that process biomass, such as agricultural waste, trees and grasses, with high efficiency to produce renewable energy, chemicals and other products.

Lignin enables trees to reach grand heights and prevents maize (corn) stalks from buckling in the wind. Plants use lignin to protect cellulose, the major part of non-edible plant matter. Cellulose consists of polymeric chains of linked glucose molecules, and assembles into long, stiff and exquisitely uniform fibres, which plants use to make their structures. Nearly all of Earth's organisms metabolize glucose for energy, and so cellulose is a much more concentrated source of energy than is glucose. There is therefore great intrinsic value in cellulose, which is why plants evolved to produce lignin to protect it.

The polymer chains in lignin are highly irregular and arranged in networks around and between cellulose fibres. The chain links are ingeniously variable, able to form multiple chemical structures. When heated in fire, or subjected to industrial processing, lignin linkages break and re-form to make stronger ones. This remarkable self-healing capacity can be either destroyed or preserved when lignin is isolated. When self-healing is destroyed, unreactive technical lignin is produced. But lignin that retains its self-healing properties holds promise for making a new glue.

Until now, the marvellous structure and chemical reactivity of lignin has frustrated scientists' attempts to use it to make high-value products. Yang *et al*. describe a surprisingly simple, and yet chemically ingenious, strategy to remove lignin from wood while preserving its self-healing properties. The authors stir ground wood, organic solvent, a little acid catalyst and strategically chosen amounts of water and formaldehyde under heating. After cooling, the cellulose is filtered away for further use, and the lignin is readily processed into a water-dispersible powder, which is used to make a water-based glue.

And not just any glue, but a structural wood adhesive (Fig. 1): the type needed to make wood-based composites, such as plywood, that are used in structures (such as walls and floors) that support heavy loads in buildings. Structural-wood adhesives must pass rigorous performance tests to satisfy national and international building codes, and the authors report results indicating that their glue could meet structural certification requirements. In North America, most people's homes contain both structural and non-structural wood composites – the latter are used to make cabinets