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BACTERIAL PHYSIOLOGY

Cropping up in an aerobic niche

> *C. puniceum* produces clostrubin A and clostrubin B to survive in oxygen-rich environments and to inhibit the growth of competing crop pathogenic bacteria

Clostridium puniceum is an important plant pathogen and causes slimy rot in potatoes. Although Clostridia are generally obligate anaerobes, *C. puniceum* grows well in the aerobic environment found in potato tubers. Shabuer, Ishida and Pidot *et al.* have now identified the metabolites that enable this bacterium to not only defend its niche against other crop pathogenic bacteria but also to tolerate oxygen.

C. puniceum produces characteristic pink pigments in infected potatoes. When the authors analysed extracts from potatoes that were experimentally inoculated with *C. puniceum* by high-performance liquid chromatography, they detected two peaks in the ultraviolet to red light spectrum corresponding to the metabolites responsible for this pigmentation. Using mass spectrometry they were able to identify one of these metabolites as clostrubin A, an aromatic polyketide that was previously found to be a product of the soil dwelling *Clostridium beijerinckii*. Nuclear magnetic resonance spectroscopy showed that the second metabolite, clostrubin B, has an aromatic polyketide backbone in addition to a sugar-like linear side chain.

As neither clostrubin A or clostrubin B showed plant toxicity and thus are unlikely to be virulence factors, the authors tested their antibacterial potential to see whether these metabolites provide a competitive advantage against other crop pathogens. Both metabolites had minimal inhibitory concentrations in the nanomolar range for all tested potato-infecting bacteria. Interestingly, these other potato pathogens were all aerobic bacteria. Why would an anaerobe such as *C. puniceum* need a competitive advantage against aerobic bacteria? As *C. puniceum* exhibited vigorous growth and strong pigment production in aerobic cultures, the authors speculated that clostrubins mediate oxygen tolerance. To test this hypothesis they inactivated the *clr* gene locus, which encodes a type II polyketide synthase, the most likely enzyme responsible for the production of clostrubins. Indeed, Δ*clr* bacteria were no longer able to synthesize clostrubins and to grow and cause potato rot under aerobic conditions. The addition of clostrubin A and clostrubin B, or to culture the bacterium under anaerobic conditions, restored the growth and pathogenic activity of the mutant strain. Flow cytometry showed that Δ*clr* cells rapidly died under aerobic conditions, whereas cells incubated with clostrubins were protected and showed similar viability to wild-type cells that were exposed to oxygen. Together these results indicate that *C. puniceum* produces clostrubins to survive in oxygen-rich environments and to inhibit the growth of competing crop pathogenic bacteria, thus providing optimal adaptation of this bacterium to its niche in potato tubers.

This study questions the classification of Clostridia as obligate anaerobes, as at least *C. puniceum* seems to be able to tolerate oxygen under certain conditions owing to the production of clostrubins. The exact mechanisms underlying tolerance still need to be determined, but targeting this process might be useful as a crop-protective measure.

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ORIGINAL ARTICLE Shabuer, G., Ishida, K., Pidot, S.J. *et al.* Plant pathogenic anaerobic bacteria use aromatic polyketides to access aerobic territory. *Science* **350**, 670–674 (2015)

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