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Microbiology

Reinventing a bacterial biopesticide: an old microbe with a fresh new look

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ABSTRACT

Growing concerns over the use of synthetic pesticides in agriculture has sparked a renewed interest in natural alternatives. Our work revisits a formerly successful bacterial biological pesticide (biopesticide) that fell out of fashion over concerns of human pathogenicity.

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In the 1980s it was discovered that some relatives of the bacterium *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) were able to form close relationships with plant roots, and also make a range of antimicrobials capable of killing plant pathogens. Several US pesticide companies exploited this bacterium in biological pesticide (biopesticide) products in the 1990s. Coating crop seeds with this soil bacterium offered protection against plant pathogens that would otherwise kill up to 60% of crops. These seed coat bacterial biopesticides offered a non-toxic alternative to man-made chemical pesticides.

Around the same time *B. cepacia* bacteria were also found to cause infections in immunocompromised individuals, such as people with cystic fibrosis. The presence of *B. cepacia* in the lungs of cystic fibrosis patients was associated with poor clinical outcomes. Concerns over the use of *B. cepacia* in agriculture and the potential for human infections prompted the US Environmental Protection Agency to hold a scientific advisory meeting to discuss these bacteria. The outcome of this meeting was a suspension on registering new biopesticides containing *B. cepacia* until they could be proven safe.

B. cepacia-based biopesticide products registered before the scientific advisory meeting could still be used in agriculture, but these eventually fell out of favour alongside a rise in the use of synthetic pesticides. Over the next 20 years our understanding

of *B. cepacia* increased dramatically. Researchers recognised that *B. cepacia* actually represented at least 20 different species, each one possessing a different pathogenicity risk in humans, but all capable of causing infections in vulnerable people. The bacterium used as a biopesticide was named *B. ambifaria* and was rarely encountered in human infections; while the species most problematic in cystic fibrosis infections were *B. multivorans* and *B. cenocepacia*. Growing concerns over the use of synthetic pesticides on agriculture, combined with a better understanding of these bacteria prompted us to re-visit *B. ambifaria* as an alternative to synthetic pesticides.

This study began by sequencing the genomes of multiple *B. ambifaria* strains to understand the genetic diversity of the bacterium. We used computer software designed to analyse bacterial DNA sequences to identify genes involved in antimicrobial production. This informed us of the widespread nature of antimicrobial synthesis in *B. ambifaria* – every strain possessed genes to produce antimicrobials. Our second goal was to screen all the strains for pathogen-killing activity and understand which genes were involved in antimicrobial production. We chose a wide range of plant pathogens: bacterial, fungal, and fungal-like organisms (oomycetes) – these pathogens cause diseases such as leaf wilt, leaf blight and damping-off. Interestingly, we found correlations between the presence of different antimicrobial production genes and the killing of different pathogens.

Some of these antimicrobial production genes identified were new and as yet uncharacterised, so we disrupted their activity to see their effect on plant pathogen killing. These genes were responsible for killing the oomycete plant pathogen *Pythium* when tested in laboratory conditions. The newly characterised genes were found to make an old antibiotic called cepacin. To test their role in a biopesticide model we coated pea seeds in *B. ambifaria* with either functioning or non-functioning (mutated) cepacin genes, and challenged the seeds with the plant pathogen *Pythium*. Seeds coated with the cepacin-producing *B. ambifaria* survived, while the *B. ambifaria* with mutated cepacin genes did not protect the germinating seeds.

The genomes of *B. ambifaria* are interesting as their DNA is spread across three chromosomes, the smallest of which can be deleted and yet the bacteria remain viable. A favourable consequence of this chromosome deletion is reduced virulence in multiple infection models. We repeated this for our *B. ambifaria* strain and deleted the smallest chromosome (knockout *B. ambifaria*) and then asked two questions: did the bacteria still protect seeds from pathogens due to the cepacin-making genes? And was the small chromosome knockout *B. ambifaria* less virulent? We were excited that the knockout *B. ambifaria* performed as well as the normal *B. ambifaria* in protecting pea seeds from *Pythium*, but was more easily cleared from the lungs of a mouse model compared to the normal *B. ambifaria*.

In summary, we identified the genes responsible for making cepacin – a key biopesticide activity in the bacterium *B. ambifaria*; and even though the normal *B. ambifaria* had low virulence, we were able to further reduce its infectious ability by deleting a part of the genome. This paves the way for using *B. ambifaria* as a substitute to synthetic pesticides in protecting crops from plant pathogens, while addressing concerns over pathogenicity.