Green Chemistry

The good, the bad and the ugly

Mould on fruit and vegetables is bad. The aggressive, destructive fungi causing it are ugly. Enter *Bacillus subtilis*, the hero that rescues fresh produce from death and decay.

> "It is only by connecting specialists in various fields of expertise that a synergistic solution to a highly complex problem can be achieved."
> Prof. Kim Clarke

EVERY YEAR, the perishable produce industry experiences considerable post-harvest crop losses. It is estimated that as much as 50% of the harvested crops are lost worldwide. Much of this is due to decay caused by microorganisms that produce post-harvest diseases.

Some of the most aggressive and destructive plant pathogens, *Botrytis* spp. (affecting table grapes) and *Penicillium* spp. (affecting citrus) induce diseases such as grey mould and black rot on table grapes, and anthracnose on strawberries, papayas, bananas, apples, potatoes, soya beans, tomatoes – indeed, almost all fresh produce can be affected. Fresh produce particularly susceptible to attacks by fungal pathogens, have high sugar levels, a high moisture content and low pH.

Effective disease control methods are essential to maintain food security. During the last decade, traditional treatment with synthetic fungicides has become increasingly undesirable due to the chemical residues they leave behind in the food chain. As a result, some of the most effective fungicides have been deregistered. Some of the pathogenic strains have also developed resistance to the fungicides.

Consequently, the need for green chemistry as an alternative to control fungal and bacterial post-harvest diseases, and to replace these commercial chemical compounds, has become more urgent in the agricultural sector.

Producers of export fruit are very aware of the increasing pressure from major export markets to fund ways of natural disease control.

HOW THE CHALLENGE WAS TACKLED

Prof. Kim Clarke, PhD in Chemical Engineering, put together a dynamic, multi-disciplinary team of specialists with expertise from life science and chemical engineering to investigate and determine the best way to produce a new bioproduct to control fruit and plant disease. Increasingly, the integration of engineering and life science disciplines is recognised as the key that unlocks the potential of new bioproducts to progress from the initial research stage to successful production and implementation. Prof. Clarke's team is uniquely competent to evaluate biological systems from a cross-disciplinary viewpoint. This enables them to consider the research challenges from multiple angles.

1 Prof. Kim Clarke.
2 Phytopathogens on table grapes.

Internationally, consumer regulations and requirements demand that alternatives to current control methods be investigated. Prof. Clarke's search for the right bio-candidate led her team to the bacterium *Bacillus subtilis* for three reasons. First is its GRAS (generally regarded as safe) status. Second is its demonstrated control against post-harvest pathogens such as *Penicillium* and *Botrytis* spp. The third reason is that it produces the lipopeptide bioproducts, surfactin, iturin and fengycin, that exhibit antibacterial and antifungal properties.

"Our research focused on the production of bioproducts from a bacterial (*Bacillus*) culture and the development of the bioproducts as an environmentally friendly alternative to current control strategies," says Prof. Clarke.

Until recently, research done on the *Bacillus*

PROJECT TITLE
The production of antimicrobial lipopeptides by *Bacillus subtilis* for biological control of post-harvest spoilage organisms

PRINCIPAL INVESTIGATOR
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DURATION
One year

PHI-2 CONTRIBUTION
R190 909

LEAD INSTITUTION
Stellenbosch University (Department of Process Engineering)

BENEFICIARY
The entire fresh fruit industry

FOCUS AREA
Green chemistry

HUMAN CAPITAL DEVELOPMENT
Two MEng students

PUBLICATIONS AND PRESENTATIONS
Six

"This research is novel in that it seeks to examine the application of the active bioproducts themselves, rather than using the entire *Bacillus* culture. In this way, it is envisaged to produce standardised and consistent bioproducts that, unlike the *Bacillus* culture, would be effective in any physical and chemical environment."
Prof. Kim Clarke

culture was limited to the application of bacterial culture directly. By examining the metabolic bioproducts of the organism, the team has identified the potential value of these products to ward off disease in post-harvest crops. In essence, *Bacillus subtilis* acts as a natural microscopic factory of bioproducts that demonstrates the amazing ability to act as antibiotics or fungicides, fighting disease and decay.

"Our research is novel in that it seeks to examine the application of the active bioproducts themselves, rather than using the entire *Bacillus* culture. By doing this, we foresee the possibility to produce standardised and consistent bioproducts which, unlike the *Bacillus* culture, would be effective in any physical and chemical environment," explains Prof. Clarke.

In pursuing their objective, the team used microbiological and genetic procedures to isolate and identify the pathogens that cause disease in South African post-harvest crops. They then applied chemical engineering and biochemistry principles to produce, extract, purify and fractionate a cocktail from the *Bacillus* cultures that contained a wide range of bioproducts, many newly discovered, which were effective against the pathogens.

"We identified two *Bacillus* candidates that produce large quantities of antifungal lipopeptides," says Prof. Clarke. By applying various microbiological techniques, the researchers confirmed that, even with crude extracts, the bioproduct cocktail was effective against all the isolated pathogens.

"The bacterially produced bioproducts have considerable potential as an effective 'green' alternative for the control of diseases in post-harvest crops, either as a multi-product cocktail or as individual fractions, tailor-made for specific plant diseases," says Prof. Clarke.

PRACTICAL IMPLICATIONS

Biological control provides a safe and ecologically preferred alternative to chemical fungicides for the control of pathogens and reduction in post-harvest crop losses since these agents require a lower effective dosage and are biodegradable.

While the results of this research refer mainly to table grapes, the findings have significant implications for a wide range of perishable crops in the South African agricultural sector.

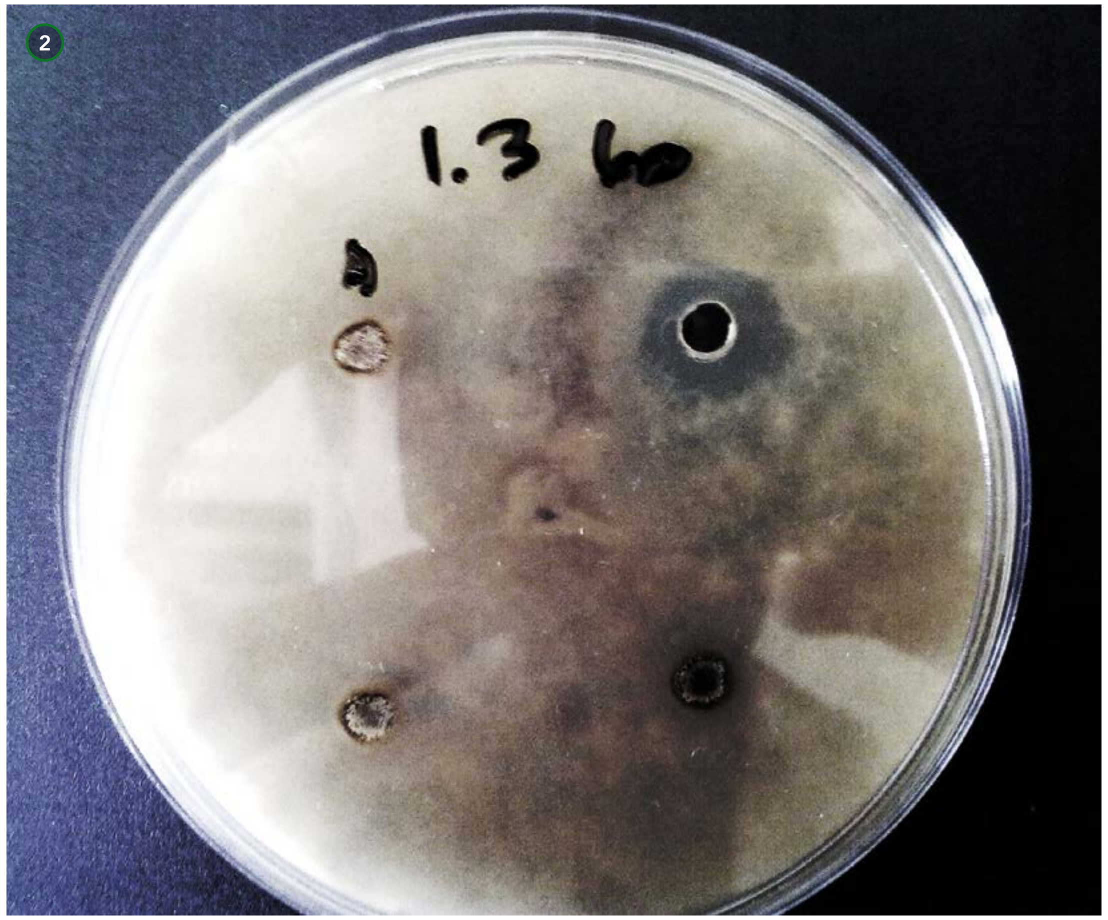
IMPACT ON THE PRODUCER'S BOTTOM-LINE

The successful production of this bioproduct can greatly reduce and even eliminate spoilage of perishable produce.

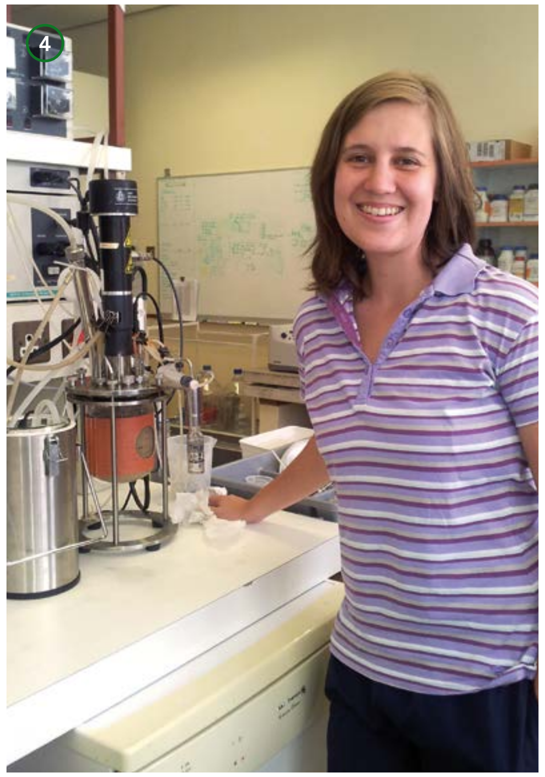
Process development would lead to enhancement of production performance and purification optimisation towards the implementation of the bioproduct as a preferred control strategy.

CONCLUSION

The bacterial production of lipopeptides, their extraction and purification from the bacterial culture and evaluation of their antagonistic activity against a range of phytopathogens causing disease in South African fruit has been investigated through an integrated approach, incorporating expertise from both engineering and life science disciplines. The findings to date suggest that the production and application of the specific bioproduct homologues to target pathogens will be a more definable, controllable and effective biocontrol strategy than direct application of the *Bacillus* culture, as was previously done.



Research focused on the production of a bioproduct from a bacterial (*Bacillus*) culture and the development of an environmentally friendly alternative to current control strategies.



1 Manda Rossouw, an analytical chemist, determines lipopeptide amounts using High-Pressure Liquid Chromatography.
2 Agar plate showing an area clear of phytopathogens around top right well containing lipopeptides, confirming fungicidal activity. (Control wells on left.)
3–4 Jaco van Rooyen, a biochemist, and Daniëlle Pretorius, a chemical engineer, are members of Prof. Clarke's research team working with bioreactors that produce lipopeptides under controlled conditions.