Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

www.plantandfood.com

Product screening test for Kasumin®, Bacstar™ and Phyton®

Part 1&2: Growth rate test and Zone of inhibition test.

Everett KR and Vergara, M.

February 2012

A draft report prepared for

Brian Smith, Etec

Everett KR

Plant & Food Research, Mt Albert

SPTS No. 6588

PLANT & FOOD RESEARCH *Pseudomonas syringae* pv. *actinidiae* (Psa) RESEARCH NOTE

KEY PROJECT DETAILS

Project Title	*In vitro* product testing
Project Leader	Kerry Everett
Research Requested / Contracted by	Etec
Date (Month, Year)	February 2012
Based on information as at	February 2012

RESEARCH QUESTION AND AIM

Treatments to limit the spread of Psa in orchards are required. Etec have requested preliminary *in vitro* testing of three products for activity against *Pseudomonas syringae* pv. *actinidiae*. This document reports on the growth rate testing of two of those products.

Aim: To carry out *in vitro* tests on products for potential efficacy for control of New Zealand isolates of *P. s.* pv. *actinidiae*

METHODOLOGY (Include brief details of experimental design, methodology and protocols)

Experimental design

P. s. pv. *actinidiae* was isolated from leaf and flower tissue collected from KPIN 7668 (RP2, KEP3) that has been haplotyped as Psa-V. Single cells from the isolation plates were used to generate the test cultures. A test measuring the growth of the bacteria in the presence of the test products was conducted. Details of the products evaluated are shown in Table 1.

Methods and protocols

Zone of inhibition test

A suspension of single cell cultures of Psa KEP3 that had been grown on King's medium B agar (KB) (King et al. 1954) for 24 hours at 25ºC was spread onto fresh Petri plates. After 48 hours at 25ºC, bacterial cells were harvested by washing with sterile deionised water (SDW) and the concentration spectrophotometrically determined. The concentration was adjusted to 10^5 cfu/ml and added to molten sterile KB. A 5-ml aliquot of this bacterial suspension was poured onto KB agar plates. Filter paper discs were soaked in 1, 10, 100 and 1000 ppm of candidate chemical products (Table 1). The biological product (Bacstar™) was used at concentrations of 10^5, 10^6, 10^7 and 10^8 cfu/ml. There was a water-soaked control disc on every plate. Plates with filter paper discs were incubated at 25ºC for 48 hours. Any zones of inhibition of bacterial growth were measured.

Growth test

Chemical products to be tested were adjusted to twice the following concentrations: 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml. The biological product (Bacstar™) was adjusted to 2 x 10^5, 10^6, 10^7 and 10^8 cfu/ml. A 2-ml aliquot of each of these concentrations of product was added to 15-ml tubes containing 2 ml of 2x concentration nutrient broth, to result in concentrations of 1x nutrient broth and 1x 1 µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml chemical products or 1 x 10^5, 10^6, 10^7 and 10^8 cfu/ml biological. A suspension of culture KEP3 that had been grown on King's medium B agar (KB) (King et al. 1954) for 24 hours at 25ºC were spread onto fresh Petri plates containing the same medium. After 44 hours at 25ºC, bacterial cells were harvested by washing with sterile deionised water (SDW) and the concentration spectrophotometrically determined. The concentration was adjusted to 10^3 cfu/ml and a 100-µl aliquot was added to the tubes containing products and nutrient broth. There was an uninoculated control for all product concentrations, and there was an inoculated control in 1x nutrient broth. The concentration of Psa cells in the presence of different concentrations of biological products was determined by removing 100 µl after 48 hours of growth, diluting in sterile deionised water and placing on King's medium B agar (King et al. 1954) in Petri plates. After 48 hours of incubation at 25°C, Psa colonies were identified by morphology and counted. The efficacy against Psa was calculated as percentage reduction in colony numbers compared with number of colonies in the unamended nutrient broth. For the other products, 100 µl was removed from each tube after 48 hours of growth at 25°C, and placed in a cuvette containing 2.4 ml of deionised water. The optical density was measured at 535 nm. The efficacy against Psa was calculated by logit transforming the OD 535 values, where logit = ln {(p/1-p)} and p = the proportion of the OD 535 values in unamended nutrient broth. Any negative values were adjusted to zero. Logit values were plotted against the logarithmic transformation of product concentration. The response was thus linearised. The slope of the linear portion of the transformed data was calculated by linear regression. The effective concentration at which growth was inhibited by 50% (EC_{50} value) was calculated from each linear regression equation for Y = 0, and when Y = - 2.77 for EC_{95} values. A constant value was added to non-transformed data to enable 0 and 100% values to be used in the calculations.

A low EC_{50} value indicates an effective product. If the EC_{95} value is also low, then the EC_{50}/EC_{95} ratio will be close to 1. This indicates total kill at a low dosage and this is a good product.

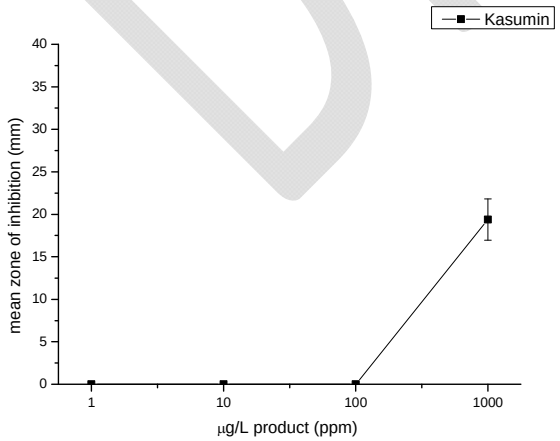
Table 1: Products tested

Product name	active ingredient	% or concentration
Kasumin® 2L	Kasugamycin hydrochloride	23 g/L
Phyton® 27AG	Copper sulphate pentahydrate	253 g/L
Bacstar™	*Bacillus subtilis* var. *amyloliquefaciens* strain D747	250 g/kg

KEY RESULTS (all results must be auditable in terms of access to raw data if required)

Zone of inhibition test

1) Bacstar™ and Kasumin® produced a zone of inhibition around the infiltrated filter paper discs on King's medium B, but Phyton® did not (Figure 1).
2) Bacstar™ produced the largest inhibitory zone at all concentrations tested (Figure 1).
3) Kasumin® produced an inhibitory zone only at the highest concentration (Figure 1).



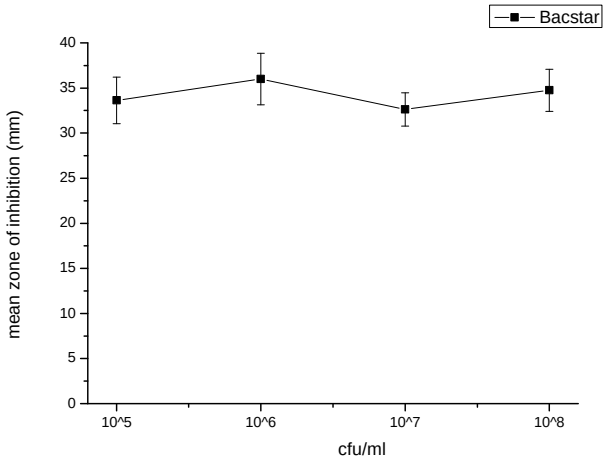


Figure 1. Zones of inhibition for all effective products tested.

Growth test

1) Psa growth was almost completely inhibited at high concentrations (100 and 1000 µg/ml) of both tested chemical products (Figure 2).
2) The EC50 and EC95 values are displayed in Table 1.

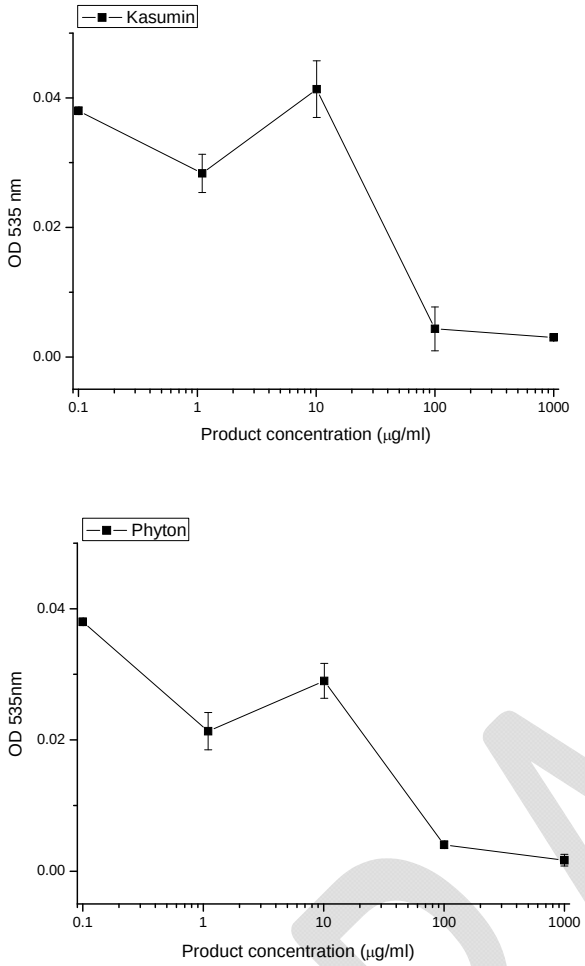


Figure 2. Optical density at 535 nm after 48 hours of *Pseudomonas syringae* pv. *actinidiae* growing in the presence of chemical products at 25°C, plotted against concentration. The value of 0.1 is zero plus a constant value so that this value can be included.

Table 2. Effective concentration (EC) in µg/ml at which bacterial cells are reduced by 50% (EC_{50}), 95% (EC_{95}) and a ratio of EC_{50}/EC_{95} as determined by absorbance at 535 nm after 48 hours of growth at 25°C.

	Effective Concentration (EC) in µg/ml		
Product name	EC_{50}	EC_{95}	EC_{50}/EC_{95}
Kasumin®	22.1	169.9	0.13
Phyton®	22.6	156.1	0.14

key to abbreviations – see Table 1.

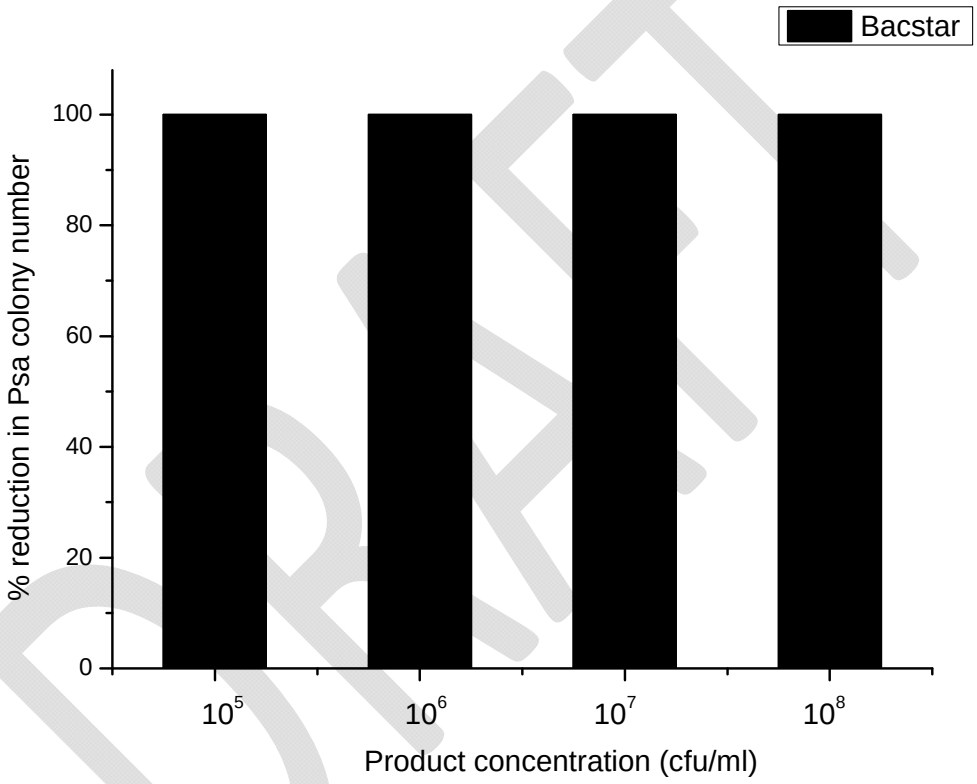


Figure 3. Percentage reduction in colony number after 48 hours of *Pseudomonas syringae* pv. *actinidiae* growing in the presence of Bacstar™ at 25°C, plotted against product concentration in colony forming units per ml.

RECOMMENDATIONS FOR INDUSTRY

In order to validate the results of these tests, field testing is required, as environmental conditions and the presence of plant material can affect the efficacy of these products.

CONCLUSIONS

All products tested had some inhibitory effect against Psa in these *in vitro* growth tests.

FUTURE RESEARCH STEPS

Small inoculated kiwifruit plants could be used to test these materials further in the laboratory or the glasshouse. Field spray trials should also be conducted to test these promising products further.

REFERENCES

King EO, Ward MK, Raney DE 1954. Two simple media for the demonstration of pyocyanin and fluorescin Journal of Laboratory and Clinical Medicine 44: 301-307.

DISCLAIMER

Unless agreed otherwise, The New Zealand Institute for Plant & Food Research Limited does not give any prediction, warranty or assurance in relation to the accuracy of or fitness for any particular use or application of, any information or scientific or other result contained in this report. Neither Plant & Food Research nor any of its employees shall be liable for any cost (including legal costs), claim, liability, loss, damage, injury or the like, which may be suffered or incurred as a direct or indirect result of the reliance by any person on any information contained in this report.

LIMITED PROTECTION

This report has been prepared by The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), which has its Head Office at 120 Mt Albert Rd, Mt Albert, Auckland.

This report has been approved by:

Kerry Everett
Scientist
Date: February 2012