Challenge plate testing to evaluate the inhibitory effects of *Bacillus amyloliquefaciens pm414* (Lolipepta) combined with 2% w/v Sodium Bicarbonate against *Alternaria* sp. on Tomato leaves.

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Summary

Lolipepta ™ - a *Bacillus amyloliquefaciens (pm414)* preparation was combined with 2% w/v Sodium Bicarbonate ($NaHCO_3$) applied *in vitro* in a challenge plating experiment against isolated from Tomato leaf matter grown in the Bundaberg area. Six application rates were chosen *in vitro* – 2% Lolipepta, 5% Lolipepta, 10% Lolipepta, 2% Lolipepta/2% w/v $NaHCO_3$, 5% Lolipepta/2% w/v $NaHCO_3$ and 2% Lolipepta/2% w/v $NaHCO_3$. The combination of Lolipepta (of either concentration) and the 2% w/v $NaHCO_3$ was highly successful in holding the suspected pathogen after 7 days incubation.

Aims

To determine if the *in vitro* addition of Lolipepta in solution with 2% w/v $NaHCO_3$ suppressed suspected *Alternaria* sp., a plant pathogen isolated from Tomato foliage.

Materials & Methods

Sample Preparation

Suspected plant pathogens were isolated from fresh Tomato leaf samples taken from an orchard in the Bundaberg region. Poly Dextrose Agar (PDA) plates were used to isolate fungi from sample material provided. Fungal colonies growing on plates were then re-isolated and grown separately on new PDA plates until pure cultures were obtained. Visual and laboratory preliminary inspections indicated pure samples similar in phenology to a leaf spot pathogen, which subsequently was morphologically identified in the laboratory as *Alternaria* sp.

Challenge Plate Preparation

Once sporulating (~ 9 days after plating, and after exposure to UV light), pure colonies were excised from the PDA plates using a scalpel to cut a plate into a regular grid pattern. Five mm square plugs were excised from the pure colonies, one of which was placed into the centre of each of 37 PDA plates for evaluation[1].

Efficacy of Lolipepta ™ and Sodium Bicarbonate

Lolipepta ™ was prepared at concentrations of 2%, 5% and 10% with sterile deionised water. Combination Lolipepta and Sodium Bicarbonate solutions were prepared by

[1] The six treatments were replicated three times (3 x 6 = 18) and two techniques were used to evaluate the efficacy of the product (18 x 2 = 36). These were compared to an untreated control.

dissolving 2.00 g of $NaHCO_3$ in 100 ml of sterilised deionised water and swirling for 10 seconds, and then pipetting into the solution either 0.2, 0.5 ml or 1.0 ml Lolipepta to make combined 2% Lolipepta/2% w/v $NaHCO_3$; 5% Lolipepta/2% w/v $NaHCO_3$ or 10% Lolipepta/2% w/v $NaHCO_3$ solutions. In order to evaluate the possible inhibitory effects of the products against the pathogen, the product and the pathogen were applied to PDA plates using the *streak plate technique,* two parallel lines were drawn along the plate, approximately 20 mm from either 'side' of the plate. The products were applied along the lines using a sterile flame loop dipped in the corresponding concentration of the product. For each concentration of the product, there were three replicate PDA plates. In the centre of each plate, a 5 mm plug of the suspected pathogen was applied.

In order to determine efficacy of the products, streak plate sample diameters were measured at the start of the trial, and growth was compared to an untreated control. Successful inhibition of the pathogen was determined from challenge plates seven days after inoculation if the pathogen had not crossed the product barrier.

Measurements were recorded at nine days after incubation; the long (7 + days) duration of incubation was necessary to obtain enough growth from the pathogen so as to be satisfied that the pathogen and product had been interacting for sufficient time to determine product efficacy (if any). The mean values of two measurements on each plate and standard deviations were presented. The percentage inhibition compared to the control was tabulated from the mean values.

Results & Discussion

Streak plate barriers were effective in inhibiting the growth of the pathogen (Table One), with the degree of inhibition increasing marginally with increasing concentrations of Lolipepta. Using plates streaked with Sodium Bicarbonate *and* Lolipepta resulted in a 25% greater inhibition of the pathogen compared to Lolipepta-only treatments. There was no significant difference in the degree of inhibition in the combined Sodium Bicarbonate/Lolipepta treatment, despite the fact that the rate of Lolipepta was varied from 2-10%. This indicates that Sodium Bicarbonate at the 2% rate was probably largely responsible for the increase in inhibition in the combined treatment – varying Lolipepta did not result in significant increases in pathogen inhibition, yet the addition of Sodium Bicarbonate consistently resulted in a >25% increase in inhibition irrespective of the concentration of Lolipepta (Figure One).

Plates *spread* with Lolipepta and/or Lolipepta + Sodium Bicarbonate yielded similar results. The 2%, 5% and 10% Lolipepta + Sodium Bicarbonate combined treatments resulted in the highest inhibition scores of approximately 90%, irrespective of the variable rate of Lolipepta across the combined treatments. Inhibition of the pathogen with Lolipepta only was lower at 67% efficacy for 10% Lolipepta, decreasing to 58% for 2% Lolipepta (Table Two).

Table One. Inhibition of *Alternaria* sp. on plates streaked with Lolipepta ™ at 2%, 5% and 10% application rates, with or without addition of 2% w/v Sodium Bicarbonate ($NaHCO_3$), as compared to a control (no treatment). Note, the negative numbers indicate the distance in mm by which the pathogen was *inhibited* from crossing the product barriers – positive numbers would have indicated the distance in mm by which the pathogen had *crossed* the barriers and had continued to grow beyond these barriers.

Treatment	9 days incubation mean ± S.E. (mm)	Percent inhibition
Control	35.0 ± 0.0	0.0[a]
2% Lolipepta ™ + 2% Carba-mate	-11.45 ± 3.69	132.71[d]
5% Lolipepta ™ + 2% Carba-mate	-12.32 ± 2.08	135.20[d]
10% Lolipepta ™ + 2%Carba-mate	-13.65 ± 2.46	139.00[d]
2% Lolipepta ™	-0.06 ± 0.80	100.01[b]
5% Lolipepta ™	-2.33 ± 1.48	106.65[bc]
10% Lolipepta ™	-3.06 ± 1.93	108.74[c]

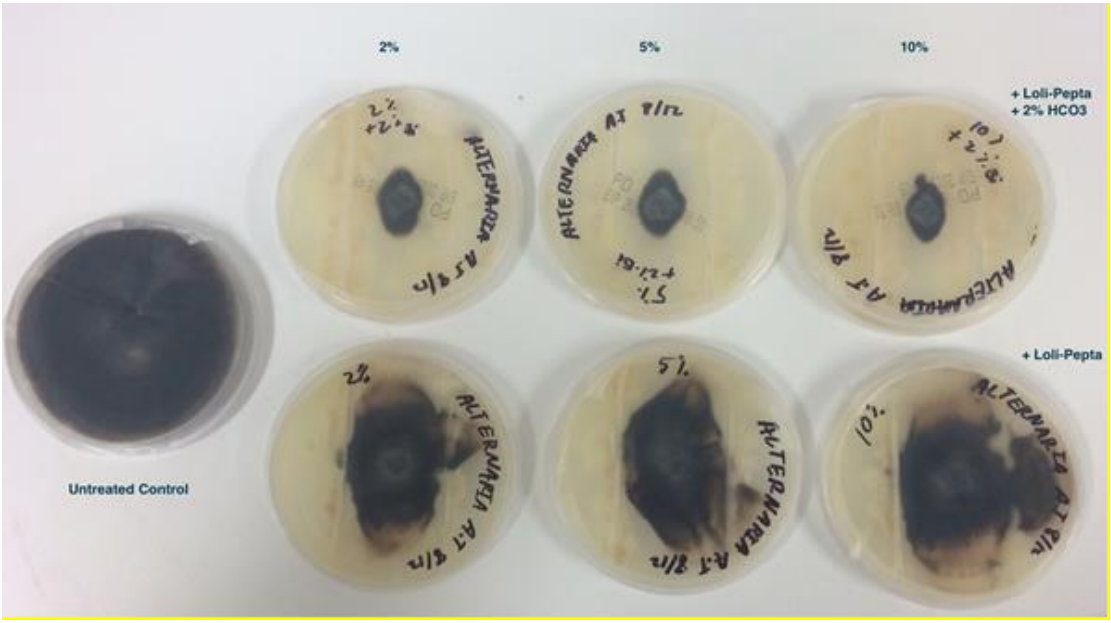

Figure One. Alternaria spread challenge plates, where the top row shows plates spread with (L-R): 2%, 5% and 10% Lolipepta plus 2% Sodium Bicarbonate, and the bottom row shows plates spread with (L-R): 2%, 5% and 10% Lolipepta only. These are compared with a control, to the far left of the image.

Table Two. Inhibition of *Alternaria* sp. on plates spread with Lolipepta ™ at 2%, 5% and 10% application rates, with or without addition of 2% w/v Sodium Bicarbonate ($NaHCO_3$), as compared to a control (no treatment).

Treatment	9 days incubation mean ± S.E. (mm)	Percent inhibition
Control	35.0 ± 0.0	0.0
2% Lolipepta ™	14.7 ± 2.5	58.0
5% Lolipepta ™	13.1 ± 2.4	62.6
10% Lolipepta ™	11.5 ± 2.9	67.1
2% Lolipepta ™ + 2% $NaHCO_3$	4.1 ± 3.6	88.3
5% Lolipepta ™ + 2% $NaHCO_3$	3.9 ± 2.3	88.9
10% Lolipepta ™ + 2% $NaHCO_3$	3.5 ± 0.7	90.0

Conclusion and recommendations

Based on the results of this trial, it appears that higher concentrations of Lolipepta do little to hold *Alternaria* sp. *in vitro,* but despite this, the product is still useful in inhibiting the growth of the pathogen. Supplementary Sodium Bicarbonate appears to have contributed more strongly to the successful inhibition of the pathogen on plates. It is recommended that $NaHCO_3$ addition to the Lolipepta be evaluated as a combined product in a commercial setting.