POTENTIAL OF *BACILLUS AMYLOLIQUEFACIENS* STRAIN D747 AS CONTROL AGENT AGAINST *PSEUDOMONAS SYRINGAE* PV. *ACTINIDIAE*

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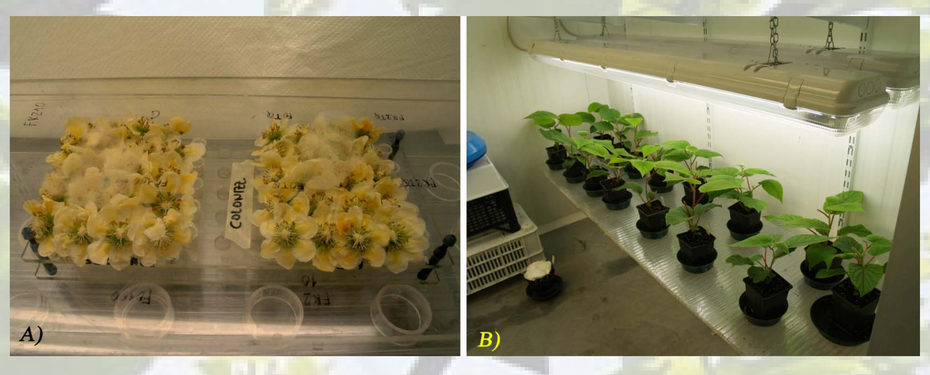

Fig. 1: A) Experiment on female flowers of *A. chinensis*; B) Experiment on leaves of *A. deliciosa*

INTRODUCTION

The strain D747 of *Bacillus amyloliquefaciens* (Amylo-X®) was assayed to evaluate its efficacy against *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of the bacterial canker of kiwifruit which gained significant relevance after the heavy epidemics that have been occurring in central-northern Italy since 2008. In this study, we investigated the ability as antagonist of this bacterium to inhibit the growth of two different Psa strains *in vitro*, its ability to survive and reduce populations of a rifampicin resistant Psa strain on female flowers of *Actinidia chinensis* and its capacity to survive on *A. deliciosa* leaves.

MATERIALS AND METHODS

Bacterial strains. *B. amyloliquefaciens* strain D747 cultured on LPG-agar at 36°C for 24h; rifampicin resistant mutants (Rif^r) of wild type strains NCPPB 3739, isolated from *A. deliciosa*, and CRA-FRU 3.1, isolated from *A. chinensis*, cultured on NSA amended with 150 ppm of rifampicin at 27°C for 48h.

***In vitro* tests.** The production of antimicrobial compounds by *B. amyloliquefaciens* was tested in agar minimal medium (Vanneste *et al.*, 1992)(Fig. 2 and Tab. 1).

Challenge on female flowers of *A. chinensis* 'Zespri Gold'. The experiment was carried out on detached flowers kept in Eppendorf tubes containing sterile distilled water (Fig. 1A). After incubation in humid chamber, an aqueous suspension of strain D747 (Amylo-X®, 2 g/L; ca. 10^7 CFU/ml) and sterile deionized water (SDW, negative control) were sprayed on newly opened flowers. Twenty-four hours after the application of the treatments, an aqueous suspension (ca. 10^6 CFU/ml) of the Psa strain CRA-FRU 3.1 Rif^r was applied on the flowers. Ten flowers per time point were then washed in 3 ml of 10 mM $MgSO_4$. The pathogen population present on each flower was determined by plating tenfold dilutions on NS – agar + 150 µg/ml of rifampicin (at 27°C for 48h), while the antagonist population was monitored plating the tenfold dilutions on LPG-agar (at 36°C for 24h). The Psa strain and D747 population were monitored for 72h (Fig. 3) and 96h (Fig. 4), respectively, by colony counting on plates. The mutant re-isolated pathogen was also identified with PCR assay.

Survival of *B. amyloliquefaciens* on leaves of *A. deliciosa* 'Hayward'. The experiment was carried out on plantlets maintained in climatized chamber (at 25°C; Fig. 1B). An aqueous suspension of strain D747 (2 g/L; ca. 10^7 CFU/ml) and SDW were applied as broadcast foliar sprays onto the plants. Twelve leaves per time point were randomly collected and washed in 250 ml of 10 mM $MgSO_4$ at 120 rpm for 45 min at 25 C°. The washing suspension was then centrifuged at 10,000 *g* for 20 min at 4°C. The pellet was resuspended in 1 ml of SDW. The bacterial population present on each set of leaves was determined by plating tenfold dilutions on LPG-agar incubated at 36°C for 24h. The population level was monitored for 21 days (Fig. 5).

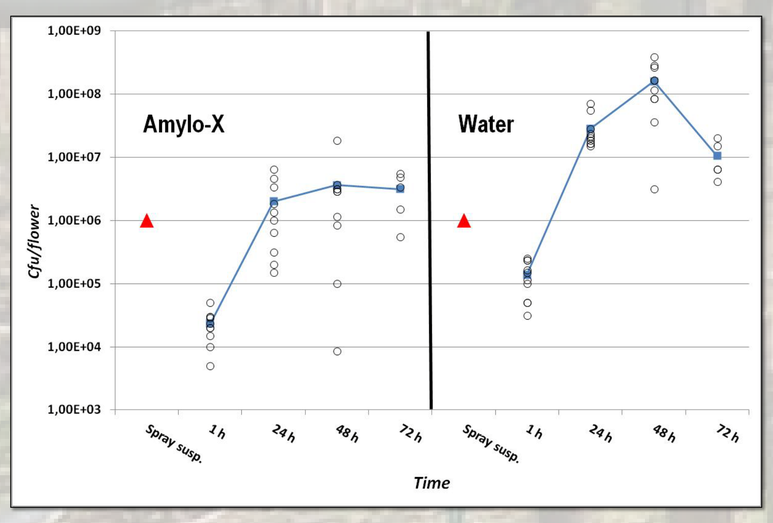

Fig. 3: Psa (strain CRA-FRU 3.1 Rif^r) mean population (CFU/flower) on *A. chinensis* female flowers pre-treated with *B. amyloliquefaciens*. The dots represent the population on each flower (10 flowers per time). ▲ = Concentration of the suspension applied at time 0 h (CFU/ml).

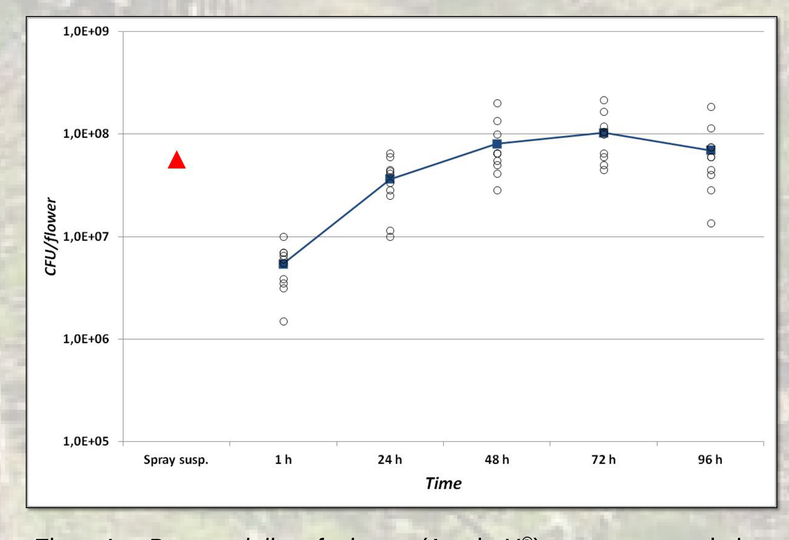

Fig. 4: *B. amyloliquefaciens* (Amylo-X®) mean population (CFU/flower) on *A. chinensis* female flowers. The dots represent the population on each flower (10 flowers per time). ▲ = Concentration of the suspension applied at time 0 h (CFU/ml).

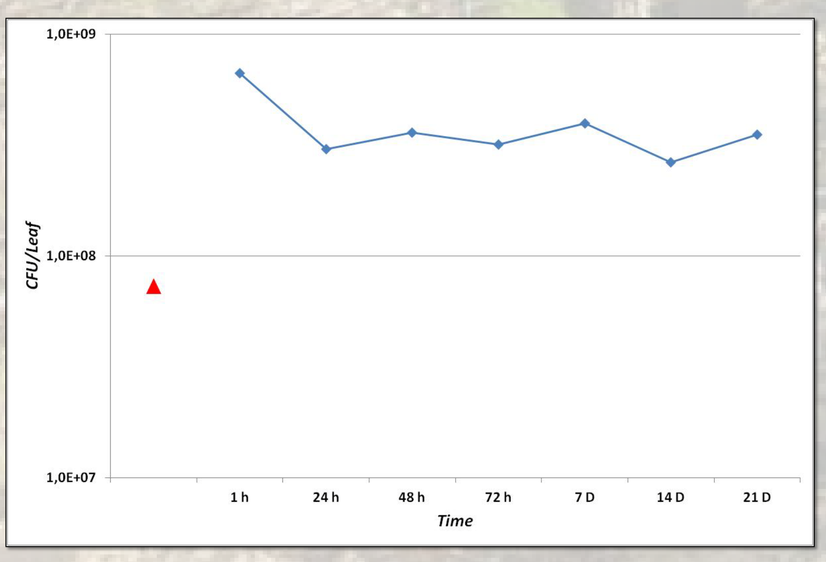

Fig. 5: *B. amyloliquefaciens* (Amylo-X®) mean population (CFU/leaf) on *A. deliciosa* leaves. ▲= Concentration of the suspension applied at time 0 h (CFU/ml).

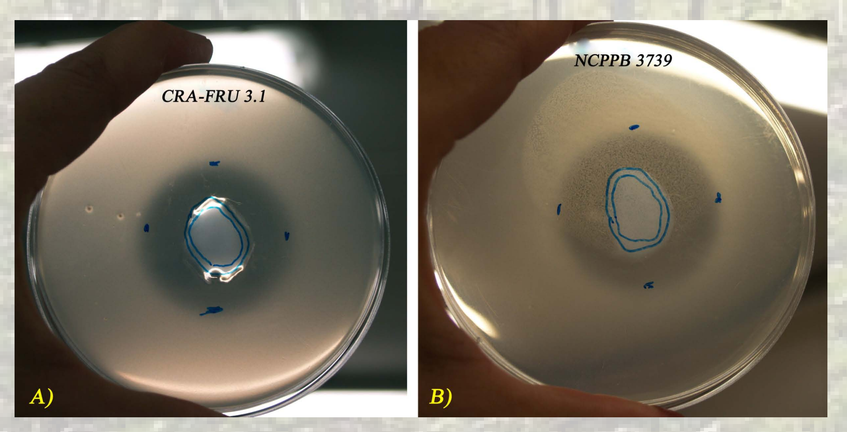

Fig. 2: *In vitro* test: inhibition of Psa strains CRA-FRU 3.1 (A) and NCPPB 3739 (B) by *B. amyloliquefaciens* (strain D747).

Tab.1: Inhibition halo (cm) of *B. amyloliquefaciens* (D747) against Psa strains NCPPB 3739 and CRA-FRU 3.1 *in vitro*.

Psa Strains	Ø of inhibition (cm) ± St. Dev.
NCPPB 3739	2.13 ± 0.68
CRA-FRU 3.1	2.25 ± 0.52

B. amyloliquefaciens strain D747 was able to colonize flowers and to survive on leaves with high level population

CONCLUSIONS

- *B. amyloliquefaciens* was able to inhibit *in vitro* both Psa wild type strains CRA-FRU 3.1 and NCPPB 3739 with similar efficacy
- The strain D747 (Amylo-X®) colonized female yellow kiwi flowers reaching a population level of approx. 10^7-10^8 CFU/flower after 96 hours
- The antagonist was able to inhibit the growth and thus reduce the population level of the pathogen strain CRA-FRU 3.1 Rif^r on *A. chinensis* flowers by almost two orders of magnitude in comparison to the negative control after 72 hours
- The biocontrol agent was able to survive on leaves of *A. deliciosa* at a high level of population (ca. 10^8 CFU/leaf) for 21 days

Literature Cited

Vanneste J.L., J. Yu, S.V. Beer. 1992 - Role of antibiotic production by Erwinia *herbicola* Eh252 in biological control of Erwinia amylovora. J. Bacteriol. 174:2785-2796.