Does HCN from *Pseudomonas fluorescens* T58 contribute to biocontrol of *Fusarium oxysporum* f.sp *lycopersici*?

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Introduction

Fusarium oxysporum f. sp. *lycopersici* is responsible for severe economic losses in tomato production world wide. Current control measures are based on soil fumigation and use of resistant cultivars which are not adequate. Alternative effective and environmentally safe methods to control Fusarium wilt are needed and biological control can provide part of the solution.

In our experiments plants treated with *Pseudomonas fluorescens* T58 before inoculating with *Fusarium* had less infection and higher shoot mass than plants infested with *Fusarium* alone. Induction of resistance was determined to be one of the mechanisms of action. *In vitro* tests showed that *P. fluorescens* T58 produces HCN, a volatile metabolite suspected to play a role in biocontrol.

The objective addressed here was to find out whether volatile metabolites from *P. fluorescens* T58 contribute to biocontrol of *Fusarium* wilt on tomato either by suppressing growth of *Fusarium*, inducing resistance in the plant or suppressing growth of competing micro-organisms in the rhizosphere.

Volatiles produced by *P. fluorescens* T58 lead to increased peroxidase activities

P. fluorescens T58 was grown in Tryptone Soy Broth (TSB) containing 4.4 g l^{-1} Glycine in tightly sealed flasks. The volatile metabolites were delivered through a PVC tube to tomato roots growing in a nutrient solution. After exposing tomato plants to the metabolites for upto 2 weeks the activities of chitinase, β-1, 3-glucanase and peroxidases in the stem were determined as parameters for resistance induction by volatile metabolites from *P. fluorescens* T58.

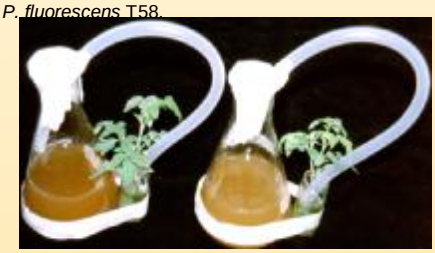


Figure 1
Production and delivery of volatile metabolites to tomato root zone.

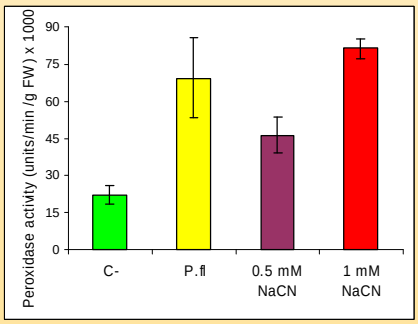
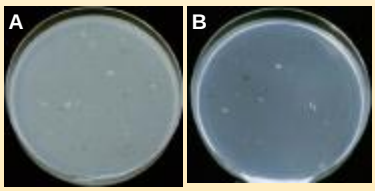


Figure 2
Exposing tomato plants to volatile metabolites as well as solutions of NaCN causes increased peroxidase activity.

Volatile metabolites from *P. fluorecens* T58 affect *Fusarium* spore germination and mycelium pigmentation

The effect of volatile metabolites on germination of *Fusarium* spores and mycelia growth was assessed by growing *P. fluorecens* T58 on Tryptone Soy Agar (TSA) containing 4.4 g l^{-1} Glycine. *Fusarium* spores or a mycelia plug was placed on TSA in another petri dish. With the covers removed the fungus and bacteria cultures were placed facing each other and tightly sealed to ensure accumulation of the volatile metabolites in the space within.

Figure 3
Effect of volatile metabolites on *Fusarium* spore germination.
A: uninhibited spore germination and subsequent mycelia growth;
B: delayed *Fusarium* spore germination after exposure to volatile metabolites



Fusarium grown in the presence of *P. fluorescens* T58

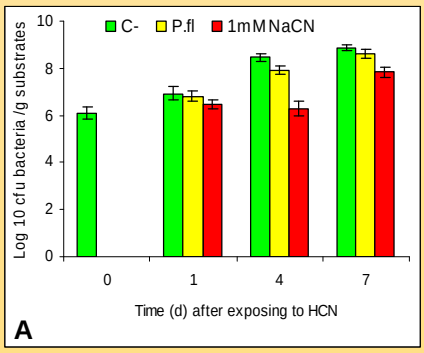
Fusarium grown in the absence of *P. fluorescens* T58

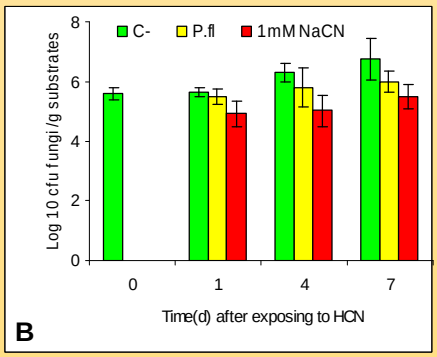
Figure4
Volatile metabolites do not stop growth of *Fusarium* mycelia (top row), but the mycelia fail to produce the pink pigment characteristic of the *Fusarium* isolate used (bottom row).

Volatiles produced by *P. fluorescens* T58 reduce bacteria and fungi populations in the soil

To assess the effect on other microorganisms volatile metabolites produced in TSB were delivered through a PVC tube into unsterilised organic substrates used for growing tomatoes in greenhouses. The population of fungi and bacteria in the organic substrates was determined for upto seven days.

Figure 5
Effect of volatile metabolites on the population of bacteria (A) and fungi (B) in organic plant growth substrates





Conclusions

Volatile metabolites from *P. fluorescens* T58 suppress *Fusarium* spore germination. Mycelia growth is not stopped but is affected by the metabolites.

Volatile metabolites induce a significant increase in peroxidase activity which can contribute to plant defence against *Fusarium*.

Volatile metabolites can significantly reduce bacteria populations in plant growth substrates while fungi populations are reduced but not always significantly.

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