Investigation on the Biocontrol of *Phytophthora* diseases on strawberry based on antagonism

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Abstract

After screening of several rhizosphere bacteria against the soilborne pathogens of red core and crown rot disease of strawberry *Phytophthora fragariae var. fragariae* and *Phytophthora cactorum* under in vitro conditions, three of the most active isolates which produced up to 63% of reduction in mycelium growth, such as *Raoultella terrigena* (G-584), *Bacillus amyloliquefaciens* (G-V1) and *Pseudomonas fluorescens* (2R1-7) were selected for further studies under in vivo conditions. In a greenhouse and field experiments, mentioned above three isolates were tested against both *Phytophthora* diseases under artificial infested soil conditions. Root dip treatment with these bacterial antagonists produced a control effect on both fungal diseases between 27 to 55 % and were in some cases comparable with the chemical fungicide Aliette.

Key words: Biocontrol, Rhizobacteria, *Phytophthora fragariae var. fragariae, Phytophthora cactorum*

Introduction

Strawberry is one of the important berry cultures in Germany, which is attacked by several viral, bacterial and fungal diseases. Of the fungi attacking strawberries two of the most important pathogens *Phytophthora fragariae var. fragariae* and *Phytophthora cactorum*, which are the cause of red core and crown rot disease of strawberry, can cause substantial economical damage in strawberry production (Seemüller 1998). In areas, where *P. fragariae var. fragariae* and *P. cactorum* are present, cultivation of strawberry depends directly on the use of chemicals. The number of chemical fungicides available for the control of both *Phytophthora* pathogens are limited. In most countries two different fungicides viz. Ridomil with Metalaxyl and Aliette with Aluminium-Fosetyl as active substance are available against these pathogens. In Germany, Aliette is the only fungicide registered for control of red stele disease of strawberry (Anonymous,1999). In some fields where Metalaxyl was used for years, resistant strains of *P. fragariae var. fragariae* have been found (Nickerson,1998), resistance of *Phytophthora* species to Metalaxyl has been documented by Seemüller et al (1989). The use of chemicals especially compounds containing mercury and copper were widely resorted to in order to achieve high levels of disease suppression. However, the persistent, injudicious use of chemicals has been discouraged owing to their toxic effects on nontarget organisms and due to the undesirable changes they inflict upon the environment. Many of the chemicals are also too expensive for the resource-poor farmers of Asia (Vasudevan et al.,2002). The problems associated with the use of agrochemicals have promoted research in the field of biological control of plant diseases as an alternative method of control also in the frame of ecological organic farming . Hence in this study, an attempt has been aimed to control both *Phytophthora* diseases by using different antagonistic bacteria

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Material and Methods

Preparation of Bacteria

After a screening of more than 100 bacterial isolates out of the rhizosphere of fruit orchards in dual culture test, three with the best inhibition effect against the two *Phytophthora* pathogens, were further used for the in vivo experiments. The following bacterial isolates were used: *Raoultella terrigena* (G-584), *Bacillus amyloliquefaciens* (G-V1) and *Pseudomonas fluorescens* (2R1-7).
Bacterial isolates G-V1, G-584 were formulated by the company E-nema, Raisdorf. The 2R1-7 isolate were prepared by growing in Tryptic Soya Broth for 24 h at 26° C, cells were harvested by centrifugation and diluted to an optical density of 0.20 at 660 nm. The bacterial concentration 10^8 to 10^9 used for the experiments.

Preparation of Pathogen Inoculum

Inocula of *P. fragariae* and *P. cactroum* were cultured in vermiculate media (composed of seeds of rye) for 4 weeks at 18° C and 3 % of inoculum used for artificial infection of soil.

Plant material

Tests were performed with strawberry (*Fragaria X ananassa* Duchesne) cv, " Elsanta " plants. This variety of strawberry is highly susceptibility to red stele and crown rot disease.

Greenhouse Experiments

Strawberry plant roots were dipped for 15 minutes into the bacterial suspension in order to have direct exposure of the roots to the antagonistic bacteria. Roots of control plants were either dipped in tap water or 0.5 % Aliette. Plants were then transferred to 14 cm plastic pots with artificially infested soil using 3 % of pathogens. The experiment was arranged in two separate blocks, each with 10 pots per treatment. 90 days of after planting, plants were removed from pots, the roots were carefully washed and disease was scored using a 0-5 scale and disease index calculated. Statistical significance was tested using Duncan's Multiple Range Test (P=0.05).

Field Experiments

Field experiments were carried out under artificially infested soil conditions (3 % of pathogen inoculum was applied to each planting hole). Plants were treated with bacteria by root dip as described above. Plants were planted in four rows at least 40 plants per treatment (arranged in randomised design). Harvesting of plants (12 weeks after planting), rating of disease severity, computerization of disease index and statistical significance were all made as described above.
Results and Discussion

In greenhouse experiment, rhizobacteria showed different levels of biocontrol activity towards both pathogens (Table .1). maximum % control of 54% against red core from

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isolate 2 R1-7 of *P. flourescens*, where as against crown rot isolate G-584 of *Raoultella terrigena* showed the highest effect of 55 % which induced nearly comparable with chemical control Aliette 64. 7 %.

Tab.1: Influence of Rhizobacteria on disease index of red core and crown rot of strawberry under artificial infested soil conditions in the greenhouse (90 days after treatment) n=20

Treatments	Red core		Crown rot	
	% Disease index	% Control	% Disease index	% Control
Control	42 a		51 a	
G-584	26 bc	38.1	23 b	55.0
G-V1	27 bc	35.7	26 b	49.0
2 R1-7	19 c	54.7	26 b	49.0
Aliette	16 c	61.9	18 b	64.7

The tested bacterial isolates also gave a considerable degree of protection against both pathogens. In a field experiment (Table. 2), the isolate 2 R1-7 of *P. fluorescens* showed also highest effect up to 50% followed by G-584 and G-V1 against red core disease but a lower degree of control against crwon rot (32 to 37 %).

Bacterial strains tested for biocontrol activity towards *Phytophthora* sps were previously shown to have biocontrol activity against different pathogens on a variety of different crops except bacterial isolate *Raoultella terrigena* of our knowledge. In our studies, all of the tested strains showed different level of protection against both *Phytophthora sps*, variation in antagonistic activity of rhizobacteria with time and in different systems is a known phenomenon (Weller 1988) and still difficult to explain. Biotic factors such as concurrence in root colonization with the soil specific microbial community or infection by pathogens may play a role. Abiotic factors such as soil type, moisture and temperature as well as variations in application technology also may of importance (Deacon 1991).

In addition, isolation and identification of *Phytophthora spp* from naturally infested soil from fields of different growers were undertaken, in order to confirm the natural infested soil status for field experiments. Isolates were identified at the genus level by using selective media, microscopic observation and plant disease detection kit (ALERT, Neogen corporation ,USA).

Further biocontrol studies are in progress on different locations of Germany under naturally infested soil conditions with strawberry farmers in order to confirm the efficacy. Investigations on the bacterial mode of action of their biocontrol activity will help in understanding the differences in activity between the bacterial strains.

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Table. 2. Influence of Rhizobacteria on disease index of red core and crown rot of strawberry under artificial infested soil conditions in the field (June 2003) (90 days after treatment) n=40

Treatments	Red core		Crown rot	
	% Disease index	% Control	% Disease index	% Control
Infected Control	37.5 a		29.0 a	
G-584	23.0 b	38.6	18.0 bc	37.9
G-V1	22.5 b	40.0	19.5 bc	32.7
2 R1-7	18.5 b	50.6	19.0 bc	34.4
Aliette	18.0 b	52.0	14.5 c	50.0
Untreated control	6.5 c		7.0 d	

The studies have been undertaken in cooperation with firm E-Nema, Raisdof with the aim to develop an antagonistic preparation against both *Phytophthor*a diseases.

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