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Influence of *Bacillus polymyxa* on the growth and development of *Fusarium oxysporum* f. sp. *tulipae*

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Summary

Antagonistic effect of *Bacillus polymyxa*, strain S13, toward *Fusarium oxysporum* f. sp. *tulipae* was evaluated *in vitro* and *in vivo*. The growth of the pathogen was greatly inhibited in dual cultures with *Bacillus polymyxa* on potato dextrose agar. Suspension of *B. polymyxa* and its filtrate substantially inhibited spore germination and development of *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs.

Key words: *Bacillus polymyxa*, *Fusarium oxysporum* f. sp. *tulipae*, tulip bulbs.

INTRODUCTION

Bacteria, strain S13, isolated during reisolation of *Phoma narcissi* (Aderh.) Boerema, de Gruyter et Noordel., comb. nov. from red spots on *Hippeastrum* x *hybr.* hort. bulb and identified as *Bacillus polymyxa*, appeared to be antagonistic to *Phoma narcissi* (syn. *Stagonospora curtisii* (Berk.) Sacc., a main pathogen of *Hippeastrum*), *Phyllosticta antirrhini* (syn. *Phoma poolensis* Taub. var. *poolensis* - de Gruyter et al., 1993), *Phytophthora cryptogea*, *Alternaria alternata*, *Fusarium oxysporum* f. sp. *callistephi* and *Pythium ultimum* on potato dextrose agar (PDA) (Saniewska et al., 1993). The largest inhibition zone was observed in dual cultures with *Alternaria alternata.* The treatment of *Hippeastrum* and *Narcissus* bulbs in suspension of *B. polymyxa* effectively limited the development bulb rot caused by *Phoma narcissi* (Saniewska et al., 1993). Bacterial suspension applied into peat infested with *Phytophthora cryptogea* immediately after gerbera planting inhibited the development of foot rot about 50 days (Saniewska et al., 1995). *B. polymyxa* or its filtrate used as spray substantially decreased the spread of rose powdery mildew (*Sphaerotheca pannosa* var. *rosae*) and snapdragon rust (*Puccinia antirrhini*) (Saniewska et al., 1998).

The purpose of this study was to determine *in vitro* and *in vivo* activity, both cell suspension and cell-free filtrate of *Bacillus polymyxa*, strain S13, toward *Fusarium oxysporum* f. sp. *tulipae*.

MATERIAL AND METHODS

In vitro growth of *Fusarium oxysporum* f. sp. *tulipae* (= *Fot*) in the presence of *Bacillus polymyxa*.

Mycelial discs together with medium 5 mm diam. taken from 10 day-old-culture of *Fot* growing on PDA at 25°C were placed on the margin of 90 mm Petri dishes containing 15 ml of PDA. On the opposite side a long streak (about 5 cm) of bacteria were made. Dual test was incubated at 25°C in the dark. Plates without bacteria served as the control. During 17 days of incubation the growth of bacteria and fungi was observed and inhibition zone of fungi mycelium was measured. Five Petri dishes constituted an experimental unit and the trial was repeated 3 times.

Influence of *Bacillus polymyxa* and bacterial filtrate on spore germination of *Fusarium oxysporum f.* sp. *tulipae*.

Spores of *F. oxysporum* f. sp. *tulipae* were taken from 7-day-old culture growing on PDA. About 0.05 ml of fungal spore suspension (3×10^2 spores/ml) were added to 10 cm^3 of suspension of *B. polymyxa*, containing $0,1 \times 10^8$ c.f.u./cm^3 , or to filtrate from potato dextrose broth (PDB), sample 66 and 67 at concentration of 10%, or only into distilled water (control). Number of germinated spores was observed under microscope after 12, 28 and 76 h incubation at 25°C in the dark. For each treatment and time of incubation observation were made in 100 fields of Bürker's camera. Percentage of germinated spores was calculated. The trial was repeated 3 times.

Influence of *Bacillus polymyxa* and filtrate on the development of *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs.

Bulbs of cv. Victor were damaged by corkborer (5 mm diam. and 1 mm depth) near the basal plate and soaked for 5 min. in bacterial suspension ($0,1 \times 10^8$; $0,25 \times 10^8$ and $0,5 \times 10^8$ c.f.u/cm^3) or filtrate at two concentrations 10%, and 25% or only in distilled water (control). After 5 h such treated bulbs were inoculated in damaged places with 10 μl of the fungal inoculum at suspension 10^6 spores/ml.

The control bulbs were inoculated directly after damage and 5 h after their soaking in water. Prochloraz at conc. 0.9 mg/ml was used as the standard fungicide. The inoculated bulbs were placed on a plastic net in a tray lined with wet filter paper and incubated at 25 – 27°C in the laboratory room. After 15 days of incubation a size of necrosis on inoculated bulbs was measured. Twenty bulbs were used for each treatment and the experiment was repeated three times. Data obtained were subjected to analysis of variance. Duncan´s multiple range t-test at 5% level of significance was used for means separation.

RESULTS AND DISCUSSION

Bacillus polymyxa inhibited *in vitro* the growth of *Fusarium oxysporum*. f. sp. *tulipae* already after 6-day-incubation in 22.8 mm distance from bacteria streak. Finally, after 17-day incubation (Fig.1, Table 1) the inhibition zone of *F. oxysporum* f. sp. *tulipae* was 10.2 mm.

Table 1
Influence of *Bacillus polymyxa* on *in vitro* growth of *Fusarium oxysporum* f.sp. *tulipae* on potato-dextrose agar after 17-day-incubation

Distance (mm) between pathogen and bacteria	Distance (mm) at which bacteria delayed the growth of mycelium	Inhibition zone (mm)
45.6	22.8*	10.2

*Bacteria delayed the growth of mycelium after 6 days of incubation

Fig. 1. Antagonistic zone between *Fusarium oxysporum* f. sp. *tulipae* and *Bacillus polymyxa* (right) and control colonies (left).

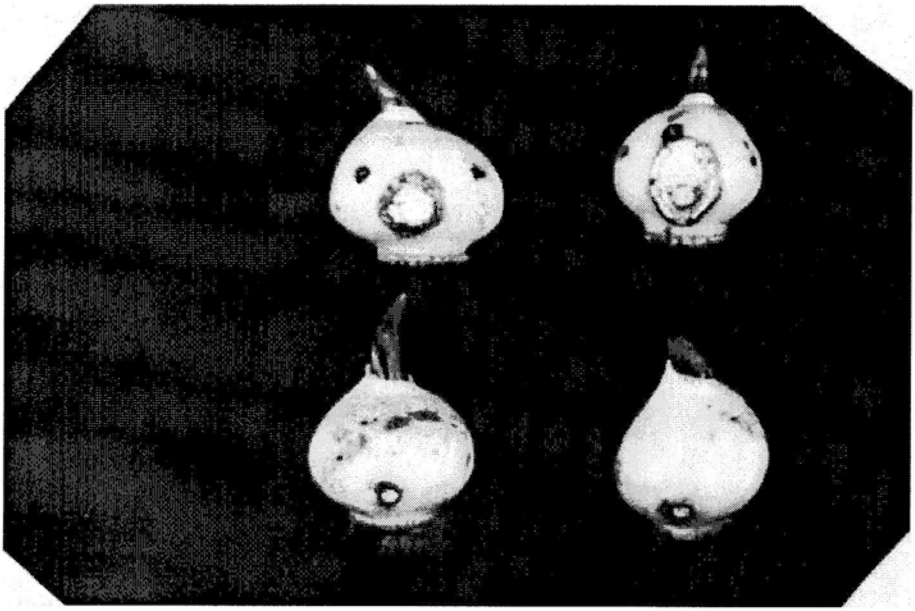
Microscopic examination of *F. oxysporum* f. sp. *tulipae* spores showed that after 12 and 28 h of incubation with *B. polymyxa* and its filtrates number of spore germination significantly decreased. Filtrate, sample 66, almost completely inhibited the germination after 76 h of incubation whereas of sample 67 and bacterial suspension were less effective (Table 2).

Table 2
Influence of *Bacillus polymyxa* and its filtrates on spore germination of *Fusarium oxysporum* f. sp. *tulipae*

Treatments	Number of cells in 1 ml or filtrate in %	Percentage of germinated spores after hrs		
Control (only in water)	–	32.0 c	40.4 b	41.5 c
B. polymyxa	0.1×10^8	15.9 b	30.8 b	66.4 d
Filtrate (sample 66)	10	0.8 a	0.0 a	0.5 a
Filtrate (sample 67)	10	3.1 a	2.6 a	22.4 b

Note: means followed by the same letter in each column do not differ at 5% level of significance

Fig. 2. Influence of *Bacillus polymyxa* (below) on the development of *Fusarium oxysporum* f. sp. *tulipae* on tulip bulbs



The development of necrosis on nontreated tulip bulbs (control) inoculated with *F. oxysporum* f. sp. *tulipae* directly after damage and 5 hours after damage of bulbs gave similar effect (Table 3). *B. polymyxa* and its filtrates inhibited the development of necrosis on tulip bulbs (Fig. 2, Table 3). Different number of bacterial cell suspension, used for soaking of tulip bulbs gave similar inhibitory effect in development of necrosis.

Filtrates were more effective in the inhibition of the pathogen spread on tulips bulbs than suspension of the bacteria (Table 3). Prochloraz completely inhibited the development of necrotic spots (Table 3).

Table 3

Influence of *Bacillus polymyxa* and its filtrates on the development of *Fusarium oxysporum* f.sp. *tulipae* (= *Fot*) on tulip bulbs; observation after 14 days of incubation

Treatments	Number of bacteria cells in 1 ml or conc. of filtrate in %	Diameter of necrosis (mm)	Depth of necrosis (mm)
Fot directly after damage	–	16.1 e	3.2 h
Fot 5 hours after damage	–	15.2 e	2.4 g
Prochloraz + *Fot*	0,9 mg/ml	0.0 a	0.0 a
Bacillus polymyxa + *Fot*	0,1 × 10^8	5.9 cd	0.6 d
	0,25 × 10^8	6.5 d	1.1 f
	0,5 × 10^8	6.3 cd	0.8 e
Filtrate (sample 66) + *Fot*	10	4.3 b-d	0.5 c
	25	3.9 bc	0.6 d
Filtrate (sample 67) + *Fot*	10	3.3 b	0.4 b
	25	2.3 b	0.3 b

Note: see table 2

The antagonistic effect of *B. polymyxa* to the previously tested pathogens (Saniewska et al., 1993, 1995, 1998) and to *F. oxysporum* f.sp. *tulipae* could be connected with production of antibiotics, changing of pH or competition between microorganisms in utilization of nutrients from the medium. Rosado and Seldin (1993) showed that *Bacillus polymyxa* produced anti-microbial substance active against fungi, yeast and different genera of Gram-positive and - negative bacteria. This substance appeared to be an antibiotic different from the polymyxin group and not identified yet. It is well known that *Bacillus subtilis* or its filtrates were effective against various fungal and bacterial pathogens and it was showed that *B. subtilis* produced many different antibiotics (Fiddman and Rossall, 1993; Gueldner et al., 1988; McKeen et al., 1986; Leifert et al., 1995; Loeffler et al., 1986). Further studies are necessary to explain of antagonistic mechanism of *B. polymyxa* toward fungal plant pathogens.

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Wpływ *Bacillus polymyxa* na wzrost i rozwój *Fusarium oxysporum* f. sp. *tulipae*

Streszczenie

Antagonistyczny wpływ *Bacillus polymyxa*, izolat S13, przeciwko *Fusarium oxysporum* f. sp. *tulipae* był badany w warunkach *in vitro* i *in vivo*. Wzrost tego patogena grzybowego w podwójnej kulturze z *Bacillus polymyxa* na pożywce ziemniaczano-glukozowej był silnie hamowany. Zawiesina *B. polymyxa* i filtraty tej bakterii zasadniczo hamowały kiełkowanie zarodników i wzrost grzybni *Fusarium oxysporum* f. sp. *tulipae* na cebulach tulipanów.