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PYOVERDINE PRODUCTION IN *PSEUDOMONAS FLUORESCENS* UTPF5 AND ITS ASSOCIATION WITH SUPPRESSION OF COMMON BEAN DAMPING OFF CAUSED BY *RHIZOCTONIA SOLANI* (KÜHN)

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Abstract: Siderophore production is an important mechanism of biological control by a number of strains of plant growth-promoting rhizobacteria. *Pseudomonas fluorescens* UTPF5 was originally obtained from onion field. Biochemical and physiological characteristics of this strain refer to biovar 3 of *P. fluorescens*. Strain UTPF5 is an effective bacterium against several phytopathogenic fungi. Pyoverdine type siderophore of this strain was isolated using XAD amberlite column. The plant growth promotion and antifungal properties of bacteria were demonstrated under greenhouse conditions in combination with Fe-EDTA, Fe-EDDHA and Zn as modulators of pyoverdine production. Amendment with zinc, Fe-EDTA and Fe-EDDHA suppressed the disease inhibition when partially used with UTPF5. 7NSK$_2$ and its pyoverdine mutant, MPFM1, were used as reference strains the inhibition percent of which was not affect by soil amendment. Iron chelates, especially Fe-EDDHA, increased growth and chlorophyll production by plants. This effect was improved in the presence of bacterial strains. The siderophore mutant MPFM1 did not exhibit satisfactory disease inhibition and growth promotion activity. *In vitro* experiments showed that purified pyoverdine could decrease the fungal growth to the same extent as pyoverdine-producing strain.

Key words: biological control, competition for iron, pyoverdine, *Rhizoctonia solani*

INTRODUCTION

Chemical fungicides often have not a satisfactory effect on soil borne phytophathogenic fungi (Vurro and Gressel 2006). Also inputs of agrochemicals used for crop protection are adversely affecting the quality of food products and the environment, thus making the development of alternative ways to control disease a high priority (Lemanceau *et al.* 1992). Some fluorescent pseudomonads are referred to as plant growth-promoting rhizobacteria (PGPR) and their effectiveness in controlling a number of plant diseases caused by soil-borne pathogens has been widely documented (O'Sullivan and O'Gara 1992; Glick 1995).The inoculation of seeds or roots with fluorescent pseudomonads to increase plant vigour and productivity has been a worldwide studied practice (Kloepper *et al.* 1980). Investigation of the cause of a beneficial effect of this kind of bacteria has implicated them in the control of a wide range of noxious root phytopathogenic fungi, amongst which *Gaeumannomyces graminis* var. *tritici* (Slinninger *et al.* 1996), *Rhizoctonia solani* (Kumar *et al.* 2007), *Phytophthora capsici* and *Fusarium oxysporum* (Lemanceau *et al.* 1992; Kumar *et al.* 2007) can be singled out. In spite of the interest devoted to understanding the protective role of PGPR, deciphering the mechanisms by which these bacteria exert their activity has remained a challenge. Mechanisms of biological control of plant pathogens by fluorescent pseudomonads generally involve production of bacterial metabolites such as siderophore (Kloepper 1980), hydrogen cyanide (Keel *et al.* 1989), antibiotics (Thomashow and Weller 1990), extra cellular lytic enzymes (Nielsen and Sorensen 1999) and induced systemic resistance (O'Sullivan and O'Gara 1992). Siderophore production was also postulated to be an important mechanism for the biocontrol activity of PGPR (Leong 1986; Becker and Cook 1988). The first contemplation was supported by the observations that treatment with purified pyoverdines had a disease-suppressive effect similar to that of treatments with the producing strain (Kloepper *et al.* 1980). Subsequent studies also showed that siderophore-negative mutants had no growth-promoting and disease suppressive activity (Schippers *et al.* 1987; Becker and Cook 1988).

Pseudomonads produce a variety of siderophore compounds such as pyoverdine, pyochelin and salycilic acid that one negatively regulated by cellular iron concentration (Budzikiewicz 1997). Siderophores protected plants through competition for iron (especially pyoverdine) and induction of plant defenses pathways (Duijff *et al.* 1993).

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Van Peer *et al.* (1990), by manipulating iron availability in soilless culture of carnation, also obtained evidence that siderophore-mediated iron competition can be involved in suppression of Fusarium wilt by fluorescent *Pseudomonas* spp.

Certain fluorescent Pseudomonas strains can further antagonize phytopathogens by using a variety of iron sources in the rhizosphere, indirectly contributing to plant protection (Visca *et al.* 2007). Maurhofer *et al.* (1994) reported that among the siderophores produced by rhizosphere bacteria, only the pyoverdines produced by the fluorescent pseudomonads had been implicated in induced systemic resistance (ISR) but further investigation showed that pyochelin and salycilic acid can similarly induce plant defenses (Buysens *et al.* 1996). Villegas research group developed a biological product GLUTICID, which is an antifungal product constituted by pyoverdine and salicylic acid produced by *P. aeruginosa* (De Villegas 2006). The main objective of this study was to determine whether or not siderophore production is a mechanism of disease suppression by UTPF5. Our goal was to apply some of the ions and chelates that modulate siderophore production and investigate their effects on disease inhibition. A siderophore mutant was used for comparison.

MATERIALS AND METHODS

Reference strains

Bacteria used were *P. aeruginosa*, reference strain 7NSK2 and its pyoverdine mutant, MPFM1, obtained from M. Hofte (University of Gent, Belgium). It was known that 7NSK2 was no the ability to produce 2, 4-diacetylphloroglucinol, pyoluteorin and HCN (Buysens *et al.* 1996). *P. fluorescens* UTPF5 was isolated from onion rhizosphere. Biochemical characterization of the bacterial strain was carried out according to Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994) and was accordingly identified as *P. fluorescens* biovar 3. The strains were maintained on kings B (King *et al.* 1954) slants and stored at 4°C. For long-term storage, cultures were maintained at –80°C in 40% glycerol-containing nutrient broth-yeast extract (NBY) (Kim *et al.* 1997).

R. solani AG-4 was isolated from roots of common beans obtained from the Department of Plant Protection, University of Tehran. Its pathogenicity was investigated on bean, and was proven.

Pyoverdine purification

For purification of pyoverdine, the method of Meyer *et al.* (1998) was used with some modification. A 0.5 l culture in Casamino acids medium of the *P. fluorescens* UTPF5 strain was grown at 25°C for 48 h on a shaker at 120 rpm. After 48 h of incubation, bacterial cells were harvested by centrifugation at 6 000 rpm for 10 min. The supernatant was adjusted to pH 6.0 and was filtered before ion exchange chromatography on a column Amberlite XAD-4 (2.5x15 cm). The pyoverdine was eluted with methanol 50%. The obtained crude pyoverdine was concentrated and then passed through a CM-Sephadex C-25 (2.6x33 cm) column, eluted with 0.2 m pyridine/acetic acid buffer (pH 6) to enhance the purity. It was then concentrated and lyophilized prior to storage at 4°C in the dark.

Cell incubation and pyoverdine determination

Samples of the stored strain were used to prepare overnight cultures in 5 ml of liquid King's B medium and incubated with agitation at 150 rpm and 28°C. Samples of 0.1 ml of this culture were used to inoculate of 25 ml of Standard Succinate Medium, SSM, (Meyer and Abdallah 1978) which were incubated for 40 h with agitation (120 rpm) at 28°C. The bacterial cells were then pelleted by centrifugation at 6 000 rpm for 15 min and the clear supernatants were utilized for analysis. The absorbance spectra at 400 nm were recorded using a PG instrument spectrophotometer ($T70^{+}$) in 1.0 cm cells, against MPFM1 supernatant as blank. Production of pyoverdine was calculated according to standard curve of purified pyoverdine. The pelleted cells were resuspended in 5 ml of distilled water to determine the turbidity of the bacterial suspension cultures at 600 nm. All glassware's were washed with 6M HCl and rinsed four times with de-ionized water (Guillermo *et al.* 2005).

Dual culture inhibition assays

Tests were performed in agar King's medium B**.** Bacterial antagonists were spotted at the sides of the Petri dish (10 µl of an overnight culture in SSM from a single colony) and preincubated at 28°C for 2 days. *R. solani* isolates were then inoculated in the center of the bacterial growth in three replicate plates and incubated as explained previously. Inhibition of fungal growth was assessed 3 days later by measuring the size of the inhibition zone (in mm).

***In vitro* antibiosis against phytopathogenic fungi by pyoverdine and a chemical chelator**

KB agar plates were modified by reducing deferated proteose peptone content to 10 g/l and adding 4 g/l deferated casamino acid for supporting fungal growth. Proteose peptone and casamino acid were deferated as Meyer and Abdallah (1978). Mycelium plugs of *R. solani*, *P. aphanidarmatum*, *S. sclerotiurum* and *F. solani* (isolated from bean rhizosphere) were inoculated in the center of plates, after one day four sides of plate was punched with 5-mm-diameter holes by using a gel puncher. Thirty-five microliters aliquots of the 100 µm pure pyoverdine, commercial defroxamine mesylate (Desferal®, Novartis, Basel, Switzerland), EDTA, DTPA and 8-hydroxyquinoline (Merck, Germany) were added to the punched holes. After observing a complete diffusion of the solutions added to the holes, the same volumes of the solutions were re-added to the holes to make the final volume 70 ml. Inhibition halo were measured after incubating at 28°C for full growth of control.

Greenhouse experiments

Preparation of pathogen and antagonist inoculums

Fungal inoculum was raised in 250 ml Erlenmeyer flasks containing pearl millet seeds and bean meal mixed in a 4 : 1 ratio, i.e. 80 g dry pearl millet seeds and 20 g of

bean meal, with 30 ml of water to moisten the mixture. All flasks were sterilized at 121°C for one hour in two consecutive days. For inoculation, mycelium plugs were taken from the periphery of 4-day-old stock cultures. Flasks were incubated at 28°C for five to seven days for uniform mycelia growth.

Seed treatment with bacteria

Seeds of common bean (cv. Naz) were disinfected with 2.5% sodium hypochlorite for three minutes and coated with bacteria by methods similar to those described by Weller and Cook (1983). Bacterial strains were grown on King's broth medium for 48 h with continuous shaking at 120 rpm at 28°C. The bacterial cells of each PGPR strain were harvested by centrifugation and suspended in 1% CMC (Carboxymethyl cellulose), adjusting their concentration to 10^9 CFU/ml. The methylcellulose suspension with vegetative cells of each bacterium were then mixed with 10 g of seeds in a Petri plate, and the coated seeds were dried for 1.5 to 2 h under a stream of sterile air at room temperature. The control was treated with 1% CMC. Bacterized and nonbacterized seeds were sown in loamy soil (0.43% total organic matter, pH 7.7, and 2.60 mg/kg iron) with 30% added acid washed quartz. Acid washing, with HCl 6N, of quartz was done to remove any iron contamination. This was followed by autoclaving three times for one hour on alternate days to remove any indigenous microbial contamination.

Evaluation of growth promotion activity

A 4x4 factorial experiment was conducted under glasshouse conditions to evaluate the effect of $ZnSo_4$ and iron chelates of bacterial strains for promotion of plant growth. The factors comprised of four bacterial applications (UTPF5, 7NSK2, MPFM1 and control without bacteria) and three soil amendments (25 ml of 1 mM Fe-EDDHA, 1 mM Fe-EDTA, and 100 µm $ZnSO_4$ solutions per pot and distilled water as control). The planted pots were placed in greenhouse with a 16/8-h photoperiod. Plants were watered twice a week from the bottom of the pots. The experiment was terminated 45 days after sowing and plant growth parameters including plant height and fresh weight of shoot and root were recorded. The leaf greenness (SPAD reading) of the upper three fully expanded leaves was measured by SPAD-502 chlorophyll meter (Minolta K. Arano & Co. Ltd., Tokyo, Japan).

Effect of soil amendments to suppress *R. solani* by antagonistic bacteria

The *R. solani* pearl millet inoculums were used for artificial infection at a concentration of 1.5 g of inoculum per g of soil. Treated bean seeds (cv. Naz) were sowed in previously described soil. Each treatment included three seeds and was replicated four times. Bacteria and soil amendment were applied in the same way as mentioned for plant growth experiment. Pots were watered daily to their starting weight to keep the soil moisture content constant at 70%. Plants were kept in a greenhouse at 24±4°C and 16/8-h photoperiod. After 3 weeks, roots were washed, and disease severity was rated using a scale of 0 to 5 (according to Kim *et al.* 1997 with modifications) where, 0 = no visible symptoms, healthy plant; 1 = < 20% roots with a single typical brown sunken lesion 2 = 50% roots with typical lesions; 3 => 70% roots with lesions; 4 = post emergence damping-off, shoot length less than 5 cm; 5 = seed rot or preemergence damping-off. Disease index percent was computed by using the formula:

$$\%DI = \frac{\sum_{i=1}^{m=3}(n_i)}{m \times 5} \times 100$$

where:
ni – disease scale for seedlings
m – sum of seedling in replication
i – number of seedlings

Statistical analyses

Data were analyzed for significance by analysis of variance, followed by Duncan's multiple range test ($p < 0.05$), with the SAS general linear model procedure (SAS Institute, Cary, NC). Normal distribution and homogeneity of variances were checked beforehand. Complete randomized design (CRD) was used in all experiments and each treatment was replicated four times.

RESULTS

Dual culture inhibition assays

In vitro antibiosis of bacterial strains were tested on King's B agar plates. Among 30 isolates only five isolate (UTPF5, UTPF76, UTPF61, 7NSK2 and UTPF24 respectively) had a satisfactory effect on *R. solani* mycelial growth. From these UTPF5 was selected because of high level of siderophore production in CAS agar, correlation of its inhibitory effect with added FeCl3 and association for inhibition with several phytopaghenic fungi (data not shown).

***In vitro* antibiosis against phytopathogenic fungi by pyoverdine and commerical iron chelators**

In this experiment five chemical and biological iron chelators with different affinity for iron were chosen. Pyoverdine and desferal obtained from UTPF5 and S*treptomyces* sp. respectively, were biological chelators. EDTA and DTPA as common agricultural chelators and 8-hydroxyquinoline that formerly was used as fungicide were synthetic iron chelators. Results showed that in case of *R. solani* and *F. oxysporum*, weak chelates (Desferal and DTPA) not only did not inhibit the growth of fungi but also increased the growth over the control. Strong iron chelats, pyoverdine and 8-hydroxyquinoline, decreased mycelial growth in all tested fungi (Table 1). In this test, *R. solani* and *F. solani* exhibited low susceptibility to iron chelates especially to linear chelates (EDTA, DTPA, Desferal) but *S. sclerotiorum* and *P. aphanidermatum* were susceptible to all iron chelates.

Plant growth promotion activity

As shown in table 2 all treatments affected the efficiency plant growth factors significantly. Bacterial siderophore could remedy deficiency of iron. Addition of iron

chelates, especially Fe-EDDHA, increased these effects. UTPF5 in the presence of 1 mM Fe-EDDHA showed the best result. Pyoverdine mutant, MPFM1, did not increase plant growth and moreover exhibited antagonistic effect on growth of bean plants. Results showed that MPFM1 decreased plant growth parameters comparing with control (Table 2), plant leaves exhibited varied at levels of deformation in the presence of this strain which seemed not to be infectious (data not shown). However, 7NSK2 and its combination with Fe-EDDHA at 1 mM significantly increased plant growth compared with the control ($p < 0.05$). In comparison to the control, all treatments significantly increased chlorophyll content of leaf. Combination 7NSK2 and Fe-EDDHA was the best treatment (Table 2).

Suppression of *R. solani* damping-off

The *R. solani* strain isolated from common beans exhibited severe pathogenicity in the greenhouse so that no seedling emergence was observed in control pots (Table 3). UTPF5 had high biological controlling activity and suitably controlled *R. solani* by 75%. All materials added to the soil decreased the biological control activity of this strain severely. Fe-EDDHA showed the highest repressing effect on disease suppression by UTPF5 among other amendment treatments. Ulterior repressing effect was observed for Fe-EDTA (Table 3).

The use of transposon mutants is a powerful tool for studying the involvement of bacterial metabolites in biocontrol of soil-borne diseases. To determine the involvement of pyoverdine production in suppression of *Rhizoctonia*

Table 1. Competition for iron mediated inhibition of *F. solani*, *R. solani*, *S. sclerotiurum* and *P. aphanidermatum* involving chemical and biological iron chelates in modified King's B medium

Chelates	Fungal growth inhibition			
	R. solani	*F. solani*	*P. aphanidermatum*	*S. sclerotiorum*
Desferal [1]	0 i	0 i	14 g	0 i
DTPA	0 i	0 i	23 f	6 g
EDTA	0 i	0 i	33 e	18 f
Pyoverdine	42 d	53 c	53 d	58 c
8-hydroxyquinoline	83 b	95 a	94 a	78 b
Water control	0 i	0 i	0 i	0 i

[1] Desferal® is commercial siderophore obtained from species of *Streptomyces*, EDTA and DTPA are commonly used agricultural chelates. 8-hydroxyquinoline, formerly used as fungicide, is a severe iron chelate.
Different letters represent significant differences according to Duncan's multiple range test ($p < 0.05$)

Table 2. Effect of soil amendments and bacterial strain on plant growth parameters of common bean in sterile condition

Treatment[1]	chlorophyll	Root dry wt. [g]	Root length [cm]	Shoot dry wt. [g]	Shoot length [cm]
UTPF5	42.66 ab	19.78 c	79.0 a	10.29 b	17.66 abc
UTPF5 + $ZnSO_4$	40.36 ab	22.53 b	68.5 a	8.28 de	16.5 abcd
UTPF5 + Fe-EDDHA	43.66 ab	25.32 a	71.29 a	11.07 a	18.52 a
UTPF5 + Fe-EDTA	39.93 b	24.412 ab	67.92 a	10.16 b	18.25 ab
7NSK2	41.80 ab	11.217 h	69.0 a	7.76 ef	15.33 cd
7NSK2 + $ZnSO_4$	41.73 ab	16.677 def	72.5 a	6.93 gh	15.5 bcd
7NSK2 + Fe-EDDHA	46.33 a	19.25 cd	74.72 a	9.51 bc	17.38 abcd
7NSK2 + Fe-EDTA	41.13 ab	18.56 cde	73.59 a	8.82 cd	16.59 abcd
MPFM1	33.30 cde	7.88 i	34 b	5.30 jk	15.33 cd
MPFM1 + $ZnSO_4$	33.50 cde	12.78 gh	50.0 ab	4.02 l	14.66 d
MPFM1 + Fe-EDDHA	37.40 bcd	15.03 fg	52.25 ab	6.27 hi	16.91 abcd
MPFM1 + Fe-EDTA	37.467 bcd	14.53 fg	51.75 ab	5.77 ij	16.41 abcd
Without Bacteria[2]	21.0 f	11.18 h	53.33 ab	4.82 k	16.0 abcd
Without Bacteria + $ZnSO_4$	27.66 ef	14.41 fg	75.0 a	5.29 jk	15.0 d
Without Bacteria + Fe-EDDHA	38.46 bc	16.57 def	77.16 a	7.45 fg	17.16 abcd
Without Bacteria + Fe-EDTA	31.33 de	16.16 ef	76.75 a	7.04 fgh	16.75 abcd

[1]amendment contain 25 ml of 1 mM Fe-EDDHA, 1 mM Fe-EDTA and 100 μm $ZnSO_4$ solutions per pot
[2]distilled water used as control.
Different letters represent significant differences according to Duncan's multiple range test ($p < 0.05$)

Table 3. Influence of integrated application of PGPR strains and ion sources on the disease severity caused by *R. solani* in common bean

Amendment	Disease severity [%]				
	MPFM1	7NSK2	UTPF5	without bacteria	without pathogen
Fe-EDDHA	68.4 e	58.33 e	63.7 e	100 a	0 i
Fe-EDTA	89 bc	57.33 e	40.33 g	93.3 ab	0 i
Zn	79 cd	47.67 fg	34 g	100 a	0 i
Control	78 d	62 e	25 h	97.3 ab	0 i

Different letters represent significant differences according to Duncan's multiple range test ($p < 0.05$)

damping-off, bacterial strain 7NSK2 and its pyoverdine-negative (Sid^-) mutant MPFM1 were compared for their ability to control bean damping-off in the absence or presence of iron and Zn sources. Results showed that 7NSK2 is more efficient than MPFM1 in disease suppression and plant growth promotion. Interestingly, disease suppression did not alter significantly in the presence of iron chelates and Zn. According to Buysens *et al.* (1996) the biocontrol activity of pyoverdine in this strain is dependent on induction of systemic resistance which is independet on quantity of this metabolite.

DISCUSSION

Fungal species exhibit different behaviour against iron chelates that refer to their siderophore stability. According to Crowley (2006) most of fungi produce hydroximate siderophore, similar to defroxamine mesylate, that have low iron affinity in comparision to pyoverdines and 8-hydroxyquinoline. Unfortunately there is less information about phytopathogenic fungi siderophores and their stability. To our knowledge, so far no report has been found on siderophore production in tested fungal species. Some Fusarium specieses produced fusarinin that includes hydroxymate with low affinity to iron, but this was not observed in phytopathogenic *Fusarium* (Das *et al.* 2006).

The concentration of free Fe^{3+} at physiological pH is about 10^{-17} m/l while about 10^{-6} m/l would be needed to maintain the necessary supply for microorganisms (Budzikiewicz 1997). In this situation severe competition for iron acquisition could be extrapolate; to circumvent this problem soil microorganisms produce a variety of compounds (siderophores) which can form water soluble Fe-complex. Naturally suppressive soils to *Fusarium* wilts are known to have physicochemical properties (high pH and CaCO3 content) contributing to a very low solubility of ferric iron, accounting for a strong iron competition in these soils (Alabouvette *et al.* 1996). In these conditions fluorescent pseudomonads produce pyoverdine type siderophore with high stability and affinity for iron that restricts growth of microorganism with low iron competition ability such as phytopathogenic fungi. Iron chelates that diminish competition for iron can remove this restriction. According to Leong (1986) Siderophore-mediated growth promoting activity of PGPR is associated with the suppression of root pathogens by competitive exclusion and the activity is reversed by the addition of Fe-EDTA. The importance of iron competition in microbial antagonism was further supported by studies on natural soil suppressiveness to *Fusarium* wilts which was ascribed at least partly to fluorescent Pseudomonads (Scher and Baker 1982; Lemanceau *et al.* 2006). In these soils, introduction of Fe-EDTA, increasing the availability of Fe (III) to pathogenic *F. oxysporum*, resulted in a lower iron competition and consequently higher *Fusarium* wilt severity. According to Lemanceau *et al.* (1992) EDDHA decreased the iron availability to *F. oxysporum* as suggested by the reduced germination of chlamydospores and germ tube length *in vitro* and in soil, whereas Fe-EDDHA did not.

In this study Fe-EDTA have less efficacy in promotion of plant growth and restriction of antagonistic activity of bacteria in comparision to Fe-EDDHA that could be explained as follow. Entisol and aridisol soils, with high bicarbonates content, comprise more than 50% of agricultural fields in Iran. Above pH 8.0, low levels of organic materials (< 0.5%) and high levels of CaCo3 make iron as a restrictive factor in productivity of these soils. Because of its low stability at pH > 6.5, Fe-EDTA could not be a suitable fertilizer for such soils (Banaei *et al.* 2005). However Fe-EDDHA can be introduced as the best iron chelate in stated situation.

The pH was 7.7 in examined soil in which Fe-EDTA had no iron chelate activity and mostly was binded divalent metal ions such as Zn^{2+} and Cu^{2+}. Whereas, Fe-EDDHA could be used as an effective iron source in such soils.

In addition to iron content, environmental conditions and composition of root exudates may influence pyoverdine (as well as other siderophores) production on/within plant tissues, thus stimulating or abolishing any effect on either plant-growth promotion or plant protection potential capabilities exerted by them (Mercado-Blanco and Bakker 2007). In this research biocontrol activity of 7NSK2 was not affected in the presence of iron chelates (Table 3), there are indications that pyoverdine production of 7NSK2 under stress situation is not due to iron-limitation and is regulated in an independent way (Hofte *et al.* 1991).

Most of the soils of Iran are alkaline, saline with high content of bicarbonates (Banaei *et al.* 2005) and therefore, *P. fluorescence* strain UTPF5 seems to be well adapted to alkaline conditions.

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POLISH SUMMARY

WYTWARZANIE PYOWERDYNY PRZEZ *PSEUDOMONAS FLUORESCENS* UTPF5 I JEJ ZWIĄZEK Z OGRANICZENIEM ZGORZELI FASOLI ZWYCZAJNEJ WYWOŁYWANEJ PRZEZ *RHIZOCTONIA SOLANI*

Wytwarzanie syderoforów jest ważnym mechanizmem biologicznego zwalczania przez wiele szczepów bakterii rizosferowych stymulujących wzrost roślin. *Pseudomonas fluorescens* UTPF5 uzyskano z pola, na którym uprawiano cebulę. Cechy biochemiczne i fizjologiczne tego szczepu znajdują odniesienie do browaru 3 *P. fluorescens*. Szczep UTPF5 jest bakterią efektywną przeciwko kilku patogenom roślin. Syderofor tego szczepu typu pyowerdyny wyosobniono przy wykorzystaniu kolumny amberlitowej. Pobudzenie wzrostu roślin i przeciwgrzybowe właściwości bakterii stwierdzono w warunkach szklarniowych, wykorzystując Fe-EDTA, Fe-EDDHA i cynk, jako czynniki modulujące wytwarzanie pyowerdyny. Dodatek cynku, Fe-EDTA i Fe-EDDHA ograniczały inhibicję, gdy były użyte częściowo z UTPF5. 7NSK2 i jego mutant pyowerdynowy MPFM1, zostały użyte jako szczepy porównawcze, w przypadku których, procent inhibicji nie był modyfikowany przez dodatki wprowadzone do ziemi. Chylaty żelaza, specjalnie F-EDDHA, stymulowały wzrost i wytwarzanie chlorofilu przez rośliny. Ten efekt był lepszy w obecności szczepów bakterii. Mutant syderoforowy MPFM1 nie wykazywał zadawalającej inhibicji choroby i stymulującego działania. Doświadczenia *in vitro* wykazały, że oczyszczona pyowerdyna mogłaby zwiększać wzrost grzyba do takiego samego stopnia jak szczep wytwarzający pyowerdynę.