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POPULATION DYNAMICS OF BACTERIA INCLUDING ANTIFUNGAL SPECIES IN THE RHIZOSPHERE OF OILSEED RAPE DURING ITS LIFE CYCLE

GABRIELE BERG, PETRA MARTEN and HUBERT BAHL

University of Rostock, Department of Biology, Microbiology, Rostock, Germany

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The population dynamics of different bacterial groups with antifungal properties was investigated in comparison with total bacteria based on colony forming units for successful biological control of the soil-borne pathogen *Verticillium dahliae* var. *longisporum* Stark causing tracheomycosis of oilseed rape. This is the first report about bacterial population densities of a plant with a winter annual life cycle. Population densities of colony forming bacteria of oilseed rape (*Brassica napus* spp. *oleifera* Metzg. Sinsk.) were significantly lower in autumn and winter than in spring and summer. The number of antifungal bacterial populations like fluorescent pseudomonads, *Bacillus spec.* (spores) and *Stenotrophomonas maltophilia* also increased during life cycle. The percentages of antifungal isolates were 7 % on average and relatively stable during time of investigation as estimated by a bioassay *in vitro*. The antifungal mechanisms of 10 different isolates of each selected taxonomic group were investigated. The mechanisms were specific for each isolate. All of these selected strains showed antifungal activity against *V. dahliae* var. *longisporum in vitro* and therefore, they were evaluated as potential biocontrol agents against this pathogen.

KEY WORDS: Population dynamics, antifungal rhizobacteria, oilseed rape, *Verticillium dahliae*

1. INTRODUCTION

Rhizobacteria are ideal for use in biological control of plant pathogens, since the rhizosphere provides the front-line defense for soil-borne pathogens (Weller, 1988). It is necessary, therefore, to identify those bacterial groups which may be abundant in the rhizosphere and then study their function with respect to prevention and reduction of plant infection by deleterious microorganisms (Miller *et al.*, 1989). The size and composition of the rhizosphere microflora is to a large degree plant-dependent (Burr and Caesar, 1984). Oilseed rape (*Brassica napus* spp. *oleifera* Metzg. Sinsk.) is an important crop with a winter annual life cycle. Two important groups of the antifungal bacterial populations of oilseed rape are fluorescent pseudomonads and isolates of *Bacillus subtilis* (Berg, 1996). Another interesting rhizobacterium of oilseed rape with antifungal properties is *Stenotrophomonas maltophilia*. This methionin-auxotrophic bacterium has been isolated in high numbers from oilseed

Dr. Gabriele Berg, Universtität Rostock, Fachbereich Biologie, Mikrobiologie, Gertrudenstr. 11a D-18051 Rostock. Tel.: 0381 494 2048, Fax: 0381 494 2244.

rape, which excrete high levels of sulphur containing compounds e.g., amino acids like methionin (Berg *et al.*, 1996).

Verticillium dahliae var. *longisporum* Stark is an important pathogen of oilseed rape causing tracheomycosis and leading to high yield losses. *V. dahliae* was reported to be suppressed by soil solarization (Tjamos, 1992). However, this method is not practicable in Central and Northern Europe due to the short time and the low intensity of sunshine. Fungicide spray treatments are ineffective against Verticillium wilt (Fravel, 1992). One reason is that plants may be infected during all developing phases. The early infections in autumn cause the highest yield losses (Zeise *et al.*, 1990). Therefore, biological control of Verticillium wilt with antifungal rhizobacteria is a very important objective.

This study reports data on the population densities based on colony forming units of fluorescent pseudomonads, *Bacillus spec.* (spores) and *Stenotrophomonas maltophilia* in the rhizosphere of oilseed rape during the growth season. Additionally, the percentage of antifungal bacteria was determined in a bioassay against *V. dahliae* var. *longisporum in vitro*. The antifungal mechanisms, including production of antibiotics (antibiosis), production of siderophores (competition), production of lytic enzymes like chitinases and β-1,3-glucanase and production of cyanide (induction of resistance of the host plant) were characterized for 10 isolates of each investigated group.

2. MATERIALS AND METHODS

Isolation of bacterial antagonists and determination of the colony forming units (cfu)

A field trial with oilseed rape cv. Wotan was established in 1994/1995 in Rostock. The trial consist of 6 plots. Samples were taken monthly at different developmental stages of plants. At each sampling time, 6 independent collective samples (based on 10 plants) were placed into plastic bags and transported to laboratory. Plant roots with adhering soil particles (10 g) were washed for 20 min in sterile 0.85 % NaCl-solution. The suspension was plated in serial dilutions on nutrient agar (Gibco, Paisley, Scotland), King B Medium (Gibco) and *Xanthomonas Maltophilia* Selective Medium XMSM (Juhnke and Des Jardins, 1989). The selective medium XMSM contained (l^{-1}): maltose (10 g) (Sigma, Deisenhofen, FRG), peptone from casein (5 g) (Serva, Heidelberg, FRG), bromthymol blue (4 ml of 2 % aqueous solution) (Sigma), and Bacto-Agar (15 g) (Difco). After the medium was autoclaved and cooled to 50°C, pH was adjusted to 7.1 with 1 N NaOH and the following antibiotics were added (in μg ml^{-1}, all from Sigma): cycloheximide (100), nystatin (50), cephalexin (50), bacitracin (25), penicillin G (25), novobiocin (10), neomycin sulfate (30), and tobramycin (1). To isolate *Bacillus* spores the suspension was heated for 20 min at 85°C in a water bath and after cooling plated on nutrient agar (Gibco). Plates were incubated for 7 days at 20°C and bacterial colonies were counted to determine the number of colonies (colony forming units = cfu). The fluorescent pseudomonads were detected on King B Medium under UV-light (254 and 366 nm). Mean population densities were calculated based on colony forming units per gram fresh weight (cfu g^{-1} fw). The calculated means for each microbial group and sampling time were

analysed for statistically significant differences using analysis of variance. Selected isolates (12 h-culture, 30°C) were stored in nutrient broth with 15 % glycerol at −80°C for other testing.

Bioassay for in vitro inhibition of V. dahliae var. longisporum

The ability of the bacterial antagonists to produce antifungal substances against *V. dahliae* var. *longisporum* was determined by a paired *in vitro* assay on Waksman agar [WA; containing: proteose-peptone (5 g) (Merck, Darmstadt, FRG), glucose (10 g) (Merck), meat extract (3 g) (Chemex, München, FRG), NaCl (5 g) (Merck), agar (20 g) (Difco), distilled water (1l), pH 6.8]. A suspension of hyphal fragments of *Verticillium* were plated on agar and bacteria were streaked as a broad band. Zones of inhibition were measured after 5 days of incubation at 20°C. *V. dahliae* var. *longisporum* strains had been isolated from oilseed rape in Rostock-Biestow (1989), were received from Dr. K. Zeise (Prophyta GmbH). 100 bacterial isolates per each sample, which were randomly selected, were investigated.

Bacterial identification

For the identification of isolates, a standardized micromethod, employing the Analytical Profile Index strip (API 20 E, API 20 NE and API 20 B) which consists of a gallery of 20 biochemical tests separated into 7 groups was used (Bio Mérieux, Marcy-L'Etoile, France). Additionally characteristics described by Balows *et al.* (1992) and Bergey's Manual of Systematic Bacteriology (Krieg *et al.*, 1984).

Bioassay Antibiosis

The relative inhibition of *V. dahliae* var. *longisporum* by extracellular products e.g. antibiotics of the bacterial strains was assayed on WA-plates (15 ml) containing 5 ml of the sterile culture filtrat (64 h-culture). The pH was measured between 7 and 8, the differences had no effect on the growth of the fungi. A 5 mm plug from agar plates of *V. dahliae* var. *longisporum* was placed in the centre of the plate. As controls, prepared WA-plates (20 ml) were similarly inoculated with mycelial plugs. Colony diameters were measured daily up for 10 days and reduction (%) in linear growth on the fungus was calculated.

Siderophore production

To analyze the ability of selected bacterial isolates to produce siderophores we used the plate assay according to Schwyn and Neilands (1987) and varied by Heupel (1992).

Lytic enzymes

Chitinase: Bacterial colonies were screened for chitinase production and excretion by plating on nutrient agar (Merck) containing 2 % colloidal chitin (Sigma). The

clear halos indicating of the enzymatic degradation were measured after 14 days of incubation at 20°C.

Glucanase:β-1,3-glucanase activity was determined by measuring the production of reducing sugars from laminarin (Fluka) according to Daugrois *et al.* (1990). The standard assay (1 ml) contains the enzyme extract, 2.5 mg laminarian, 100 mM acetate buffer pH 5.2. The laminarin substrate was dissolved in acetate buffer by heating at 60°C before use. The reaction mixture was incubated 1 h at 50°C. Total reducing sugars were assayed by colorimetric method and expressed as glucose equivalents.

Cyanide

To analyse bacterial production of cyanide we used the "Aquaquant 14417-Testsystem" (Merck). Culture broth (nutrient broth, 64-h) of the isolates was investigated.

Indole-3-acetic acid (IAA)

Production of IAA was investigated with the method of Gordon and Weber (1991) in which IAA present in culture filtrate (3 ml) was reacted with Salkowski reagent (2 ml) to yield a pink coloured product after 30 min incubation, was used. The contents of IAA in the medium was qualitatively measured on a spectrophotometer at 530 nm.

3. RESULTS

Bacterial populations dynamics

The total number of bacteria in the rhizosphere of oilseed rape increased during the plant's life cycle (Fig. 1). There were significant differences of the bacterial populations during the plant phase leaf-rosette in autumn and winter (October–March) and the phase for flowering and fruiting in spring and summer. In September, one month after sowing, the population of total bacterial cells was 4.3×10^7 cfu g^{-1} fw (colony forming units per gram root, fresh weight). In July, the highest number of bacteria was detected: 7.5×10^8 cfu g^{-1} fw. It is not possible keep the influence of soil temperature and development stage of plants seperate because of the interdependence of growth and temperature in the field. The numbers of fluorescent pseudomonads also increased (Fig. 1). In January, the lowest number was determined with 6×10^8 cfu g^{-1} fw and the highest number in July with 5.2×10^8 cfu g^{-1} fw. The percentage of fluorescent pseudomonads in the rhizosphere was high (21 – 96 %) during all phases of life cycle (Tab. 1) and the percentage of pseudomonads accounted for 56 % on average. Population densities of *Bacillus spec.* were low in general but also increased at the last sampling time (Fig. 2). The number of isolates of *Bacillus spec.* was variable and represent more than about 3 % on average of the total bacterial populations in the rhizosphere of oilseed rape. *S. maltophilia* was found in low numbers in

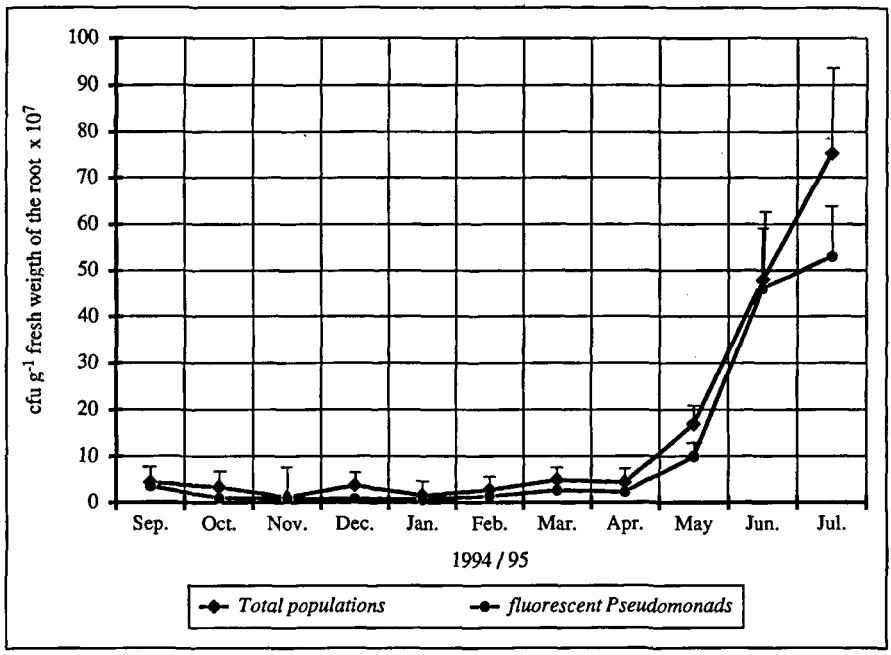

Fig. 1 Total bacterial populations and populations of fluorescent pseudomonads in the rhizosphere of oilseed rape during the vegetation period. (Note: Data are given as a number of bacteria (colony forming units on nutrient agar) per gram root calculated on a fresh weight basis).
Bars show standard deviation.

Table 1 Fluorescent pseudomonads, *Stenotrophomonas maltophilia*, *Bacillus* spec. (spores) and antifungal bacteria as percentages of bacteria found in the rhizosphere of oilseed rape. Antifungal bacteria were detected in an *in vitro* bioassay (dual culture against *Verticillium dahliae*)

Date	*Percentages of (%)*			
1994/95	*Pseudomonads*	*S. maltophilia*	*Bacillius spec.*	*Antifungal isolates*
September	70,7	0,095	1,405	2,7
October	28,4	1,1	N.D.	15,2
November	74,3	1,5	13,2	3,9
December	21,4	0,07	1,8	7,3
January	41,1	0,2	10,3	5,2
February	50,2	0,1	3,3	11,8
March	52,7	0,05	1	5,2
April	51,9	0	1,2	3,8
May	59,2	0	0,2	7,2
June	96	1,4	0,04	6,7
July	70,3	1	0,6	5,4

the winter months and higher numbers in months with higher temperatures like September (3.7×10^4 cfu g^{-1} fw), October (3.5×10^5 cfu g^{-1} fw), November (1.3×10^5 cfu g^{-1} fw), June (6.6×10^6 cfu g^{-1} fw), and July (7.3×10^6 cfu g^{-1} fw, Fig. 2). Population densities of *S. maltophilia* were low from all samples.

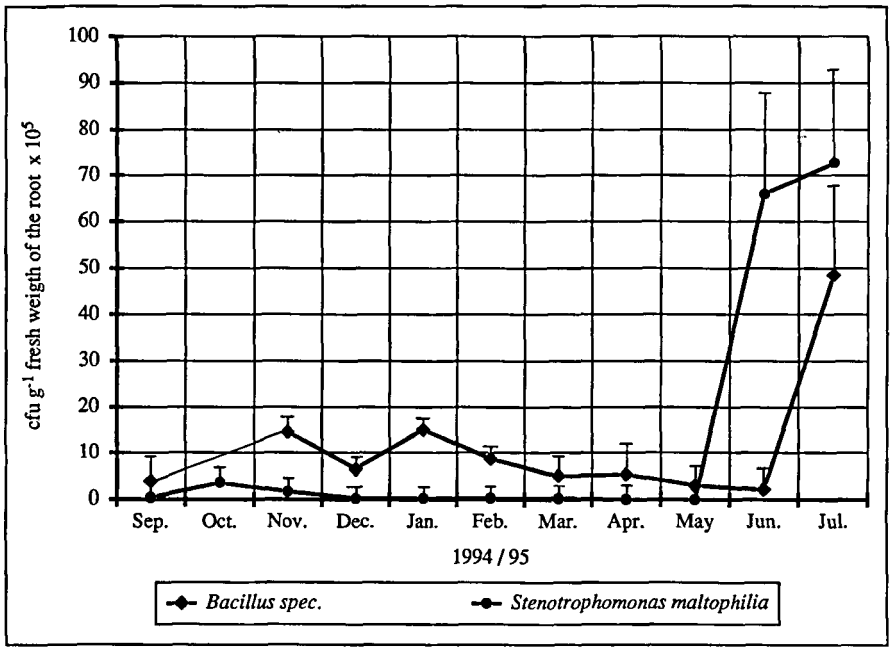

Fig. 2 Bacterial populations of *Bacillus spec.* (spores) and *Stenotrophomonas maltophilia* in the rhizosphere of oilseed rape during the vegetation period. (Note: Data are given as a number of bacteria (colony forming units on nutrient agar after heating for *Bacillus spec.* and on *Xanthomonas maltophilia* Selective Medium XMSM according to Juhnke and Des Jardins, 1989 for *Stenotrophomonas maltophilia*) per gram root calculated on a fresh weight basis).
Bars show standard deviation.

The percentages of antifungal isolates were relatively stable during the plant's life cycle (Tab. 1). There were no significant differences at anthesis or at maturity like the other investigated parameters. The percentage was 7 % on average.

Antifungal and plant growth promoting mechanisms of selected isolates

Of each investigated bacterial group with potential antifungal properties, 10 isolates were selected and screened for their activity in dual culture against *V. dahliae* var. *longisporum* and their antifungal and plant growth promoting mechanisms *in vitro* (Tab. 2). Seven isolates of the group of fluorescent pseudomonads belonged to *Pseudomonas fluorescens*, the other to *P. aureofaciens*. All of the isolates, exceptionally isolate *C36*, showed a very high activity against *V. dahliae* var. *longisporum* in dual culture assay. The antifungal effect based on a combination of several mechanisms. Antibiosis is involved in all interactions between *Pseudomonas* species and fungal pathogens. Most of the isolates produced siderophores in high quantity. Only four isolates were able to produce the lytic enzyme β-1,3-glucanase and no *Pseudomonas* isolate showed chitinolytic activity. Production of cyanide was proved for all isolates. Six isolates were able to excreted indole-3-acetic acid into supernatant. The efficiency of antifungal activity in bioassay and involved mechanisms of the isolates of *Bacillus subtilis* were different (Tab. 2). Only three isolates produced

Table 2 Antifungal and plant growth promoting mechanisms of the isolates

No. isolates	*Activity V. dahliae*	*Bioassay Antibiosis*	*Siderphores*	*Lytic enzymes*		*HCN*	*IAA*
				Glucanase	*Chitinase*		
Isolates of fluorescent pseudomonads							
C 21[1]	+++	+	+++	–	–	+++	+
C 36[1]	++	++	+++	–	–	+++	+
PS 18[1]	+++	++	+++	+	–	+	–
PS 235[1]	+++	++	+++	–	–	+	–
PS 861[1]	+++	++	++	+	–	+	–
PS 1182[1]	+++	++	–	+	–	+	–
PS 1657[1]	+++	++	+++	–	–	+	+
PS 1097[2]	+++	++	–	+	–	+	+
PS 1106[2]	+++	++	+++	–	–	+	+
PS 1106[2]	+++	++	++	–	–	+	+
Isolates of Bacillus							
PS 40	+++	+	–	+	+	–	–
PS 62	+++	+	–	+	+	–	–
PS 75	+++	+	–	+	+	–	–
PS 108	+	+	–	+	+	–	–
PS 111	+	–	–	+	+	–	–
PS 892	+++	+	++	+	–	–	–
PS 909	+++	++	+	+	+	–	–
PS 992	+++	++	–	+	–	–	–
PS 1441	+	++	–	+	–	–	–
PS 1475	+++	+++	+	+	+	–	–
Isolates of Stenotrophomonas maltophilia							
R 3089	+++	+++	+	+	+	–	+
C 4	+++	+	+	–	–	–	–
PS 1	+++	+	–	–	–	–	+
PS 2	+	++	–	+	–	–	+
PS 373	+++	+	–	–	–	–	+
PS 538	+++	+	–	–	–	–	+
PS 671	+++	+++	–	–	–	–	+
PS 672	+++	+++	–	–	–	–	+
PS 683	+++	+++	–	–	–	–	+
PS 1328	+++	+	–	–	–	–	+

Activity against *Verticillium dahliae*: Dual culture assay against *V. dahliae* var. *longisporum*, + represents 0–5 mm wide zone of inhibition, ++ represents 5–10 mm wide zone of inhibition, +++ represent > 10 mm wide zone of inhibition; Bioassay Antibiosis: Sterile filtrate test against *V. dahliae* var. *longisporum*; inhibition zones: +++ 100–60 % ++ 60–30 %, 30–1 %; Siderophores according to Schwyn & Neilands 1987 (+++ represents 20 mm wide orange zone, ++ represents 5–20 mm wide orange zone, + represents 5–3 mm orange zone); Chitinase-Activity: plate assay (+represents hydrolysis, – represents no hydrolysis); β-1,3-Glucanase-Activity: according to Daugrois *et al.*, 1990 (+++ > 10 IE ml^{-1}, ++ 10–1 IE ml^{-1}, +0,1–1 IE ml^{-1}); Production of HCN (=Cyanide): Merck. Schnelltest 14417: – represents <0001 mg/l; Production of IAA (=Indole-3-acetic acid) according to Gordon and Weber (1991).

[1]Species belong to *Pseudomonas fluorescens*. [2] Species belong to *Pseudomonas aureofaciens*.

siderophores while most isolates produced substances able to inhibit fungal growth. The isolates of *Bacillus* showed a high lytic activity and most of the isolates produced both lytic enzymes. No isolate was able to produce cyanide or indole-3-acetic acid. Also, the isolates of *S. maltophilia* showed a high antifungal activity *in vitro* to (Tab. 2). The main antifungal effect observed was based on antibiosis. Only two isolates produced siderophores. The isolate R 3089 produced β-1,3-glucanase and chitinase.

The combination of antifungal and plant growth promoting mechanisms was specific for the taxonomic group, e.g., *Pseudomonas* strain produced chitinase, no *Bacillus* strain cyanide or indole-3-acetic acid and no *Stenotrophomonas* strain cyanide. However, within the bacterial groups the intensity of produced substances was different.

4. DISCUSSION

Marked changes were found to take place in the bacterial populations in the rhizosphere of oilseed rape during its life cycle. It is not possible keep the influence of soil temperature and development stage of plants seperate because of the interdependence of growth and temperature in the field. On the examination of total bacteria counted, significant differences were found between the phase leaf-rosette in autumn/winter and at anthesis/maturity in spring/summer. The fluorescent pseudomonads have the highest percentage of potential antifungal bacteria. The group is identical with the RNA-homology group 1 and comprises strains of *Pseudomonas aeruginosa*, *P. fluorescens*, *P. chlororaphis*, *P. putida*, *P. aureofaciens*, *P. syringae* and *P. viridiflava* (Palleroni *et al.*, 1973). In further investigations, isolates of *P. fluorescens*, *P. chlororaphis*, *P. putida* and *P. aureofaciens* were found in the rhizosphere of oilseed rape and characterized as beneficial part of rhizobacteria (Berg, 1996). The selected isolated of this investigation belonged only to *P. fluorescens* and *P. aureofaciens*. All of the investigated isolates showed antifungal activity *in vitro*.The antifungal activity based on a combination of mechanisms and was specific for each strain. Pseudomonads with fluorescent properties are typical colonizers of the root (Miller *et al.*, 1990). Not all of the fluorescent pseudomonads belong to beneficial part of rhizobacteria e.g., the phytopathogenic species *Pseudomonas syringae*. Van Peer *et al.* (1990) reported also some deleterious isolates in *Pseudomonas fluorescens* and *Pseudomonas putida* depending on the secondary metabolism and intensity of production of antifungal and plant growth promoting compounds. The percentage of *Bacillus spec.* in the rhizosphere of oilseed rape was variable. *Bacillus* was discovered on account of their ability of sporulation and this give no evidence of activity in the rhizosphere. The percentage of Gram-positive isolates in the rhizosphere is generally low (Lambert *et al.*, 1987; Berg, 1996). *Bacillus subtilis* is not a typical colonizer of the rhizosphere, it was isolated in the same abundances from the surrounding soil (Miller *et al.*, 1989; Kloepper and Bowen, 1991). There are many reports about antifungal properties of this bacterial species (Berg *et al.*, 1994). The antifungal activity is based on antibiosis (Winkelmann *et al.*, 1983; Loeffler *et al.*, 1990), on production of siderophores and β-1,3-glucanases (Berg and Ballin, 1994). All of our selected isolates showed antifungal activity. The mechanisms included production of antibiotics, siderophores and lytic enzymes as reported in literature.

Stenotrophomonas maltophilia is a wide spread bacterium in the rhizosphere of many plants such as *Zea mays* (Lambert *et al.*, 1987), *Brassica oleracea*, *Sinapis alba*, *Triticum vulgare*, *Beta vulgaris* (Hedges and Messens, 1990), *Cucumis sativus* (Sugimoto *et al.*, 1990) and *Cichorium intybus* var. *foliosum* (Juhnke *et al.*, 1987). *S. maltophilia* is auxotrophic for methionin and was especially abundant in some rhi-

zospheres, such as those of cruciferous plants, which produce particularly high levels of sulphur-containing compounds (Debette and Blondeau, 1980). Direct antifungal activity of *S. maltophilia* may be based on the production of siderophores. It is not known if *S. maltophilia* produce siderophores itself, but Jurkevich *et al.* (1991) showed that an isolate of this species was able to utilize the Fe-complexes of the pseudobactin siderophores as a Fe-source. On the other hand, the antifungal activity may be based on the production of lytic enzymes (Berg *et al.*, 1996). *S. maltophilia* produces the antifungal antibiotics alteramid A and maltophilin (Jacobi *et al.*, 1996). Additionally isolates of *S. maltophilia* were able to produce plant growth hormones, such as indol-3-acetic acid (Berg and Ballin, 1994). The isolates investigated in this work showed antifungal activity too. The main activity was based on antibiosis, only some of the isolates possed the ability to produce siderophores and lytic enzymes.

The percentage of antifungal bacteria was stable during vegetation period, although bacterial populations were low in general during early plant growth. These investigations show that in the infection time of *V. dahliae* var. *longisporum* the abundances of rhizobacteria were not optimal and possible not enough for a defence against soil-borne pathogens. This offers the possibility for application of beneficial rhizobacteria, may be one of the investigated isolates, for a better protection of the root of oilseed rape.

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References

Balows, A.; Trüper, H. G.; Dworkin, M.; Harder, W. and Schleifer, W. K. H.: The Prokaryotes, a Handbook on the Biology of Bacteria. Springer-Verlag Berlin, Heidelberg, New York (1992).

Berg, G.: Rhizobacteria of oilseed rape antagonistic to *Verticillium dahliae*. *Journal of Plant Diseases and Protection*, **103**, 20–30 (1996).

Berg, G. and Ballin, G.: Bacterial antagonists of *Verticillium dahliae* KLEB. *Journal of Phytopathology*, **141**, 99–110 (1994).

Berg, G.; Marten P. and Ballin, G.: *Stenotrophomonas maltophilia* in the rhizosphere of oilseed rape – occurrence, characterization and interaction with phytopathogenic fungi. *Microbiological Research*, **151**, 19–27 (1996).

Berg, G.; Knappe, C.; Ballin, G. and Seidel, D.: Biological control of *Verticillium dahliae* KLEB. by naturally occuring rhizosphere bacteria. *Arch. Phytopath. Pflanz.*, **29**, 249 – 262 (1994).

Brown, M. F. and Wyllie T. D.: Ultrastructure of microsclerotia of *Verticillium albo-atrum*. *Phytopathology*, **60**, 538 – 542 (1969).

Burr, T. J. and Caesar, A.: Beneficial plant bacteria. *CRC Critical Review*, **2**, 1–20 (1984).

Debette, J. and Blondeau, R.: Présence de *Pseudomonas maltophilia* dans la rhizosphére de quelque plantes cultivée. *Canadian Journal of Microbiology*, **26**, 460–463 (1980).

Fravel, D. R.: Systems for efficient delivery of microbial biocontrol agents to soil. In *Biological Control of Plant Diseases. Progress and Challenges for the Future* (Tjamos, E. C., Papavizias, G. C., Cook, R. J., eds.) NATO ASI Series, A, Vol. **230** (pp. 399–406) Plenum Press, New York (1992).

Gordon, S. A. and Weber, R. P.: Colorimetric estimation of indoleactic acid. *Plant Physiology*, **26**, 192–195 (1951).

Hedges, R. W. and Messens E.: Genetic aspects of rhizosphere interaction. In *The Rhizosphere* (Lynch, J. M., ed.). Wiley & Sons (pp. 129–176) Chichester, England (1990).

Heupel, M.: Biologische Bekämpfung bodenbürtiger Wurzelbranderreger der Zuckerrübe (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* DOELL) durch den Einsatz mikrobieller Antagonisten. Diss. Univ. Göttingen, 191 p. (1992).

Jacobi, M.; Kaiser, D.; Jung, G.; Winkelmann, G.; Berg, G. and Bahl, H.: Maltophilin – a new antifungal compound produced by *Stenotrophomonas maltophilia* R3089. *Journal of Antibiotics*, in press (1996).

Juhnke, M. E. and Des Jardin, E.: Selective Medium for Isolation of *Xanthomonas maltophilia* from Soil and Rhizosphere Environments. *Applied and Environmental Microbiology*, **55**, 747–750 (1989).

Juhnke, M. E.; Mathre, D. E. and Sands, D. C.: Identification and characterization of rhizosphere-competent bacteria of wheat. *Applied and Environment Microbiology*, **53**, 2793–2799 (1987).

Jurkevitch, E.; Hadar, Y. and Chen, Y.: Differential siderophore utilization and iron uptake by soil and rhizosphere bacteria. *Applied and Environmental Microbiology*, **58**, 119–124 (1991).

Kloepper, J. W. and Bowen, K. L.: Quantification of the geocarposphere and rhizosphere effect of peanut (*Arachis hypogaea* L.). *Plant and Soil*, **136**, 103–109 (1991).

Kreig, N. R.; Holt, J. G.; Murray, R. G. E.; Brenner, D. J.; Bryanth, M. P.; Moulder, J. W.; Pfenning, N., Sneath, P. H. A.; Staley, J. T.: (eds.) *Bergey's Manual of Systematic Bacteriology*, Vol. **1**, The Williams and Wilkins Co., Baltimore (1984).

Lambert, B.; Frederik, L.; Van Rooyen, L.; Gossele, F.; Papon, Y. and Swings, J.: Rhizobacteria of maize and their antifungal activities. *Applied and Environmental Microbiology*, **53**, 1866–1871 (1987).

Leoffler, W.; Katzer, W.; Kremer, S.; Kugler, M.; Petersen, F.; Jung, G.; Rapp, C. J. and Tschen, S. M.: Antifungal antibiotics of *Bacillus subtilis* and related species. *Forum Microbiology*, **3**, 156–163 (1990).

Miller, H. J.; Henken, G. and Van Veen, J. A.: Variation and composition of bacteria population in the rhizospheres of maize, wheat and grass cultivars. *Canadian Journal of Microbiology*, **35**, 656–660 (1989).

Miller, H. J.; Liljeroth, E.; Willemsen-De Klein, M. J. E. M. and Van Veen, J. A.: The dynamics of actinomycetes and fluorescent pseudomonads in wheat rhizoplane and rhizosphere. *Symbiosis*, **9**, 389–391 (1990).

Palleroni, N. J.; Kunisamwa, R.; Contopoulou, R. and Doudorff, M.: Nucleic acid homologies in the genus *Pseudomonas*. *International Journal of Systematic Bacteriology*, **43**, 606–609 (1973).

Tjamos, E. C.: Selective elemination of soilborne plant pathogens and enhancement of antagonists by steaming, sublethal fumigation and soil solarisation. In *Biological Control of Plant Diseases. Progress and Challenges for the Future* (Tjamos, E. C., Papavizas, G. C., Cook, R. J., eds.) NATO ASI Series, Sereis A, Vol. 230 (pp. 1–17), Plenum Press, New York (1992).

Schwyn, B. and Neilands, J. B.: Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, **160**, 47–56 (1987).

Sugimoto, E. E.; Hoitink, H. A. J. and Touvinen, O. H.: Oligotrophic pseudomonads in the rhizosphere: Suppressiveness to Pythium damping-off of cucumber seedlings (*Cucumis sativus* L.). *Biology and Fertility of Soils*, **9**, 231–234 (1990).

Van Peer, R.; Punte, H. L. M.; De Weger L. A. and Schippers, B.: Characterization of root surface and endorhizosphere *Pseudomonas* in relation to their colonization of roots. *Applied and Environmental Microbiology*, **56**, 2462–2470 (1990).

Weller, D. M.: Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology*, **26**, 379–407 (1988).

Winkelmann, G.; Allgaier, H.; Lupp, R. and Jung, G.: Iturin AL – a new long chain iturin a possessing an unusual high content of C16-ß-amino acids. *Journal of Antibiotics*, **36**, 1451–1457 (1983).

Zeise, K.; Seidel, D. and Amelung, D.: Zum Befallsverlauf von *Verticillium dahliae* KLEB. an Winterraps. *Archiv für Phytopathologie und Pflanzenschutz*, **26**, 205–207 (1990).