Microbiol. Res. (1996) **151**, 19–27

Microbiological Research

© Gustav Fischer Verlag Jena

Stenotrophomonas maltophilia in the rhizosphere of oilseed rape – occurrence, characterization and interaction with phytopathogenic fungi

Gabriele Berg, Petra Marten, Günter Ballin

University of Rostock, Department of Biology, Microbiology, Justus-von Liebig-Weg 8, D-18051 Rostock, Germany

Accepted: October 31, 1995

Abstract

Isolates of *Stenotrophomonas maltophilia* have been isolated from the rhizosphere of oilseed rape. The percentage of *S. maltophilia* in this habitat was determined using a selective medium according to Juhnke and Des Jardin (1989). The average number of *S. maltophilia* per gramme root (wet weight) was 10^7 cfu, 3.7% of the total bacterial number. Selected strains were screened for their ability to inhibit in vitro the growth of *Rhizoctonia solani* and *Verticillium dahliae* var. *longisporum*, important soil borne pathogens of rape. The majority of isolates showed an antifungal activity in vitro but the efficiency of strains was different. The antifungal activity was achieved by a combination of several known antifungal mechanisms. Direct antifungal effect may be based on antibiosis and production of lytic enzymes. Siderophores were secreted by rhizobacteria to improve the availability of iron. The disease incidence of several plant pathogens could be reduced in greenhouse by the rhizobacterium significantly.

Key words: *Stenotrophomonas maltophilia* – rhizobacteria of oilseed rape – biological control

Introduction

The possibility of replacing chemical control of plant root diseases with a biological alternative remains an exiting and challenging objective (O'Sullivan and O'Gara 1992). Chemical pesticides have been the object of substantial criticism in recent years, due to the environmental effects causing health hazards to humans and other nontarget organisms, including beneficial natural enemies (Chet *et al.* 1994). Therefore, biological control of soil borne pathogens using naturally occurring antagonistic rhizosphere bacteria is an interesting alternative to the use of pesticides. *Verticillium dahliae* var. *longisporum* Stark and *Rhizoctonia solani* Kühn are two important soil borne pathogens causing diseases of oilseed rape. The increasing importance of oilseed rape for renewable biological raw material resulted in a marked expansion of the rape growing area in Europe. Over the years very short rotations have led to a build-up of *Verticillium* wilt caused by *V. dahliae* var. *longisporum* and post emergence damping off and brown girdling root rot caused by *R. solani* followed by yield losses.

Stenotrophomonas maltophilia (Hugh) Palleroni and Bradbury (syn. *Pseudomonas maltophilia* and *Xanthomonas maltophilia;* Palleroni and Bradbury 1993) is an ubiquitous bacterium. It occurs free living in nature, in animals, and in various areas of the human body, where it is sometimes associated with opportunistic infections (Swings *et al.* 1983). It has been recovered from plants (cotton seeds, bean pods, rhizosphere of wheat, oat, cucumber, maize, cabbage and different weeds (Swings *et al.* 1983). *S. maltophilia* has been isolated from the rhizosphere of several plant species especially cruciferous plants, which produce particularly high levels of sulphur-containing compounds, f. e. amino acids like methionine (Debette and Blondeau 1980). *S. maltophilia* requires methionine (Ikemoto *et al.* 1980). The species is also one of the four dominant bacterial species in the rhizosphere of european cereals (Lam-

Corresponding author: G. Berg

bert and Joos 1989). Little is known about the antagonistic mechanisms of this species. *S. maltophilia* has been suggested to have properties useful in the biological control of soil borne plant pathogens (Elad *et al.* 1987; Lambert *et al.* 1987). Gregory *et al.* (1986) and Kwok *et al.* (1987) used them as biocontrol agent. Observations suggest an important ecological role for this species in the element cycle in nature (Ikemoto *et al.* 1980). Studies of *S. maltophilia* are required for a better understanding of the function of this species in nature.

Bacterial antagonism responsible for the benefit of plants and biological control may be due to the production of antibiotics, competition or parasitism (Chet *et al.* 1990). The relative importance of these mechanisms may differ considerably among strains. Antibiotics play a major role in disease suppression by some bacteria and often act in concert with other mechanisms such as competition and parasitism (Fravel 1988). The results by Thomashow and Weller (1988) with defective mutants in phenazine synthesis and by Hill *et al.* (1994) with defective mutants in pyrrolnitrin synthesis support the importance of antibiotics in disease suppression by *Pseudomonas fluorescens* in the rhizosphere. Competition for nutrients supplied by root and seed exudates probably occurs in most interactions between bacteria and pathogens (Elad and Chet 1987; Weller 1988). Kloepper *et al.* (1980) were the first who demonstrate the importance of siderophore production as a mechanism of competition. Hamdan *et al.* (1991) acknowledged the role of siderophores in disease suppression. Pyoverdin siderophores produced by *Pseudomonas* spp. contribute significantly to biological control of *Pythium ultimum* of cotton (Loper 1988) while aerobactin production by *Enterobacter cloacae* was not essential for biological control of the same pathogen (Loper *et al.* 1993). Parasitism relies on lytic enzymes for the degradation of cell walls of pathogenic fungi. Microorganisms capable of lysing other organisms can serve as a powerful tool for biological control (Chet *et al.* 1990). Many rhizobacteria are classified as chitinolytic microorganisms, f. e. *Aeromonas caviae* (Inbar and Chet 1991), *Serratia marcescens* (Ordentlich *et al.* 1988) and *E. coli* cloned with the *chi*A gene from *Serratia marcescens* (Shapira *et al.* 1989). Induced systemic resistance, broadly defined as activation of latent defense mechanisms in plants prior to pathogenic attack, has been hypothesized in recent years to be an oparable mechanism in several rhizobacterial systems (Kloepper *et al.* 1992).

The objective of our investigations was to determine the occurrence of *S. maltophilia* in the rhizosphere of oilseed rape and to characterize antifungal mechanisms and efficiency of selected isolates in vitro and in planta.

Material and methods

Isolation of bacterial antagonists and determination of the colony forming units (cfu). Bacteria were isolated from roots of oilseed rape. The material was rinsed in sterile water and the suspension was plated in serial dilutions on nutrient agar (Gibco, Paisley, Scotland) and Xanthomonas Maltophilia Selective Medium XMSM (Juhnke and Des Jardin 1989). The selective medium XMSM contained (l^{-1}): maltose (10 g) (Sigma, Deisenhofen, FRG), Tryptone (Gibco, Paisley, Scotland), 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, following antibiotics were then added (in $\mu g\,ml^{-1}$, all from Sigma): cycloheximide (100), nystatin (50), cephalexin (50), bacitracin (25), penicillin G (25), novobiocin (10), neomycin sulfate (30), and tobramycin (1). Plates were incubated 5 days at 20 °C and bacterial colonies were counted to determine the amount of colonies (colony forming units = cfu). Colonies on XMSM representative of different yellow pigmented types present were selected and purified. Selected isolates (24 h-culture, 30 °C) were stored in nutrient broth with 15% glycerol at −80 °C.

Bioassay for in vitro inhibition of V. dahliae and R. solani. The ability of the bacterial antagonists to produce antifungal substances against *Verticillium dahliae* and *Rhizoctonia solani* was determinated by the paired *in vitro* assay on Waksman agar [WA; containing: 5 g proteose-peptone (Merck, Darmstadt, FRG), 10 g glucose (Merck), 3 g meat extract (Chemex, München, FRG), 5 g NaCl (Merck), 20 g agar (Difco), 1 l destilled water, pH 6.8]. A suspension of hyphal fragments of *Verticillium* or *Rhizoctonia* 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. The isolates of *Verticillium dahliae* var. *longisporum* and *Rhizoctonia solani,* which where isolated from oilseed rape in Rostock-Biestow (1989), were received from Dr. K. Zeise (University of Rostock, Institute of Phytomedicine).

Bacterial identification. For the identification of isolates, a standardized micromethod, emloying the

Analytical Profile Index strip which consists of a gallery of 20 biochemical tests separated into 7 groups was used (Bio Meriéux, Marcy-L'Etoile, France). API 20 NE tests and API 20 E tests were used for identifying the isolates. Additionally characteristics described by Balows *et al.* (1992) and Bergey's Manual of Systematic Bacteriology (Krieg *et al.* 1984), Van Den Mooter and Swings (1990) and Palleroni and Bradbury (1993) were checked.

Strain characterization by SDS-PAGE (sodium dodecyl sulfate-polyarylamide gel electrophoresis). Cells were sampled from stationary-phase cultures (50 ml nutrient broth, 30 °C, 24 hours). The samples were centrifuged, washed in 2 ml of HEPES-buffer (10 mM, pH 7.3) and recentrifuged. Cell pellets were solved in 0.5 ml HEPES-buffer (with 10 mM phenylmethansulfonylfluorid). Cells were destroyed by ultrasound for 1 minute (Sonoplus HD 60, Bandelin Electronics). The suspensions were centrifuged and the supernatants were stored at −20 °C. The amounts of proteins in the samples were determined according to Bradford (1976). For each sample 150 µg protein was loaded on a discontinous polyacrylamide gel (7.5−15%) similar to the one described by Laemmli (1970). After a run of approximately 4 hours the gel was fixed and stained in Coomassie blue (0.1% brilliant blue R, Sigma) in 50% methanol- 10% acetic acid for 30 minutes. It was destained in methanol-acetic acid water (25 : 10 : 65 vol/vol/vol).

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 (1988). The ternary complex chrome azurol S/iron(III)/hexacyltrimethyl-ammonium bromide (Sigma) served as an indicator.

Lytic enzymes. Chitinase: Bacterial colonies were screened for chitinase production and excretion by plating on nutrient agar (Merck) containing 5% colloidal chitin (Fluka, Buchs, Switzerland). The clear halos indicating 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 laminarin, 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.

Antibiotics. Fermentation broth of *S. maltophilia* (300 ml nutrient broth, 2 d, 30 °C, 120 rpm) containing antifungal substances was centrifuged at 10000 g for 10 minutes. The centrifugate was then passed through a SERVACHROM XAD-2 column (Serva, Heidelberg, FRG). After washing with two volumes of distilled water and one volume acetone-water (1 : 1) the active substances were desorbed with one volume methanol. The eluate was concentrated under vacuum (50 °C) and further purified by gel filtration on Sephadex LH-20 (Pharmacia, Uppsala, Sweden) using ethanol as eluting solvent. The active fractions were concentrated under vacuum (50 °C) and then the dry residue was dissolved in 1 ml of ethanol. The eluate was tested for antifungal substances by thin-layer chromatography on silica gel plates (Merck) using butanol-ethyl acetate-distilled water (3 : 1 : 1 vol/vol/vol) for development. The thin-layer chromatogram was autoclaved and covered with a thin agar layer (Czapek-Dox-Agar, GIBCO, Paisley, Scotland) and with conidia of *Verticillium* or with hyphal fragments of *Rhizoctonia* (bioautography assay). The assay was developed at 20 °C for 5 days.

Greenhouse experiments. Soil samples (sandy loam, pH 7.2) were infested with *R. solani* (4.5 g sclerotia of *R. solani* kg^{-1} soil) or by *V. dahliae* (10 g microsclerotia of *V. dahliae* kg^{-1} soil). Polypropylene boxes (7 × 19 × 14 cm) were filled with infested soils and planted with cotton seeds (*Gossypium barbadense* L. "Pima") or with rape seedlings (*Brassica napus* "Accord"). Bacteria were applicated suspended in distilled water (10^6-10^7 cells g^{-1} soil) (assay 1, 2), endophytic (assay 3) and in methylcellulose (10^7 cells per seed) (assay 4). The experiments were conducted under greenhouse conditions at a temperature of 28−32 °C with daily irrigation. The disease incidence was determined according to Inbar and Chet (1989) and Zeise (1992).

Results

Quantification of S. maltophilia in the rhizosphere of oilseed rape

The numbers of *S. maltophilia* and total bacteria obtained from the rhizosphere of oilseed rape are given in Table 1. The population of cells of *S. maltophilia* (cfu) in the rhizosphere of oilseed rape in Mecklenburg-Western Pomerania was 1.1×10^7 on

Table 1. Total number of colony forming units and number of *Stenotrophomonas maltophilia* from root samples isolated with selective medium XMSM (Xanthomonas Maltophilia Selective Medium according to Juhnke & Des Jardin 1989)

No.	origin[1]	Total bacteria (cfu)[2]	*S. maltophilia* (cfu)[2]	Percentage *S. maltophilia* (%)
1	Rostock, 4/93	7.30×10^5	–	–
2	Biestow, 4/93	4.00×10^6	–	–
3	Biestow, 4/93	4.66×10^6	–	–
4	Bentwisch, 4/93	1.41×10^6	–	–
5	Bentwisch, 4/93	1.04×10^6	–	–
6	Rostock, 5/93	1.71×10^6	–	–
7	Rostock, 5/93	1.73×10^6	–	–
8	Rostock, 5/93	1.65×10^6	3.00×10^4	1.80
9	Rostock, 5/93	4.60×10^5	–	–
10	Rostock, 5/93	4.15×10^7	4.50×10^6	10.80
11	Rostock, 5/93	6.70×10^6	2.60×10^5	3.80
12	Rostock, 5/93	1.77×10^7	–	–
13	Rostock, 5/93	3.60×10^6	–	–
14	Rostock, 5/93	6.10×10^7	1.00×10^4	0.02
15	Rostock, 5/93	1.46×10^8	–	–
16	Rostock, 5/93	9.10×10^7	–	–
17	Rostock, 5/93	1.14×10^8	–	–
18	Rostock, 5/93	1.03×10^8	6.00×10^6	5.80
19	Rostock, 5/93	7.40×10^7	3.00×10^4	0.04
20	Rostock, 5/93	1.18×10^8	–	–
21	Rostock, 5/93	1.14×10^8	3.00×10^6	2.60
22	Rostock, 5/93	7.30×10^7	–	–
23	Rostock, 5/93	1.49×10^8	–	–
24	Biestow, 5/93	9.70×10^7	3.00×10^4	0.03
25	Biestow, 5/93	1.55×10^8	–	–
26	Biestow, 5/93	1.80×10^8	–	–
27	Biestow, 5/93	2.03×10^8	–	–
28	Biestow, 5/93	1.94×10^8	1.00×10^7	5.10
29	Biestow, 5/93	1.39×10^8	–	–
30	Biestow, 5/93	1.17×10^8	–	–
31	Biestow, 5/93	1.22×10^8	–	–
32	Biestow, 5/93	2.31×10^8	1.10×10^8	47.60
34	Admannshagen, 5/93	2.40×10^6	–	–
35	Admannshagen, 5/93	6.18×10^8	1.45×10^8	23.50
36	Admannshagen, 5/93	9.85×10^7	6.00×10^5	0.30
37	Biestow, 6/93	3.24×10^8	7.80×10^7	24.00
38	Biestow, 6/93	1.46×10^7	1.00×10^6	6.80
39	Biestow, 6/93	1.06×10^8	2.60×10^5	0.24
40	Biestow, 6/93	1.34×10^8	2.00×10^6	1.40
41	Biestow, 6/93	3.40×10^8	3.00×10^7	8.80
42	Biestow, 6/93	1.66×10^7	8.00×10^4	0.48
43	Biestow, 6/93	1.18×10^7	–	–
44	Biestow, 6/93	1.56×10^8	8.00×10^5	0.50
45	Biestow, 6/93	3.62×10^8	4.20×10^7	11.60
46	Biestow, 6/93	5.00×10^7	4.00×10^5	0.80
47	Rostock, 6/93	2.62×10^8	2.40×10^7	9.10
48	Rostock, 6/93	2.26×10^8	8.00×10^6	3.50
49	Rostock, 6/93	1.84×10^8	4.60×10^6	2.50
50	Rostock, 6/93	8.06×10^8	6.80×10^7	8.40

[1] Locality (town in Mecklenburg-Western Pomerania) and date (month and year) of the sample
[2] per gramme root (wet weight)

Table 2. Characteristics of selected isolates of *S. maltophilia*

Characteristic	No. of positive strains	in %	Comparison with literature*
Colonies mucoid	1, 27	20	14%
Colonies yellow	all	100	86%
Rods	all	100	100%
multitrichous	all	100	100%
motile	all	100	100%
Acid produced from			
Maltose	all	100	100%
D-Glucose	all	100	43%
D-Mannose	all	100	43%
Xylose	42, 44, 47	27	0%
Sucrose	1, 44, 47, 74, 78	45	14%
Stimulatory effect by			
L-Methionine	30, 32, 35, 44	45	100%
L-Cysteine	30, 32, 35, 37, 41, 44	64	100%
Growth at 28 °C	all	100	100%
Growth at 37 °C	1, 27, 30, 35, 74, 78	55	71%

* Van Den Mooter & Swings (1990)

Table 3. Identification of selected isolates of *S. maltophilia*
API 20 NE: Identification system of non-enteric Gram-negative rods (bioMérieux)
API 20 E: Identification system of Enterobacteriaceae and other Gram-negative rods

Identification system	API profile	% id.[1]	Isolates
API 20 NE	1-4-7-2-3-4-1	99,9%	1, 27, 74
	1-2-7-2-3-4-5	99,8%	30, 32, 35, 37, 41, 42, 44, 78
API 20 E	1-2-0-2-0-0-0	96,8%	1, 27, 32, 35, 41, 42, 44, 74, 78
	0-2-0-2-0-0-0	89,9%	30

[1] Quality of identification selected on the value of the percentage of identification

average (g^{-1} wet weight root). The proportion of *S. maltophilia* growing on selective medium (XMSM) to total bacteria growing on nutrient agar was determined for 50 plants and ranged from 0–48%, 3.7% on average. The proportion of this bacterium varied with each plant from which the bacteria were isolated, the differences between single plants was high. In 49% of the root samples no colony of *S. maltophilia* could be detected. Only in 10% of the samples the percentage of *S. maltophilia* was higher than 10%.

Identification of the isolates

The *S. maltophilia* isolates produced orange-yellow colonies with a yellow halo in selective medium (XMSM) because of acid production from maltose. 10 isolates of over 600 were selected for taxonomic investigations. Isolates were identified by using API-system, the scheme outlined in Bergey's Manual of Systematic Bacteriology (Krieg *et al.* 1984) compared with informations from Swings *et al.* (1983) and Palleroni and Bradbury (1993). All isolates were aerobic, Gram-negative, catalase-positive, motile rods with multitrichous polar flagella (Table 2). All isolates were able to produce acid from maltose, D-glucose and mannose. Only a few isolates produced acid from xylose or sucrose (Table 2). Not all isolates were stimulated by the sulfur-containing amino acids L-methionine or L-cysteine. While all of the isolates were grown at a temperature of 28 °C only half could grow at 37 °C (Table 2), some of them very weakly.

A comparison of the protein pattern of isolates (Fig. 1) showed that the isolates were heterogenous in their protein pattern, too. The isolates represent different electrotypes and may be differenciated into groups: (1) isolate 30, 32, 35, 37; (2) isolate 41, 44; (3) isolate 47, 42 and (4) isolate 74, 78.

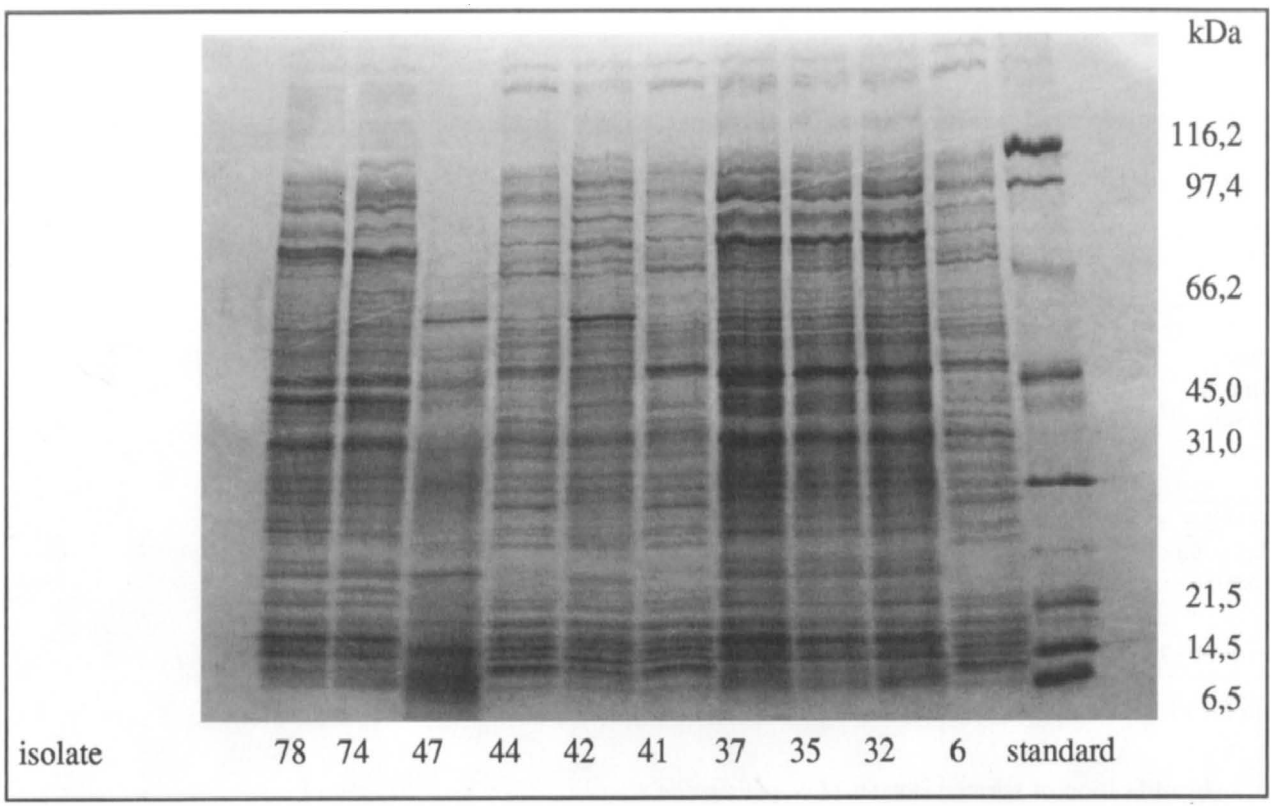


Fig. 1. Protein pattern of isolates of *S. maltophilia* in SDS-page-analysis (Standard: Broad Range, BioRad)

Table 4. Antifungal activity of the isolates in bioassay (dual culture in vitro)

Zone of inhibition	Number of isolates active against *V. dahliae*	Number of isolates active against *R. solani*
> 10 mm	1	1
5–10 mm	15	7
0–5 mm	29	40
no inhibition	21	18

Antifungal effects

A total of 66 isolates were screened for their ability to suppress *V. dahliae* and *R. solani* in an in vitro bioassay (Table 4). The efficiency of the isolates was different. Only one isolate showed a strong effect in vitro with an inhibition zone about 10 mm. Most isolates showed a low inhibition against both of the pathogens, but the majority of the isolates had an antifungal effect.

Antagonistic mechanisms

We have tried to prove the involvement of antagonistic mechanisms in the inhibition of fungal pathogens (Table 5). Some isolates of *S. maltophilia* were able to produce antifungal substances. We proved the production of antifungal compounds in culture filtrates in a bioautography assay. An antifungal effect was detected for the isolate 1, 30, 32 and 35 (Table 5). All isolates were able to produce siderophores on a plate assay in different amounts. The production of lytic enzymes was investigated. Five isolates (1, 27, 30, 42, 74) produced β-1.3-glucanase in culture medium while only the isolate 1 excreted chitinase when grown on chitin.

Biocontrol experiments

The isolate 1 (with a high efficiency in vitro and a combination of antifungal mechanisms) was applicated and their influence on diseases caused by *V. dahliae* and *R. solani* in several crops was examined in the greenhouse. Use of *S. maltophilia* strain 1 to reduce the incidence of disease caused by *R. solani* in cotton and by *V. dahliae* in oilseed rape revealed a significant biocontrol effect (Fig. 2). When applied as a suspension, *S. maltophilia* was effective in reducing disease incidence caused by *R. solani* in cotton (15–18% disease reduction). The bacterial cells reduce the incidence of disease caused by *V. dahliae* in oilseed rape (23%). The efficiency of the endophytic applicated bacterial cells was lower, the reduction of the disease incidence of *R. solani* was only 9%.

Tab. 5. Antifungal mechanisms of the isolates of *S. maltophilia*

No.	Bioassay 1	Antibiosis	Siderophores	β-1,3-Glucanase	Chitinase
1	+++	+	+	++	+
27	+	−	++	+++	−
30	++	+	++	+	−
32	++	+	++	−	−
35	++	+	++	−	−
37	++	−	++	−	−
41	++	−	++	−	−
42	++	−	+++	+	−
44	++	−	+	+	−
74	+++	−	+++	+	−
78	+++	−	+	−	−

Bioassay 1: dual culture assay against *V. dahliae;* +++ represents > 10 mm wide zone of inhibition; ++ represents 5−10 mm wide zone of inhibition, + represents 0−5 mm wide zone of inhibition
Antibiosis: proof with DC-chromatography (bioautography test)
Siderophores according to Schwyn & Neilands 1987 (+++ represents 20 mm wide orange zone, ++ represents 5−20 mm wide orange zone, + represents 5−3 mm wide 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, ++ 10−1 IE/ml, + 0,1−1 IE/ml)

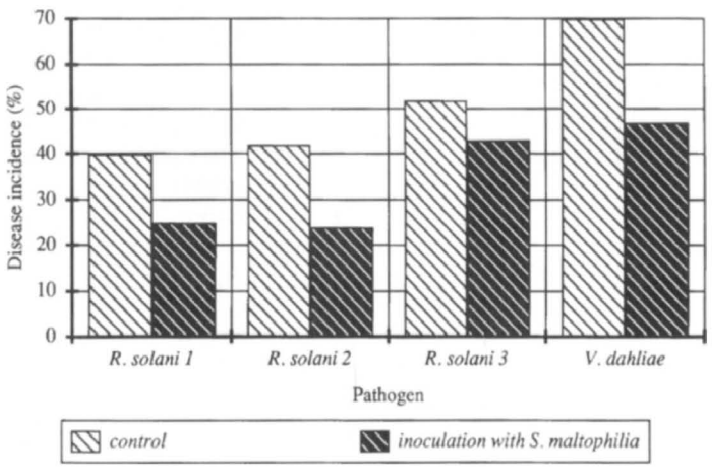


Fig. 2. Biological control of *R. solani* strain 1, 2, 3 in cotton and *V. dahliae* in oilseed rape by *S. maltophilia* under greenhouse conditions. Cells of *S. maltophilia* were applicated in soil (1, 2, *V. dahliae*) or endophytic (3)

Discussion

Of the 50 investigated plants only at near of the half *S. maltophilia* could be detected. Lambert *et al.* (1987) showed that there were large differences between bacterial populations from different plants within one field and that only a few species of rhizobacteria were widely spread throughout one field. This percentage of 3.7% *S. maltophilia* in the rhizosphere of rape is comparable with abundances of *S. maltophilia* in the rhizosphere of other plants known from literature. Juhnke and Des Jardin found a percentage of *S. maltophilia* from 0−100% (38% on average) for diverse grasses. Debette and Blondeau (1980) calculated 3.8% *S. maltophilia* for the rhizosphere of rape.

S. maltophilia is a member of naturally occurring rhizosphere communities of many plants f. e. *Zea mays* (Lambert *et al.* 1987), *Brassica oleracea, Sinapis alba, Triticum vulgare, Beta vulgaris* (Hedgens and Messens 1990), *Cucumis sativus* (Sugimoto *et al.* 1990), *Cichorium intybus* var. *foliosum* (Juhnke *et al.* 1987). All of the selected isolates from selective medium (XMSM) according Juhnke and Des Jardin (1989) belonged to *S. maltophilia.* The physiological features of the strains were heterogeneous. According to API-identification systems all of the isolates belonged with a high percentage to *S. maltophilia.* Van Den Mooter and Swings (1989) reported numerical analysis of 295 phenotypic trail 266 *Xanthomonas* strains of which 7 were labeled as *S. maltophilia.* We compared these informations with characteristics of our strains (Table 2). We noted differences in the

production of acids from various carbon sources (Table 2). Stimulation of growth by the sulphur-containing amino acids was present in 100% of *S. maltophilia* strains by Van Den Mooter and Swings (1989) while only half of our isolates were stimulated. These findings were confirmed by the results of Ikemoto *et al.* (1980) who differentiate methionine-requiring and non-requiring strains of *S. maltophilia* into 2 biovars. Several observations have suggested that methionine may have a special important role in the rhizosphere (Hedges and Messens 1990). This observations was at least partly explained by the observations that *S. maltophilia* was especially abundant in some rhizospheres, such as those of cruciferous plants, which produce particularly high levels of sulphur-containing compounds (Debette and Blondeau 1989). The requirement for methionine or other sulphur-containing amino-acids by *S. maltophilia* was first reported by Iizuka and Komagata (1963). The heterogenity of isolates of *S. maltophilia* will be supported by different protein pattern of the isolates.

The antifungal efficiency of the isolates was also very different. The majority of isolates showed an antifungal activity in bioassays in vitro against *V. dahliae* and *R. solani*. Our investigations about antifungal mechanisms indicate that the antifungal effect of *S. maltophilia* isolates is a combination of several mechanisms (Table 5). Direct antifungal activity may be based on the production of siderophores (all isolates). It is not known that *S. maltophilia* produce siderophores itself, but Jurkevich *et al.* (1992) 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 based on the production of lytic enzymes. Some of the isolates produced an antifungal substance which supports the antifungal effect. The chemical structure of the active compound is still unknown (Berg and Ballin 1994). Additionally isolates of *S. maltophilia* were able to produce plant growth hormones f. e. indol-3-acetic acid (Berg and Ballin 1994).

Kwok *et al.* (1987) reported first the biocontrol ability of *S. maltophilia* against *R. solani* especially in combination with *Trichoderma hamatum*. Strains of *S. maltophilia* were able to suppress *Pythium ultimum* (Chen *et al.* 1987) in situ and in vitro (Heupel 1992) and also other soil borne pathogens too (Elad *et al.* 1987). Compared with results of other authors for other antagonistic bacteria, f. e. *Pseudomonas cepacia* (Fridlender *et al.* 1993), *Aeromonas caviae* (Inbar and Chet 1989), *Streptomyces ochraceisleroticus* (Turhan 1981) the reduction of disease incidence by *S. maltophilia* was low. But a continuous reduction of the soil inoculum of *V. dahliae* will be a success.

Acknowledgements

We would like to thank Prof. I. Chet (The Hebrew University of Jerusalem, Faculty of Agriculture) for biocontrol experiments and Prof. G. Winkelmann (University of Tübingen) for helpful discussion. We thank Hella Goschke for valuable technical assistance and Dr. Karen Zeise (University of Rostock) for handing over of *Verticillium* strains.

References

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

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

Bradford, M. M. (1976): A rapid and sensitive method for quantification of microgram quantities of protein using the principle of protein-dye binding. Analyt. Biochem. **72,** 248–254.

Chen, W., Hoitink, H. A. J., Schmitthenner, A. F. (1987): Factors affecting suppression of *Pythium* damping-off in container media amended with composts. Phytopathology **77,** 755–760.

Chet, I., Ordentlich, A., Shapira, R., Oppenheim, A. (1990): Mechanisms of biocontrol of soil-borne plant pathogens by rhizobacteria. Plant and Soil **129,** 85–92.

Chet, I., Barak, Z., Oppenheim, A. (1994): Genetic engeering of microorganisms for improved biocontrol activity. In Chet, I. (ed.): Biotechnology in Plant Disease Control. Wiley and Sons, Chichester, England, 211–235.

Daugrois, H. J., Lafitte, C., Barthe, J. P., Touze, A. (1990): Induction of β-1,3-Glucanase and chitinase activity in compatible and incompatible interactions between *Colletotrichum lindemuthianum* and bean cultivars. J. Phytopathol. **130,** 225–234.

Debette, J., Blondeau, R. (1980): Présence de *Pseudomonas maltophilia* dans la rhizosphère de quelque plantes cultivée. Can. J. Microbiol. **26,** 460–463.

Elad, Y., Chet, I. (1987): Possible role of competition for nutrients in biocontrol of *Pythium* damping-off by bacteria. Phytopathology **77,** 190–195.

Elad, Y., Chet, I., Baker, R. (1987): Increased growth response of plants induced by rhizobacteria antagonistic to soilborne pathogenic fungi. Plant and Soil **98,** 325–339.

Fridlender, M., Inbar, J., Chet, I. (1993): Biological control of soilborne plant pathogens by a β-1,3-glucanase producing *Pseudomonas cepacia*. Soil Biol. Biochem. **25,** 1211–1221.

Fravel, D. R. (1988): Role of antibiosis in the biocontrol of plant diseases. Ann. Rev. Phytopathology **26,** 75–91.

Hamdan, H., Weller, D. M., Thomashow, L. S. (1991): Relative importance of fluorescent siderophores and other factors in biological control of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2-79 and M4-80R. Appl. Environm. Microbiol. **57,** 3270–3277.

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

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

Hill, D. S., Stein, J. I., Torkewitz, N. R., Morse, A. M., Howell, C. R., Pachlatkow, J. P., Becker, J. O., Lignon, J. M. (1994): Cloning of genes involved in the synthesis of pyrrolnitrin from *Pseudomonas fluorescens* and role of pyrrolnitrin synthesis in biological control of plant disease. Appl. Environm. Microbiol. **60,** 78–85.

Iizuka, H., Komagata, H. (1964): Microbiological studies on petroleum and natural gas. I. Determination of hydrocarbon-utilizing bacterida. J. Gen. Appl. Microbiol. **10,** 207–221.

Ikemoto, S., Suzuki, K., Kaneko, T., Komagata, K. (1980): Characterization of strains of *Pseudomonas maltophilia* which do not require methionine. Int. J. Syst. Bacteriol. **30,** 437–447.

Inbar, J., Chet, I. (1991): Evidence that chitinase produced by *Aeromonas caviae* is involved in the biological control of soil-borne plant pathogens by this bacterium. Soil Biol. Biochem. **23,** 973–978.

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

Juhnke, M. E., Mathre, D. E., Sands, D. C. (1987): Identification and characterization of rhizosphere-competent bacteria of wheat. Appl. Environm. Microbiol. **53,** 2793–2799.

Jurkevich, E., Hadar, Y., Chen, Y. (1991): Differential siderophore utilization and iron uptake by soil and rhizosphere bacteria. Appl. Environm. Microbiol. **58,** 119–124.

Kloepper, J. W., Leong, J., Teintze, M., Schroth, M. N. (1980): Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature **286,** 835–836.

Kloepper, J. W., Wei, G., Tzun, S. (1992): Rhizosphere population dynamics and internal colonization of cucumber by plant growth-promoting rhizobacteria which induce systemic resistance to *Colletotrichum orbiculare*. In: Tjamos, E. C., Papavizas, G. C., Cook, R. J. (eds.): Biological Control of Plant Diseases. Progress and Challenges for the Future. NATO ASI Series, Series A, Vol. **230,** Plenum Press, New York, 185–192.

Krieg, 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.) (1984): Bergey's Manual of Systematic Bacteriology, Vol. 1, The Williams and Wilkins Co., Baltimore.

Kwok, O. C. H., Fahy, P. C., Hoitink, H. A. J., Kuter, G. A. (1987): Interactions between bacteria and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in bark compost media. Phytopathology **77,** 1206–1212.

Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227,** 680–685.

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

Lambert, B., Joos, H. (1989): Fundamental aspects of rhizobacterial plant growth promotion research. Trends Biotechnol. **7,** 215–219.

Loper, J. E. (1988): Role of fluorescent siderophore production in biological control of *Pythium ultimum* by a *Pseudomonas fluorescens* strain. Phytopathology **78,** 166–172.

Loper, J. E., Ishimaru, C. A., Carnegie, S. R., Vanavichet, A. (1993): Cloning and characterization of aerobactin biosynthesis genes of the biological control agent *Enterobacter cloacae*. Appl. Environm. Microbiol. **59,** 4189–4197.

Ordentlich, A., Elad, Y., Chet, I. (1988): The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. Phytopathology **78,** 84–88.

O'Sullivan, D. J., O'Gara, F. (1992): Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol. Rev. **56,** 662–676.

Palleroni, N. J., Bradbury, J. F. (1993): *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings *et al.* 1983. Int. J. Syst. Bacteriol. **43,** 606–609.

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

Shapira, R., Ordentlich, A., Chet, I., Oppenheim, A. B. (1989): Control of plant diseases by chitinase expressed from cloned DNA in *Escherichia coli*. Phytopathology **79,** 1246–1249.

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

Swings, J., De Vos., P., Van Den Mooter, M., J. De Ley (1983): Transfer of *Pseudomonas maltophilia* Hugh 1981 to the genus *Xanthomonas* as *Xanthomonas maltophilia* (Hugh 1981) comb. nov. Int. J. Syst. Bacteriol. **33,** 409–413.

Tomashow, L. S., Weller, D. M. (1988): Role of phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J. Bacteriol. **170,** 3499–3508.

Van Den Mooter, M., Swings, J. (1990): Numerical analysis of 295 phenotypic features of 266 *Xanthomonas* strains and related strains and an improved taxonomy of the genus. Int. J. Syst. Bacteriol. **40,** 348–369.

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

Zeise, K. (1992): Gewächshaustest zur Resistenzprüfung von Winterraps (*Brassica napus* var. *oleifera Metzger*) gegen den Erreger der Rapswelke *Verticillium dahliae* Kleb. Nachrichtenbl. Deut. Pflanzenschutzdienst **44,** 125–128.