

Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community

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Abstract

In a field release experiment, rifampicin resistant mutants of two antagonistic plant-associated bacteria were used for seed tuber inoculation of transgenic T4 lysozyme expressing potatoes, transgenic control potatoes and non-transgenic parental potatoes. The T4 lysozyme tolerant *Pseudomonas putida* QC14-3-8 was originally isolated from the tuber surface (geocaulosphere) of T4 lysozyme producing plants and showed in vitro antibacterial activity to the bacterial pathogen *Erwinia carotovora* ssp. *atroseptica*. The T4 lysozyme sensitive *Serratia grimesii* L16-3-3 was originally isolated from the rhizosphere of parental potatoes and showed in vitro antagonism toward the plant pathogenic fungus *Verticillium dahliae*. The establishment of the inoculated bacteria in the rhizosphere and geocaulosphere of the different plant lines was monitored over one growing season to assess the effect of T4 lysozyme produced by transgenic potato plants on the survival of both inoculants. Both introduced isolates were able to colonize the rhizo- and geocaulosphere of transgenic plants and non-transgenic parental plants, and established in the rhizosphere at levels of ca. \log_{10} 5 colony forming units g^{-1} fresh weight of root. During flowering of plants, significantly more colony counts of the T4 lysozyme tolerant *P. putida* were recovered from transgenic T4 lysozyme plants than from the transgenic control and the parental line. At this time, the highest level of T4 lysozyme (% of total soluble protein) was detected. Effects of the inoculants on the indigenous microbial community were monitored by analysis of PCR-amplified fragments of the 16S rRNA genes of the whole bacterial community after separation by denaturing gradient gel electrophoresis (DGGE). At any sampling time, the DGGE pattern of rhizosphere and geocaulosphere communities did not show differences between the inoculated and non-inoculated potatoes. Neither of the introduced strains became a dominant member of the bacterial community. This work was the first approach to assess the establishment of plant growth promoting rhizobacteria and potential biocontrol agents on transgenic plants. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: T4 lysozyme; Transgenic potato; Antagonistic plant-associated bacteria

1. Introduction

The heterologous expression of T4 lysozyme and secretion into the apoplast enhanced the resistance of potato plants to *Erwinia carotovora* ssp. *atroseptica* [1,2], which causes blackleg disease and soft rot of tubers. This approach is applicable to various crops where conventional breeding methods have so far been unsuccessful, and thus may become economically important [2,3]. Many other

phytopathogenic bacteria and fungi and also plant beneficial bacteria are sensitive to T4 lysozyme in vitro [4–6], and evidence was found that T4 lysozyme is also active in the phyllosphere [7] and the rhizosphere of the transgenic potato plants [8]. Therefore, there is public concern about potential adverse effects towards plant beneficial rhizobacteria which might counteract the intended resistance of the transgenic plants or affect follow-up crops. In a previous study, bacterial isolates from the rhizosphere and the tuber surface (geocaulosphere) of transgenic T4 lysozyme producing potatoes and non-transgenic parental plants were screened for their potential to promote plant growth by production of indole-3-acetic acid (IAA), and to antago-

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nize *E. carotovora* ssp. *atroseptica* and/or *Verticillium dahliae* [8,9]. Two of the strains were selected for seed tuber inoculation of T4 lysozyme producing and control plants in the field: the first, L16-3-3, represented a group of strains identified as *Serratia grimesii* which was exclusively isolated from control plants [8]. The strain L16-3-3 was in vitro sensitive against T4 lysozyme. The second, *Pseudomonas putida* QC14-3-8, was isolated from T4 lysozyme producing plants and was in vitro tolerant against T4 lysozyme. The objectives were to investigate under field conditions (i) whether these two strains, representing antagonistic plant-associated bacteria, are affected by plant-produced T4 lysozyme, (ii) whether they establish in the phytosphere, giving further evidence for their potential as biocontrol agents (BCAs), and (iii) whether T4 lysozyme expression of the plants can improve the establishment of T4 lysozyme tolerant inoculants.

Another aspect of our study was to analyze the impact of the introduced bacteria on the indigenous rhizosphere and geocaulosphere bacteria, because the introduction of exogenous microorganisms may affect the natural microflora [10,11], including plant beneficial bacteria. For fingerprinting the bacterial communities, the denaturing gradient gel electrophoresis (DGGE) of 16S rDNA amplicons was applied to monitor shifts in the structure of the plant-associated microbial communities due to seed tuber inoculation over one growth season.

2. Materials and methods

2.1. Plants and experimental field design

The transgenic potato line DL5 constitutively expressed and secreted T4 lysozyme, a transgenic control line DC1 without T4 lysozyme gene and the non-transgenic parental potato line (*Solanum tuberosum* L. cv. Désirée = DESI) were provided by Dr. K. Düring (Federal Centre for Breeding Research on Cultivated Plants, Quedlinburg, Germany). In 1998, rhizobacteria inoculated and non-inoculated potato tubers were grown in a field trial in Groß Lüsewitz (Germany). The trial consisted of three replicates per plant line per treatment and nine replicates of fallow plots. The plots were arranged in a complete randomized block design containing 12 plants each. Samples were collected at three times during the vegetation period corresponding to the growth stages of plants: young plants (growth stage 11–19), flowering plants (growth stage 61–69) and senescent plants (growth stage 91–97), according to Hack et al. [12].

2.2. Characterization of bacterial strains

The strains *P. putida* QC14-3-8 and *S. grimesii* L16-3-3 were characterized on the basis of their carbohydrate utilization profile by the API system (bioMérieux, France),

their fatty acid profiles (Microbial Identification System, MIDI Inc., Newark, USA) and sequencing of the 16S rRNA genes. The sequence data were analyzed by the MWG-Biotech software package BaseImagIR v 4.1. and the whole sequences of each strain were aligned with reference 16S rRNA gene sequences using the BLAST algorithm [13]. The sensitivity of QC14-3-8 and L16-3-3 and their respective rifampicin resistant spontaneous derivatives towards purified T4 lysozyme were determined by a survival assay according to de Vries et al. [6]. Both antagonists were tested for their ability to produce the plant growth hormone IAA (auxin) in vitro [8].

In the field experiment, spontaneous mutants of *P. putida* QC14-3-8 and *S. grimesii* L16-3-3 resistant to rifampicin (Fluka, Buchs, Switzerland) were used. To test if the mutant strains were similar to the wild-types, L16-3-3^{Rif^r} and QC14-3-8^{Rif^r} were tested in colony morphology, antimicrobial properties and growth rate. Stock cultures of both isolates were grown in nutrient broth II (NBII, Sifin, Berlin, Germany) amended with 100 µg ml⁻¹ rifampicin at 30°C for 12–24 h. Aliquots of 1 ml were cryopreserved at –70°C in 15% glycerol.

2.3. Preparation of inocula and potato tuber treatment

Both strains were precultured in Erlenmeyer flasks (2 l) containing 500 ml NBII (Sifin) amended with 100 mg l⁻¹ of rifampicin on a rotary shaker at 50 rpm at 30°C for 12 h. Precultured cells of each isolate were transferred into 10-l fermenters (Biostat, B. Braun, Melsungen AG, Germany) both containing 9.5 l NBII amended with rifampicin (100 mg l⁻¹). After fermentation for 12 h at 30°C, the bacterial cell suspensions (for titers see legend to Fig. 2) were diluted with 10 l of drinking water. Prior to planting in soil, the seed tubers were submerged in the bacterial cell suspension for 10 min. Seed tubers of all three plant lines were treated with corresponding volumes of water instead of bacterial suspension as control treatment. The potatoes were planted on 23 April 1998 and harvested on 5 August 1998.

2.4. Sampling procedures and enumeration of introduced rifampicin-marked bacteria

Plant roots with adhering soil taken from three randomly selected plants from each plot were sampled into sterile Stomacher bags and treated as one sample (27 samples). Prior to cell extraction, 5 g of each sample was transferred into a new Stomacher bag. Samples were extracted in a Stomacher laboratory blender (BagMixer, Interscience, St. Nom, France) with different sterile solutions as described previously [8]. In order to investigate the colonization of the tuber surface at the end of the growing season, six tubers from three plants from each plot were sampled into sterile Stomacher Bags and treated as one sample. The extraction procedure was described previously

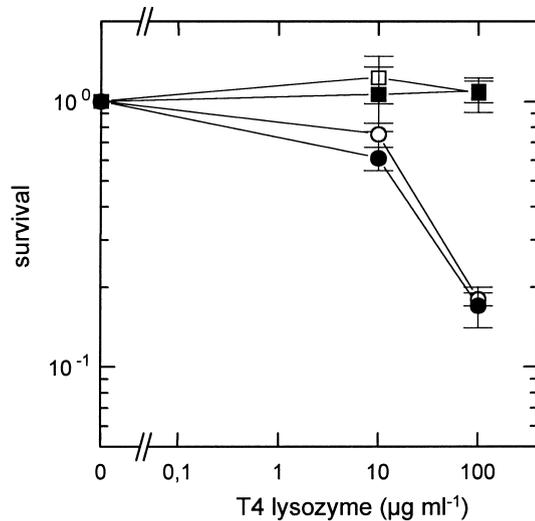


Fig. 1. Survival of cells following exposure to T4 lysozyme for 1 h at 25°C (○, *S. grimesii* L16-3-3; ●, *S. grimesii* L16-3-3Rif⁺; □, *P. putida* QC14-3-8; ■, *P. putida* QC14-3-8Rif⁺). The data (±S.D.) are means of three independent experiments.

[8]. To estimate the colony counts of both inoculants, each sample was serially diluted and 0.1-ml aliquots of each dilution were plated onto nutrient agar II (NAII, Sifin) amended with 100 µg ml⁻¹ rifampicin and 100 µg ml⁻¹ cycloheximide (Sigma, Steinheim, Germany) using a spiral plater (Autoplate 4000, Exotech, Inc., Gaithersburg, MD, USA). Plates were prepared in duplicate and incubated for 24–48 h at 30°C. Bacterial colonies were enumerated by scanning the agar plates (CASBA 4, Spiral Biotech Inc., USA). The analysis of the spiral plate counts was performed by a software programme (M.A.P./NT, BIOSYS, Karben, Germany).

2.5. Molecular analysis by DGGE

Fingerprinting of the bacterial rhizosphere and geocaulosphere communities by DGGE was carried out as described previously [14]. Briefly, 16S rDNA fragments (positions 968–1401 (*Escherichia coli* rDNA sequence)) were

amplified by PCR from rhizosphere and geocaulosphere DNA extracts with the primer pair F984GC-R1378. The amplicons were separated at 60°C in a denaturant gradient of 40–58% of 7 M urea and 40% (v/v) formamide. Acid silver staining was used for the detection of DNA in DGGE gels [15].

2.6. Expression monitoring by Western blot analysis

For expression monitoring, leaf samples of six plants per line randomly distributed over the field were taken. T4 lysozyme expression was evaluated by Western blots using a polyclonal anti-T4 lysozyme antibody (T4-pAB) made in rabbit. Western blotting has been performed according to Düring et al. [1] with the following modifications. Unspecific binding sites were blocked over night at 4°C. Membranes were incubated with primary antibody (T4-pAB) for 3 h, biotinylated secondary antibody (anti-rabbit biotin-IgG F(ab')₂ fragment, Boehringer Mannheim, Germany) for 45 min and streptavidin-alkaline phosphatase conjugate (Life Technologies, Karlsruhe, Germany) for 20 min. Before chemiluminescent detection, membranes were equilibrated, 2×5 min in DEA buffer (0.1 M diethanolamine, 1 mM MgCl₂, pH 10). The substrate CSPD® (Boehringer Mannheim) was replaced by CDP-Star (Boehringer Mannheim). Each Western blot was repeated at least twice. Films were evaluated densitometrically and the amount of T4 lysozyme was estimated by comparing the density of the plant-encoded T4 lysozyme band with a reference band of 1 ng T4 lysozyme.

2.7. Statistical analysis

Statistical analysis was performed at $P < 0.05$ level with the SAS software package (Proc GLM or Proc GENMOD in SAS release 6.12; SAS Institute, Cary, N.C.). Data of bacterial plate counts of introduced isolates were log₁₀ transformed before they were studied by two-factor analysis of variance (rhizosphere: plant lines and growth stages). The bacterial counts of the two antagonists recov-

Table 1
Characteristics of the introduced bacterial isolates

Isolates	QC14-3-8	L16-3-3
Origin	geocaulosphere of transgenic potatoes	rhizosphere of parental potatoes
Antagonistic to ^a :		
● <i>E. carotovora</i> ssp. <i>atroseptica</i>	active	inactive
● <i>V. dahliae</i>	inactive	active
Production of IAA ^b	+	+
Lysozyme survival assay ^c	tolerant	sensitive
Identification/characterization		
● API 20NE/API 20E	<i>P. aeruginosa</i> (91.8%)	<i>S. liquefaciens</i> (81.6%)
● FAME	<i>P. fluorescens</i> (SIM: 0.14)	<i>S. grimesii</i> (SIM: 0.93)
● Sequencing of 16S rRNA	<i>P. putida</i> (99%)	<i>S. grimesii</i> (99%)

^aDual culture assays [8].

^bColorimetric determination by a microplate method [8].

^cSurvival assay according to de Vries et al. [6].

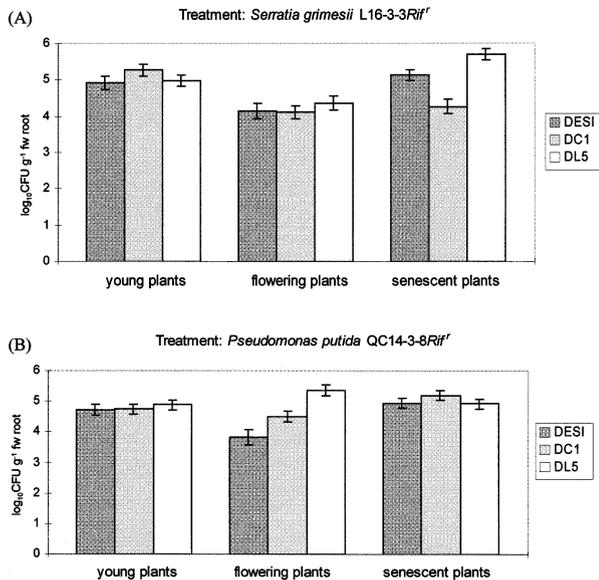


Fig. 2. Mean values \pm confidence interval of spiral plate counts of the introduced bacteria recovered from the rhizosphere. Colonization of roots of transgenic (DL5), transgenic control plants (DC1) and non-transgenic potato plants (DESI) by *S. grimesii* L16-3-3Rif⁺ (A) and *P. putida* QC14-3-8Rif⁺ (B) at different stages of plant development after inoculation of seed tubers. (A) The inoculation densities were log₁₀ 9.6 CFU ml⁻¹ for *S. grimesii* L16-3-3Rif⁺ and log₁₀ 9.4 CFU ml⁻¹ for *P. putida* QC14-3-8Rif⁺. At the last sampling, significantly more CFU ($P < 0.0001$) of L16-3-3Rif⁺ could be recovered from DL5 plants than from control plants (DESI, DC1). At the time of flowering, significantly more CFU ($P = 0.001$) of QC14-3-8Rif⁺ could be recovered from DL5 plants compared to both controls.

er on NAI were analyzed for differences in colonizing the transgenic (DL5), the non-transgenic (DESI) and the transgenic control plants (DC1).

3. Results

3.1. Characterization of antagonistic bacteria

The strain QC14-3-8 was originally isolated from the tuber surface (geocaulosphere) of transgenic potato plants (DL5). QC14-3-8 was selected from an in vitro screen as antagonist to *E. carotovora* ssp. *atroseptica* and was found to produce IAA in vitro [8]. According to the API 20NE test, the strain QC14-3-8 appeared as a strain of *Pseudomonas aeruginosa*, whereas FAME analysis suggested that the isolate was a member of the *Pseudomonas fluorescens* group. The 16S rDNA sequence of strain QC14-3-8 revealed 99% identity to that of the strain *P. putida* DAM 1236 (GenBank 4433340). *P. putida* QC14-3-8 as well as its spontaneous rifampicin resistant mutant QC14-3-8Rif⁺ were resistant to lysozyme treatment (up to 100 μ g ml⁻¹; Fig. 1).

The second strain L16-3-3 was originally isolated from the rhizosphere of the parental plant line (DESI). L16-3-3 was picked during an in vitro screen as antagonist to

V. dahliae and was able to produce IAA in vitro [8]. According to the API 20E test, it appeared as a strain of *Serratia liquefaciens*. The FAME analysis suggested that the isolate was a strain of *S. grimesii*. Analysis of sequenced 16S rDNA revealed 99% identity to that of the type strain *S. grimesii* DSM 30063, (GenBank 4582212). *S. grimesii* L16-3-3 and its spontaneous rifampicin resistant derivative L16-3-3Rif⁺ were affected by T4 lysozyme, particularly at 100 μ g ml⁻¹ (Fig. 1). The results of characterization of the isolates are summarized in Table 1.

3.2. Colonization of the rhizo- and geocaulosphere by *S. grimesii* L16-3-3Rif⁺

In April 1998, *S. grimesii* strain L16-3-3Rif⁺ was released as seed potato inoculant. The strain colonized the rhizosphere of transgenic plants (DL5) as well as of control plants (DESI, DC1) (Fig. 2A). About log₁₀ 5 colony forming units (CFU) g⁻¹ fw (fresh weight) of root was recovered from the rhizosphere of young plants. No statistically significant difference in plate counts of L16-3-3Rif⁺ was found between the three plant lines. Colony counts of L16-3-3Rif⁺ decreased by almost one order of magnitude (log₁₀ 4.2 CFU g⁻¹ fw root) from the first sampling (34 days after planting) to the second sampling (70 days after planting) (Fig. 2A). At the time of harvest, significantly more CFU of L16-3-3Rif⁺ were recovered from transgenic DL5 plants than from parental (DESI) or transgenic control plants (DC1). Thus the T4 lysozyme sensitivity of L16-3-3 found in vitro did not affect its oc-

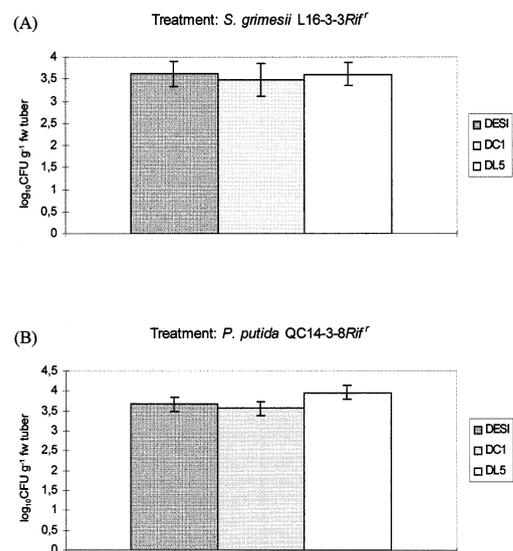


Fig. 3. Mean values \pm confidence interval of spiral plate counts of the introduced bacteria recovered from the geocaulosphere. Colonization of the tuber surface of transgenic (DL5), transgenic control plants (DC1) and non-transgenic potato plants (DESI) by *S. grimesii* L16-3-3Rif⁺ (A) and *P. putida* QC14-3-8Rif⁺ (B) at the end of the growing season (senescent plants) after inoculation of seed tubers (see legend to Fig. 2). No significant differences between the three plant lines were found over the time of sampling ($P = 0.631$).

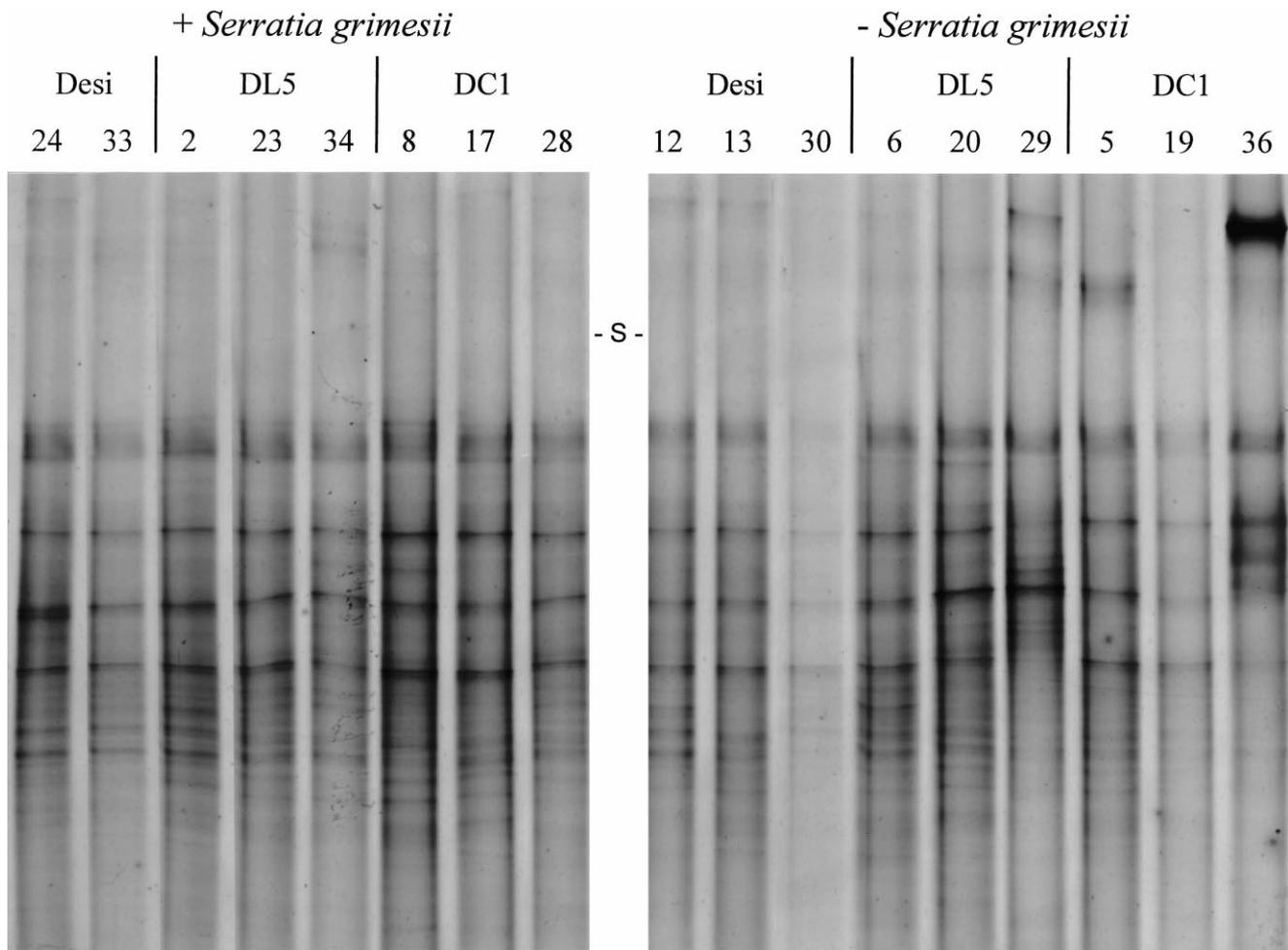


Fig. 4. Similar DGGE fingerprints of bacterial communities in the rhizosphere of senescent potato plants grown from seed tubers with or without *S. grimesii* L16-3-3Rif^r. DESI: non-transgenic parental potato plants, DL5: T4 lysozyme expressing potato plants, DC1: transgenic control plants without T4 lysozyme gene, S: electrophoretic mobility of the 16S rDNA fragment from L16-3-3Rif^r. On top of the lanes, plot numbers of the samples are shown.

currence on the surface of T4 lysozyme producing plants under field conditions.

The strain L16-3-3Rif^r was also able to colonize the geocaulosphere and established in this microenvironment at levels of approximately \log_{10} 3.6 CFU g⁻¹ fw of tuber (Fig. 3A). No significant differences in colony counts could be observed between the three plant lines DESI, DC1 and DL5.

3.3. Colonization of the rhizo- and geocaulosphere by *P. putida* QC14-3-8 Rif^r

P. putida QC14-3-8Rif^r was able to colonize the rhizosphere of transgenic plants (DL5) as well as of control plants (DESI, DC1). At the first sampling time, levels of about \log_{10} 4.8 CFU g⁻¹ fw root could be recovered from the rhizosphere of the three plant lines (Fig. 2B). At the second sampling, significantly more colony counts of QC14-3-8Rif^r were recovered from transgenic DL5 plants than from DC1 and DESI. At the time of harvest, the inoculant was recovered from the rhizosphere of all three

plant lines at similar levels of ca. \log_{10} 5 CFU g⁻¹ fw root (Fig. 2B). The strain QC14-3-8Rif^r was also able to colonize the geocaulosphere and established in this microhabitat at levels of ca. \log_{10} 3.7 CFU g⁻¹ fw of tuber without significant differences between the plant lines (Fig. 3B).

3.4. Molecular analysis of bacterial communities

Total DNA was extracted from all cell extracts of rhizosphere and tuber surface samples. A fragment of the 16S rRNA gene was amplified by PCR and separated in DGGE. For each sampling, the DGGE fingerprints were compared between three replicate root samples of inoculated and non-inoculated plants of the plant lines DESI, DL5 and DC1. In addition, DGGE analysis was performed to monitor seasonal shifts of the rhizosphere bacterial communities. For the tuber surface, one composite sample per treatment was analyzed in DGGE for the last sampling in comparison to the rhizosphere samples. Representative DGGE fingerprints are shown in Figs. 4–6. The bacterial community fingerprints showed a stable pat-

tern for young plants as well as flowering and senescent plants. Only when the plants became senescent, some exceptional fingerprints increased the variability (Fig. 5: plot 36, Fig. 6: plot 29). No differences were apparent between rhizosphere fingerprints of inoculated and non-inoculated plants. Seasonal community shifts (Fig. 6) and differences between rhizosphere and geocaulosphere communities (Fig. 5) were evident, regardless of inoculation or plant lines. *S. grimesii* L16-3-3Rif^r could only be detected in some samples from young roots of all three plant lines (DESI: weak band from plot 24, DL5: weak band from plots 2 and 23, DC1: strong band from plot 8). It decreased below detection limit on roots of flowering and senescent plants (Fig. 3) and on tubers (Fig. 5). *P. putida* QC14-3-8Rif^r was not detected in any DGGE fingerprint, but a weak band, which corresponded in electrophoretic mobility, was apparent in all fingerprints including inoculated and non-inoculated plants, throughout the season (Figs. 4–6).

3.5. Expression level of T4 lysozyme in transgenic potatoes

T4 lysozyme could be detected in protein extracts of

leaves at the following amounts (in % of total soluble protein): 1.04×10^{-4} (28 May 1998), 8.26×10^{-4} (11 June 1998), 7.16×10^{-4} (1 July 1998) and 5.77×10^{-4} (28 July 1998).

4. Discussion

In this study, the effects of plant-produced T4 lysozyme on the establishment of two potentially beneficial bacteria in the rhizo- and geocaulosphere of transgenic and non-transgenic potatoes and the impact of the introduced bacteria on the indigenous rhizosphere and geocaulosphere bacteria were investigated. Both inoculants established in the rhizosphere of transgenic and non-transgenic potatoes at levels of approximately \log_{10} 4–5 CFU g⁻¹ fw root. This corresponds well with other successful attempts for the establishment of fluorescent pseudomonads in the rhizosphere [16,17]. Scher et al. [18] defined colonizers of corn roots as bacteria that attain CFU more than \log_{10} 3.7 CFU g⁻¹ root. Weller [19] suggested that so far as introduced bacteria are concerned, in general, a root colonizer is a bacterium that when introduced becomes dis-

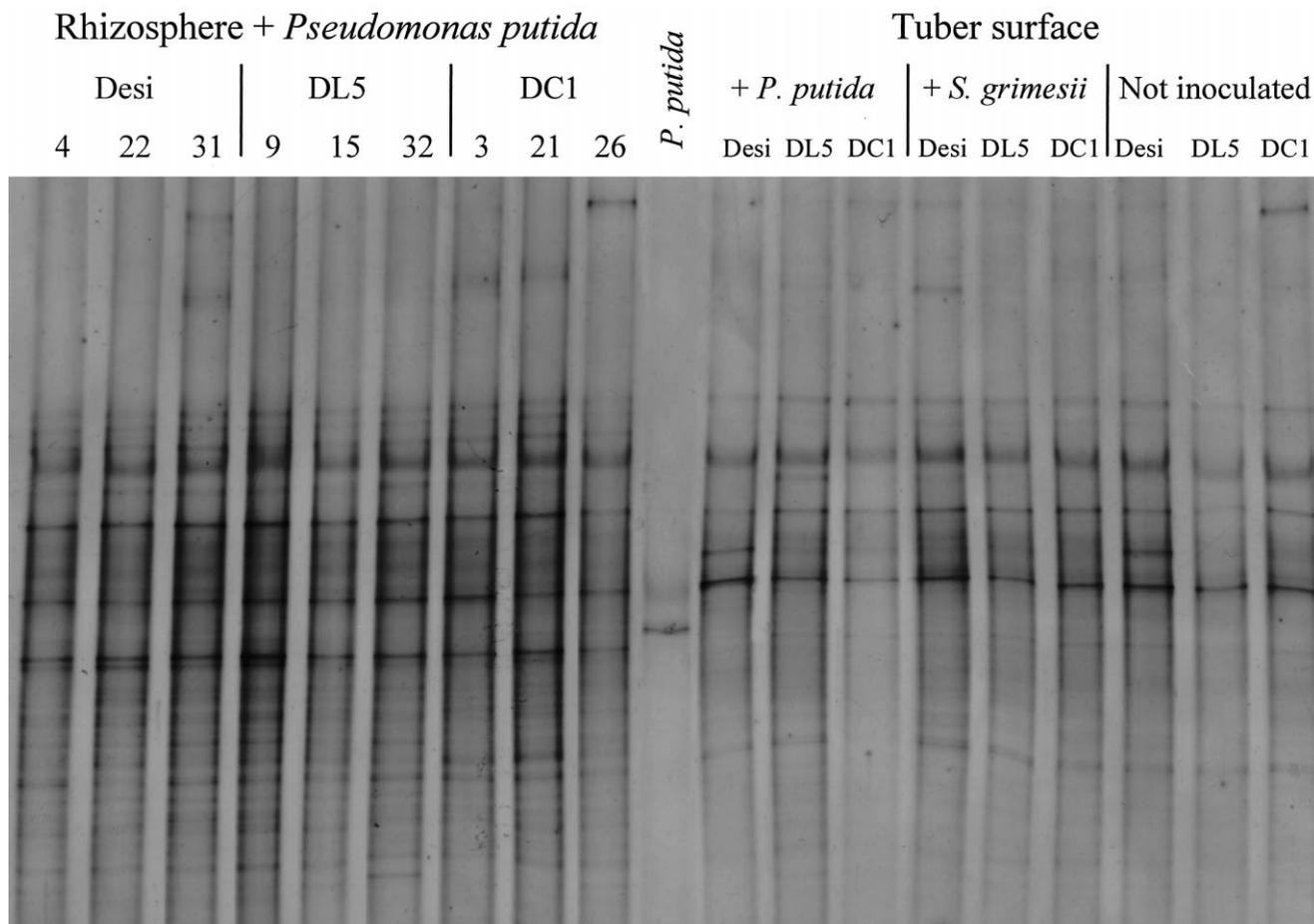


Fig. 5. DGGE fingerprints of bacterial communities from the rhizosphere of senescent potato plants inoculated with *P. putida* QC14-3-8Rif^r, and from the tuber surface of inoculated and uninoculated plants. DESI: non-transgenic parental potato plants, DL5: T4 lysozyme expressing potato plants, DC1: transgenic control plants without T4 lysozyme gene. On top of the lanes, plot numbers of the samples are shown.

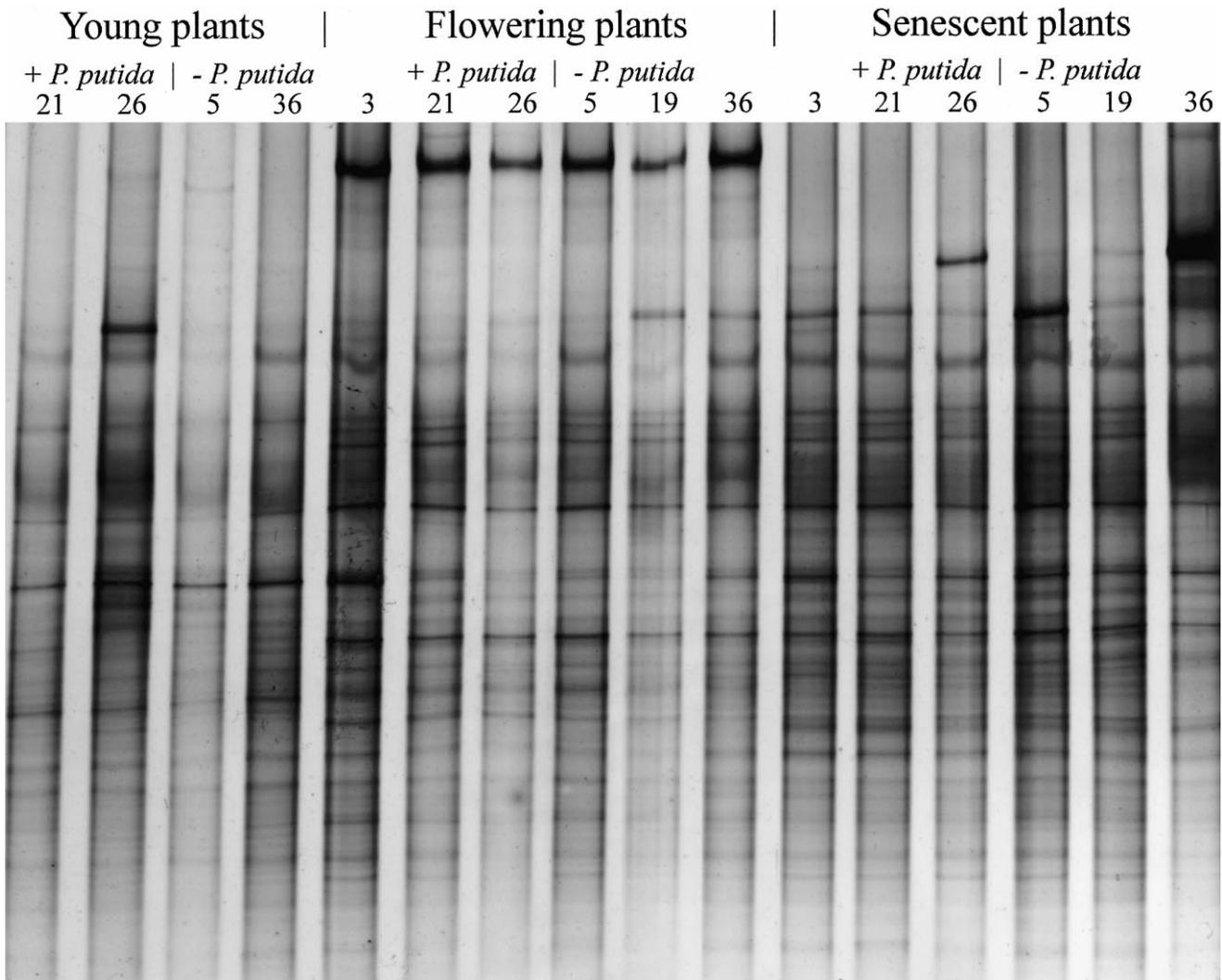


Fig. 6. DGGE fingerprints showing seasonal shifts of bacterial rhizosphere communities from plants (DC1) inoculated or uninoculated with *P. putida* QC14-3-8Rif^r. On top of the lanes, plot numbers of the samples are shown.

tributed along the root in natural soil, propagates, and survives for several weeks in the presence of competition from the indigenous rhizosphere microflora. In this work, colony counts showed that after tuber inoculation, the root was colonized, and the culturable populations of both inoculants were stable in the rhizosphere over the growing season. In contrast, Troxler et al. [20] observed a significant decrease of CFU recovered from the rhizosphere after seed inoculation of wheat with a BCA (*P. fluorescens* CHA0).

In this work, over the vegetation period, expression of the foreign T4 lysozyme gene was monitored by Western blotting analysis. The highest level of lysozyme expression was detected during flowering of plants. At this sampling time, significantly higher amounts of the T4 lysozyme tolerant *P. putida* QC14-3-8Rif^r could be recovered from the transgenic plant line DL5. It is possible that this strain could have an advantage in colonizing T4 lysozyme producing potatoes because of its tolerance towards lysozyme. In this case, transgenic potato plants, producing T4 lyso-

zyme, might enhance the establishment of lysozyme tolerant strains and if such strains have antagonistic properties, a synergistic effect can occur. In a previous study, all antagonists which belonged to the species *S. grimesii* were obtained only from non-transgenic parental or control plants [8]. However, the results of this study indicate that this species was able to colonize transgenic potato plants as well as control plants. At the last sampling (senescence), significantly higher amounts of the T4 lysozyme sensitive *S. grimesii* L16-3-3Rif^r were recovered from the transgenic plant line DL5. The level of T4 lysozyme expression decreased from the time of flowering of plants until senescence. Smalla and Heuer [21] showed that the presence of enterics in the rhizosphere decreased at the end of the growing season when plants became senescent.

It has been demonstrated that both isolates were able to colonize the tuber surface of transgenic as well as that of non-transgenic potatoes, but the culturable population of both inoculants was about one exponential unit lower in the geocaulosphere than in the rhizosphere. This was ex-

pected, because the rhizosphere represents a more attractive habitat compared to the tuber surface due to the exudation of nutrients [8].

The structures of the bacterial rhizosphere and geocaulosphere communities of inoculated plants as revealed by molecular fingerprinting (DGGE) were not significantly altered. Due to target competition in PCR, the relative abundance of a 16S rRNA gene must exceed 1% of the total targets to give a clearly visible band in the DGGE fingerprint [22]. Therefore, the strains disappeared from the fingerprints or appeared only as minor bands, in accordance with the selective plate counts. For the same reason, only prominent populations could be monitored for inoculation effects. Nevertheless, community shifts due to the introduced strains were also not apparent when the sensitivity of DGGE fingerprinting was increased by applying primers specific for alpha-Proteobacteria, beta-Proteobacteria or *Actinomycetales*, as previously described [14] (data not shown).

In contrast to genetically engineered plants with enhanced resistance to pathogens, the biological control of plant pathogens by naturally occurring plant-associated bacteria evaluated as BCA or genetically modified bacteria [19,20] represents another strategy of disease control. In recent years, there has been an increasing interest in using bacterial inoculants because of their ability to antagonize plant pathogens and to promote plant growth, but there is a lack of knowledge on the establishment of introduced antagonists on transgenic plants. If antagonistic bacteria were able to colonize the rhizosphere of a transgenic plant as well as of the non-transgenic parental plant, a synergistic effect to the enhanced resistance due to the genetical modification could be expected. The described field experiment was the first attempt to combine both methods.

In conclusion, no negative effect of T4 lysozyme on the establishment of both antagonistic plant-associated strains in the rhizo- and geocaulosphere was observed under field conditions. In fact, during the time of flowering of potato plants, the T4 lysozyme tolerant strain *P. putida* QC14-3-8*Rif^r* was recovered in significantly higher amounts from T4 lysozyme producing plants than from control plants. The stable establishment of both strains in the microhabitats is necessary for potential evaluation of the strains as biological control agents.

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