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Diversity and community structure of culturable *Bacillus* spp. populations in the rhizospheres of transgenic potatoes expressing the lytic peptide cecropin B

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Abstract

A greenhouse experiment was carried out with transgenic potato plants expressing the antibacterial lytic peptide cecropin in order to determine whether non-target *Bacillus* communities in the rhizosphere are affected by such plants. Two cecropin expressing lines, a line containing only vector sequences and the parental variety Achirana Inta (AI) were included in the experiment. At the flowering and the tuber production stage a total of 621 *Bacillus* isolates was obtained from the potato rhizospheres, and strains were analysed by PCR–RFLP analysis of the 16S rRNA gene and the 16S–23S rDNA intergenic spacer. Representative isolates were further analyzed by partial 16S rDNA sequence analysis. At the flowering stage the cecropin expressing lines caused a transient, but significant effect on the diversity and community structure of culturable *Bacillus* spp. Populations associated with the plasmid-containing potato line showed diversity values comparable to the *Bacillus* communities found in the rhizospheres of wildtype plants, but community structures were highly different. At the tuber production stage the rhizosphere *Bacillus* populations showed only few differences. The observed effects can be partly explained by different sensitivities of the *Bacillus* community members towards cecropin. In addition, unintentionally altered plant characteristics seem to be responsible for the different population structures found in the rhizospheres of transgenic plants.
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Keywords: *Bacillus* isolates; Rhizosphere; Transgenic potato; Cecropin; 16S rRNA gene; 16S–23S rRNA intergenic spacer

1. Introduction

Bacterial diseases of potato (*Solanum tuberosum* L.) account world-wide for high production losses, with developing countries being particularly affected. Resistance traits have not been introduced into cultivars by conventional breeding and chemical control of bacterial pathogens is not feasible. Genetic transformation offers novel ways to obtain disease resistance by introducing foreign genes into plants of agricultural importance. The addition of genes encoding lytic peptides to the potato genome is a promising strategy for suppressing bacterial pathogens as such peptides have been found to exhibit both strong antimicrobial activity in vitro, and significant activity in transgenic tobacco and potato plants in vivo (Jaynes et al., 1993; Allefs et al., 1996; Huang et al., 1997). Various peptides have been isolated from bacteriophages, insects, crabs and plants (Hultmark et al., 1980; Nakamura et al., 1988; García-Olmedo et al., 1989)

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that may confer resistance against plant pathogenic bacteria.

Cecropins are a family of small, highly basic lytic peptides that were first isolated from the hemolymph of pupae of the giant silk moth, *Hyalophora cecropia* (Hultmark et al., 1980). All cecropins exhibit lytic and antibacterial activity against several gram-negative and gram-positive bacteria in vitro (Hultmark et al., 1982). In particular, cecropin B proved to be highly toxic to a number of plant pathogenic bacteria (Jaynes et al., 1987; Nordeen et al., 1992). Recently, for the generation of cecropin-expressing potatoes (Kopper, 1999; Keppel, 2000) two modified cecropin B genes were employed, cecropin C38 that lacks the N-terminal signal peptide and C4 carrying a barley hordothionin signal peptide, which was found to improve post-translational folding (Florack et al., 1995).

Limited information is available on the consequences of introducing genes for the production of antimicrobial substances into the plant genome. As such substances are rarely specific and exhibit lytic activity against a wide range of bacteria, transgenic plants producing antimicrobial substances may affect the soil or rhizosphere microflora. Potentially they may have detrimental effects on plant growth-promoting bacteria. Heuer et al. (1997) analysed the rhizosphere microbial species composition of transgenic potatoes, which produced T4 lysozyme against bacterial infection, by temperature gradient gel electrophoresis, a cultivation-independent approach. No apparent differences between lysozyme-expressing and parent plants were found in the actinomycete community patterns, whereas the total bacterial community patterns differed slightly. Similarly, Heuer et al. (2002) reported that the effects due to the T4 lysozyme release from potato roots are minor compared to environmental factors. In addition, the phyllosphere bacterial communities of T4 lysozyme-expressing potatoes were characterized by their sole-carbon-source utilization profiles using BIOLOG GN microplates as well as by fatty acid analysis of individual isolates (Heuer and Smalla, 1997). Significantly, fewer gram-positive bacteria were isolated from leaves of the lysozyme expressing potatoes. Recently, Lottmann et al. (1999) studied the influence of T4 lysozyme-producing potato plants on potentially beneficial and antagonistic bacteria in the rhizo- and geocaulosphere. Only minor differences could be detected between transgenic and control plants. Ahrenholtz et al. (2000) reported an increased killing of *Bacillus subtilis* on the root hairs of T4 lysozyme-producing potatoes.

The aim of this study was to determine the effect of cecropin-producing potato plants that were developed for the control of bacterial pathogens such as *Ralstonia solanacearum* or *Erwinia* spp. on non-target culturable *Bacillus* spp. populations in the rhizosphere. This bacterial group is a common soil and rhizosphere inhabitant, and several strains have been found to contribute positively to plant growth and health (Asaka and Shoda, 1996; Wilhelm et al., 1998). Two cecropin-expressing lines, a line containing only vector sequences, and the parental variety Achirana Inta (AI) were included in our analysis.

2. Materials and methods

2.1. Transgenic plant lines and greenhouse experiment

Two cecropin-expressing transgenic potato clones were analysed, Achirana Inta (AI) C38 6/1 and AI C4–C4 1/2. They differ slightly in that AI C38 6/1 contains cecropin 38 under the control of the *Ca2-NOS3* promoter–terminator system whereas AI C4–C4 1/2 contains the cecropin C4 double construct *mas2*C4–*mas1*C4 (Keppel, 2000). The *Ca2*-double promoter is derived from the cauliflower mosaic virus and causes constitutive expression. The *mas1–mas2* double promoter regulates the expression of enzymes involved in the biosynthesis of mannopin in *Agrobacterium tumefaciens* (Ni et al., 1995). The promoter *mas1* is a weak constitutive promoter, whereas the *mas2* promoter is auxin- and wound inducible. In addition, the transgenic line AI pMOG 1/1 containing only empty vector sequences and the parental line AI were included.

Potatoes were grown in tissue cultures on MS medium (Murashige and Skoog, 1962). At a plant height of about 10 cm, plants were transferred to the greenhouse for adaptation. After four weeks they were transplanted into pots filled with standard growth substrate (Frux soil substrate ED63; 100–250 mg l^{-1} N, 45–90 mg l^{-1} P 80–210 mg l^{-1} K). Five replicates, each consisting of one plant, were used and the plants were arranged in a randomized complete block design.

Plants were harvested after 5 weeks at the flowering stage and after 10 weeks at the tuber production stage. Transgenic and non-transgenic lines showed the same plant growth development. The rhizospheres of five plants (replicates) per potato line were analysed at each sampling time.

2.2. Isolation of bacilli from the rhizosphere

Plant roots with tightly adhering soil were shaken individually in 100 ml of 1/4 strength Ringer's solution and 10 g of sterile sand for 1 h at room temperature at 200 r.p.m. After settling of sand and root material the supernatant was taken and used for the isolation of bacteria. For the isolation of *Bacillus* spp., the samples were heated at 80 °C for 20 min and subsequently the spores were plated on ATCC medium 552 (Atlas, 1993) supplemented with 7% NaCl. The plates were incubated for 2 days at 29 °C. A maximum of 16 colonies were picked at random for each treatment and replicate resulting in 621 isolates, which were analysed.

2.3. Generation of template DNA for PCR

For DNA amplification cell extracts were prepared by resuspending freshly grown cells in 100 μl TE and adjusting the OD at 600 nm to 2.6. Then, the samples were boiled for 10 min, centrifuged for 2 min at 12,000 × *g* and the supernatant was used for PCR.

2.4. PCR–RFLP analysis of the 16S rRNA gene and of the 16S–23S rRNA intergenic spacer

PCR amplification of the 16S–23S intergenic spacer region (IGS) was carried out with the *Bacillus* isolates by using the primers pHr and p23SROI (Massol-Deya et al., 1995). PCR reactions were performed in a total reaction volume of 50 μl containing 1 × PCR reaction buffer (Gibco, BRL), 200 μM each of dATP, dCTP, dGTP and dTTP (Gibco, BRL), 3 mM $MgCl_2$, 0.2 μM primers, 1 μl cell extract or 50 ng pure DNA, and 2 U Taq DNA Polymerase (Gibco, BRL). All amplifications were performed with a MJ Research Inc., thermocycler (PTC-100™). The following temperature cycle was used: an initial denaturation step of 5 min at 95 °C followed by 35 cycles of 30 s denaturation at 95 °C, 1 min annealing at 52 °C and 2 min extension at 72 °C and a final extension step of 4 min at 72 °C. Aliquots (6–15 μl) were digested with *Alu*I, *Rsa*I, *Dde*I or *Hae*III (Gibco, BRL) and the resulting fragments were analysed by horizontal agarose gel electrophoresis in 2.3% agarose gels. Bands were scanned and recorded using the program RFLPscan Plus (version 3.0, Scanalytics). In order to analyse diversity at the species level, PCR amplification was performed as described above by using the pimers rD1 and fD1 (Weisburg et al., 1991). RFLP analysis of 16S rRNA genes was carried out using *Dde*I and *Hae*III (Gibco, BRL) as restriction enzymes.

2.5. Sequence analysis

For partial sequence analysis, 16S rRNA gene PCR products were purified with the NucleoTraPCR kit (Macherey-Nagel) according to the manufacturer's instructions and used as templates in sequencing reactions. Sequencing was performed by the dideoxy chain termination method with the primer 8f (Weisburg et al., 1991) using an ABI 373A automated DNA sequencer and the ABI PRISM Big Dye Terminator Cycle Sequencing Kit (PE Applied Biosystems Inc., Faster City, CA, USA). Sequences (approximately 490 bp) were subjected to BLAST analysis (Altschul et al., 1990) with the NCBI database.

2.6. Antibacterial assays of cecropin B

Antibacterial activity of cecropin B against the most abundant *Bacillus* isolates (B1, B2 and B6) and B11 was assayed by measuring zones of growth inhibition in thin agar plates as described by Hultmark et al. (1982) testing 2, 4 and 6 μg cecropin B (Sigma). Data based on two separate measurements were subjected to an analysis of variance and means were separated by Duncan's multiple range test.

2.7. Data analysis

The indices of diversity (H) and equitability (J) proposed by Shannon and Weaver (1963) were calculated in order to describe phylotype diversities of *Bacillus* communities. They are defined by the equations: $H = -\Sigma\, p_i \ln p_i$, and $J = H/\ln S$; where p_i represents the number of individuals from one phylotype, divided by the total number of individuals in the community

sample, and *S* represents the phylotype richness in the sample.

In order to determine treatment and replicate effects on the whole population structures the AMOVA procedure was used. AMOVA is a method for analysing molecular variance that produces estimates of variance components and F-statistic analogs reflecting the correlation of haplotypic diversity at different levels of hierarchical subdivision. The significance of the variance components is tested by using a permutational approach (Excoffier et al., 1992). The vectors for the presence of RFLP bands (1 for the presence of each band on a gel; 0 for the absence of each band on a gel) for each strain were used to compute the genetic distance for each pair of strains. The parameter used was the Euclidian metric measurement of Excoffier et al. (1992) as defined by Huff et al. (1993). The AMOVA analysis was performed with the Arlequin 2.000 program (Schneider et al., 2000), which is available at the following URL: http://anthro.unige.ch/arlequin.

In order to compare the abundances of individual RFLP types in the various treatments the data were $\log_2$ transformed and subjected to an analysis of variance. If significant differences were found, means were separated using Duncan's multiple range test.

2.8. Nucleotide sequence accession numbers

The nucleotide sequences determined in this study have been deposited in the NCBI database under accession numbers AF497247–AF497259.

3. Results

3.1. 16S and IGS PCR–RFLP analysis

Among the rhizosphere isolates a total of 8 16S rRNA (Table 1) and 13 IGS RFLP types was found (Table 2). The majority of bacteria belonged to IGS RFLP type B1, B2 and B6, and type B1 and B2 had identical 16S RFLP patterns.

At the flowering stage the Shannon–Weaver diversity indices based on the RFLP analysis of the IGS region were in the range from 0.54 with AI C4–C4 isolates to 1.08 with isolates obtained from the non-transgenic parental plants (Table 2). The isolated bacilli obtained from the cecropin expressing transgenic lines showed significantly ($P < 0.01$) lower diversity indices than those from other treatments. Similar observations were made with the equitability values (Table 2). Diversity indices of the *Bacillus*

Table 1
Distribution and data analysis of *Bacillus* 16S rRNA RFLP types

Bacillus 16S RFLP type	Flowering stage				Tuber production stage			
	AI	C38	C4	MOG	AI	C38	C4	MOG
BI	3[a]	2	4	0	0	0	0	0
BII	26	14	9	16	68 a	50 b	53 ab	40 b
BIII	0 b	0 b	0 b	3 a	0	0	0	0
BIV	44 b	63 a	66 a	17 c	11 b	24 a	26 a	34 a
BV	1	0	0	0	0	0	0	0
BVI	0 b	0 b	0 b	41 a	1	0	0	0
BVII	2	0	1	0	0	0	0	0
BVIII	2	0	0	0	0	0	0	0
Totals	78	79	80	77	80	74	79	74
Richness	6	3	4	4	3	2	2	2
Shannon–Weaver diversity (*H*)	0.90[b] aA	0.48 b	0.52 b	0.97 aA	0.36 B	0.57	0.60	0.64 B
Shannon–Weaver equitability (*J*)	0.84[b] a	0.69 ab	0.46 bB	0.87 a	0.45 b	0.82 ab	0.86 aA	0.78 ab

Means within a column which are not significantly different from each other at $P < 0.01$ share the same small letters. Different capital letters indicate significant differences between different plant growth stages at $P < 0.01$. AI, non-transgenic line Achirana Inta; C38 and C4, cecropin-expressing lines Achirana Inta C38 6/1 and Achirana Inta C4–C4 1/2.

[a] Data from five replicates are summarized.

[b] Data are the means of five replicates.

Table 2
Distribution and data analysis of *Bacillus* IGS-RFLP types

Bacillus IGS RFLP type	Flowering stage				Tuber production stage			
	AI	C38	C4	MOG	AI	C38	C4	MOG
B1	9[a] a	7 a	1 b	6 a	15	28	23	23
B2	17	7	8	11	48 a	22 b	30 ab	16 b
B3	0	0	0	2	0	0	0	0
B4	0	0	0	1	0	0	0	0
B5	3	2	3	0	0	0	0	0
B6	44 b	63 a	66 a	16 c	11	24	27	32
B7	0	0	0	0	0	0	0	1
B8	0	0	1	0	0	0	0	0
B9	0 b	0 b	0 b	41 a	0	0	0	0
B10	1	0	0	0	0	0	0	0
B11	2	0	1	0	0	0	0	0
B12	2	0	0	0	0	0	0	0
B13	0	0	0	0	5a	0b	0b	0b
Totals	78	79	80	77	79	74	80	72
Richness	7	4	6	6	4	3	3	4
Shannon–Weaver diversity (*H*)	1.08[b] a	0.58 bB	0.54 bB	1.04 a	0.91	0.98 A	1.02 A	0.75
Shannon–Weaver equitability (*J*)	0.81[b] ab	0.62 bc	0.46 cB	0.79 ab	0.79 ab	0.84 ab	0.93 aA	0.54 b

Means within one column which are not significantly different from each other at $P < 0.01$ share the same small letters. Different capital letters indicate significant differences between different plant growth stages at $P < 0.01$. AI, non-transgenic line Achirana Inta; C38 and C4, cecropin-expressing lines Achirana Inta C38 6/1 and Achirana Inta C4–C4 1/2.

[a] Data from five replicates are summarized.

[b] Data are the means of five replicates.

isolates obtained at the tuber production stage ranged from 0.75 (bacilli obtained from AI pMOG plants) to 1.02 (AI C4–C4 isolates) and did not differ significantly (Table 2). Isolates from cecropin-expressing plants showed higher diversities at the tuber production stage than at the flowering stage, whereas the diversity of remaining isolates decreased or remained constant.

The community structures among *Bacillus* isolates found at the flowering stage were highly different. AMOVA indicated that the ceropin-expressing lines, the parental plant and AI pMOG showed significantly different community structures, whereas the AI C4–C4 and AI C38 rhizospheres possessed comparable *Bacillus* populations (Table 3). Although the populations of AI pMOG rhizospheres showed similar diversity and equitability values to those obtained from wildtype plants, community structures were highly different. IGS type B9 was only found in the rhizosphere of AI pMOG plants at the flowering stage, where 53% of the AI pMOG isolates belonged to this group (Table 2). Furthermore, IGS type B6 showed significantly reduced abundances in that treatment. This phylotype showed higher abundance in the rhizospheres of cecropin-expressing lines than in other treatments. At the tuber production stage differences were less pronounced. IGS RFLP type B2 showed lower abundances in association with cecropin-expressing plants as well as with AI pMOG, whereas B13 was exclusively found in the rhizospheres of wildtype plants (Table 2). *Bacillus* isolates that contributed to the high diversities in the non-transgenic rhizospheres of the first sampling time, and which represented minor groups, were not found in samples collected at the tuber production stage.

3.2. Sequence analysis of Bacillus isolates

Representative isolates of all IGS RFLP types were analysed by partial 16S rDNA sequencing. BLAST analysis revealed that all isolates showed high similarity (97–100%) to *Bacillus* species (Table 4), and in total nine different species were found. The IGS RFLP type B6, which was highly abundant in the potato

Table 3
AMOVA analysis of *Bacillus* communities

Growth stage	Variance component	Variance among treatments (%)	Variance among replicates (%)	Variance within populations (%)	P[a] treatments	P[a] replicates
Flowering stage	AI vs. C38 vs. C4 vs. MOG	23.63	0.87	75.70	0.000	0.196
	AI vs. C38	5.83	0.30	93.87	0.044	0.389
	AI vs. C4	7.95	0.62	91.43	0.008	0.311
	AI vs. MOG	24.02	0.01	75.97	0.000	0.449
	C38 vs. C4	−0.79	3.19	97.60	0.103	0.091
	C38 vs. MOG	38.14	0.97	60.90	0.000	0.211
	C4 vs. MOG	40.57	1.17	58.26	0.000	0.159
Tuber production stage	AI vs. C38 vs. C4 vs. MOG	2.18	11.89	85.93	0.000	0.000
	AI vs. C38	6.78	5.70	87.52	0.000	0.007
	AI vs. C4	2.84	4.86	92.29	0.003	0.011
	AI vs. MOG	8.41	17.93	73.66	0.000	0.000
	C38 vs. C4	−1.11	4.66	96.45	0.035	0.034
	C38 vs. MOG	−3.50	20.39	83.11	0.000	0.000
	C4 vs. MOG	−2.19	18.45	83.74	0.000	0.000
Flowering vs. tuber production stage	AI	15.49	2.45	82.06	0.000	0.075
	C38	24.37	3.53	72.10	0.000	0.038
	C4	27.24	2.82	69.94	0.000	0.065
	MOG	19.77	13.88	66.35	0.000	0.000

[a] Probability of a more extreme variance distribution.

rhizosphere, showed 99% sequence homology to a *B. megaterium* 16S rRNA gene. Types B1 and B2 were frequently found at the tuber production stage in the rhizosphere of all potato lines and proved to belong to *B. pumilis*. *B. thuringiensis* strains (B3, B4) and a *B. macroides* strain (B9) were only detected in the rhizospheres of the plasmid containing transgenic plant, whereas a *B. endophyticus* (B10), a *B. firmus* (B12) and a *B. subtilis* (B13) strain were exclusively associated with the rhizospheres of the wildtype potato plant.

Table 4
Partial 16S rDNA sequence (approximately 490 bp) analysis and tentative phylogenetic affiliation of *Bacillus* isolates

IGS-phylotype	16S-phylotype	Closest NCBI match	Accession number	Similarity (%)
B1	BII	*B. pumilis* KL-052	AY030327	100
B2	BII	*B. pumilis* M1-9-1	AB048252	100
B3	BIII	*B. cereus* ATCC43881	AF290550	99
		B. thuringiensis ATCC33679	AF290549	99
B4	BIII	*B. thuringiensis* 4Q281	AF155954	100
B5	BI	*B. marisflavi* TF-11	AF483624	100
B6	BIV	*B. megaterium*	AF142677	99
B7	BII	*B. subtilis* O9	AF287011	99
B8	BI	*B. marisflavi* TF-11	AF483624	99
B9	BVI	*B. macroides* NCDO 1661	X70312	100
B10	BV	*B. endophyticus*	AF295302	100
B11	BVII	*B. cereus* ATCC43881	AF290550	99
		B. thuringiensis ATCC33679	AF290549	99
B12	BVIII	*B. firmus*	X60616	97
B13	BII	*B. subtilis* N10	AF318900	99

Table 5
Lethal concentrations of cecropin B for selected *Bacillus* IGS-RFLP types

IGS RFLP type	Lethal concentration of cecropin (μM)
B1	18 d
B2	55 cd
B6	123 ab
B11	166 a

Data are the means of two replicates. Means which are not significantly different from each other at $P \leq 0.05$ share the same letters as superscripts.

3.3. Antibacterial assays of cecropin B

The sensitivity of four *Bacillus* (B1, B2, B6, B11) towards the lytic peptide cecropin B was assessed, and the lethal concentrations are listed in Table 5. Significantly different lethal concentrations were observed ranging from 18 to 166 μM.

4. Discussion

The lytic peptide cecropin has been introduced into a variety of plants in order to enhance resistance to phytopathogenic bacteria (Nordeen et al., 1992; Jaynes et al., 1993; Florack et al., 1995; Huang et al., 1997; Kopper, 1999; Keppel, 2000). Cecropin expression of the transgenic potato lines used in this study, AI C38 6/1 and AI C4–C4 1/2, was verified by Northern blot analysis in which the selected clones showed high expression (Kopper, 1999). Higher cecropin contents were found in AI C38 6/1 than in the line AI C4–C4 1/2 (Keppel, 2000), and in resistance tests using *Erwinia chrysanthemi* inoculants both cecropin-expressing lines showed comparable resistance levels towards the pathogen (Keppel, 2000).

Due to the secretion of root exudates, the rhizosphere exhibits high microbial activity, where not only pathogens, but also plant-growth promoting bacteria as well as antagonists of plant pathogens proliferate. At the flowering stage, plants expressing the antibacterial peptide had a highly significant effect ($P < 0.01$) on the diversity of culturable bacteria belonging to the genus *Bacillus*. Diversity indices are based on relative frequencies of different strains and consider non-identical phenotypes as equally distant, but do not consider similarities of different strains. Therefore, the AMOVA procedure was additionally applied in order to compare whole community structures taking genetic distances between phenotypes into account. At the flowering stage the culturable bacilli showed also significantly altered community structures in the rhizospheres of the cecropin-expressing and non-transgenic potato lines. Several strains were only found in association with the wildtype cultivar but their prevalence in association with transgenic and non-transgenic lines did not differ significantly. However, the most frequently detected IGS type at the flowering stage, a *B. megaterium* strain (B6), showed significantly increased abundance in the rhizospheres of the cecropin-producing lines. This strain was also more frequently associated with the cecropin-expressing potato clones at the tuber production stage. This can be explained by the high tolerance of this microbe for the antibacterial peptide in comparison with other strains tested in this study or with previously reported results (Boman and Hultmark, 1987; Boman and Boman, 1989) indicating the enrichment of cecropin tolerant strains in rhizospheres of plants expressing the bacterial peptide. Similar observations were made by Lottmann et al. (2000). In that study, T4-lysozyme expressing potatoes and the parental line were inoculated with a lysozyme tolerant *Pseudomonas putida* strain that showed in vitro antagonistic activity against *Erwinia carotovora* ssp. *atroseptica*. During flowering significantly more colony counts of the biocontrol strain were recovered from transgenic plants than from the parental line. Furthermore, we found that two *B. pumilis* strains, which tolerated lower concentrations of cecropin than other bacilli, showed decreased abundances in the rhizosphere of cecropin-expressing lines. However, differences were only partly significant. During flowering, the bacilli isolated from transgenic plants, which contain only vector sequences, showed significantly different community structures as compared with the parental line as well as with the cecropin-expressing plants. Nevertheless, the diversity of these populations was comparable with those of the wildtype plant. A *B. macroides* strain, which was not found in the rhizospheres of the non-transgenic plants and cecropin-expressing lines, was highly abundant in the rhizospheres of the transgenic vector-containing potato plants.

At the tuber production stage rhizospheres of all plants had comparable *Bacillus* community structures and diversities, indicating the transient nature of the earlier observed effects. As compared with the flowering stage, the *Bacillus* populations in the rhizospheres of cecropin-expressing potatoes showed significantly increased diversities, whereas the bacilli associated with wildtype plants showed highly similar diversity values at both sampling times.

The impact of transgenic plants on the culturable spore-forming *Bacillus* populations at the flowering stage may be at least partly explained by the toxic effects of cecropin that was released from plant cells. The release of antibacterial substances such as T4-lysozyme from transgenic potato roots has been demonstrated as well as that the secreted enzyme showed bacteriolytic activity (de Vries et al., 1999; Ahrenholtz et al., 2000). The transgenic potatoes used in those studies carry the T4-lysozyme fused to a barley α-amylase signal peptide, which allows secretion of the enzyme in the intercellular space. The cecropin-expressing potato lines analysed in this investigation either lack an N-terminal signal peptide or contain a plant-specific hordothionin N-terminal signal peptide. In both cases, cecropin is supposed to remain within the cell. However, a release of the lytic peptide from roots into the rhizosphere may be due to dying root cells, which are discharged into soil. Nevertheless, such a release has not been detected yet probably due to the rapid degradation of cecropin in soils (Berenyi M., unpublished results) and due to the lack of highly sensitive methods to quantify the lytic peptide. In addition to potentially toxic effects of the lytic peptide, unintentionally altered plant characteristics due to the interruption of particular genes or somaclonal variation may be responsible for the observed shifts in the *Bacillus* community structure. Such side-effects may lead to an altered composition of root exudates, which represent an important source of substrates available for root-associated bacteria and selectively influence the microbial community structure (Grayston et al., 1998). Significant but transient effects on microbial communities due to unintentionally altered plant characteristics by genetic engineering were also reported by Donegan et al. (1995). Furthermore, the observation of altered eubacterial community structures in the root interior and rhizosphere of transgenic, herbicide-tolerant oilseed rape was explained by unintentionally changed root exudation (Siciliano et al., 1998; Dunfield and Germida, 2001; Siciliano and Germida, 1999; Gyamfi et al., 2002).

Our results demonstrate that genetically modified plants producing an antibacterial compound may at least transiently affect the diversity and community structure of a bacterial group that is frequently found in soils and rhizospheres. Field experiments will assess the agronomic performance of the transgenic potato lines under natural conditions and investigate the role of transiently affected bacteria on plant growth and health. Further research using cultivation-dependent as well as cultivation-independent approaches will elucidate whether other microbial groups, and in particular plant growth promoting bacteria are affected.

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