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Molecular fingerprints reveal that the effects of genetically modified potato plants with increased zeaxanthin content on bacterial and fungal rhizosphere communities do not exceed natural cultivar variability

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Effects of genetically modified (GM), zeaxanthin-accumulating potato plants on microbial communities in the rhizosphere were compared to natural variability among different potato cultivars. Two GM lines, their parental cultivar as well as four other potato cultivars were grown in randomized field plots at two sites and in different years. Rhizosphere samples were taken at three developmental stages of plant growth and analyzed based on denaturing gradient gel electrophoresis (DGGE) fingerprints of *Bacteria*, *Actinobacteria, Alpha-* and *Betaproteobacteria*, *Bacillus*, *Streptomycetaceae*, *Pseudomonas, gacA*, *Fungi* and *Ascomycetes*. Of the bacterial DGGE gels analyzed, significant differences between the parental and the two GM lines were mainly detected for *Actinobacteria*, but also for *Betaproteobacteria* and *Streptomycetaceae*, yet they occurred only at one site and in one year. For *Fungi*, especially for *Ascomycetes*, significant differences occurred more frequently than for bacteria. Comparing all seven plant genotypes, DGGE analysis revealed that different cultivars had a stronger effect on both bacterial and fungal communities than the genetic modification. Effects of the genetic modification were mostly detected at the senescence stage of the plants. The site was the overriding factor affecting microbial community structure compared to the plant genotype. In general, the fingerprints of the two GM lines were more similar to the parental cultivar and observed differences did not exceed natural cultivar variability.

INTRODUCTION

Microorganisms play a key role in agriculture because they are important for plant growth and health, the turnover of organic material, and the maintenance of ecosystem services. In the rhizosphere, defined as the soil influenced by the plant roots (37), microorganisms benefit from nutrients provided by root exudates and form close relationships with the plants. The plant species but also plant genotypes have been reported to influence microbial communities in the rhizosphere (14, 16, 21, 27, 28, 36). Despite their importance for soil and plant health, the response of soil microbes to the large-scale cultivation of genetically

modified (GM) crops is still poorly understood. Gene technology offers the possibility for a more targeted modification of the plant compared to classical breeding approaches, what might limit effects on associated microbes. Therefore, it needs to be assessed whether such a single genetic modification indeed correlates with a less pronounced effect on microbial communities in the rhizosphere.

Potatoes with increased zeaxanthin levels in their tubers were designed as a functional food to counteract the disease of age-related macula degeneration, which is a major cause of the visual impairment of elderly people. It could be shown that a high dietary intake of zeaxanthin significantly reduces the risk of suffering from this disease (9, 35). Zeaxanthin is naturally produced in potato plants but further modified to violaxanthin via the enzyme zeaxanthin epoxidase. Down-regulation of the zeaxanthin epoxidase gene resulted in the accumulation of zeaxanthin in tubers of GM potato plants (34). However, it cannot be excluded that additional plant metabolism processes as well as root exudation patterns are affected by these genetic modifications.

While many studies aimed to investigate potential impacts of GM plants on their associated microbial communities (reviewed in 3, 25), the majority of studies conducted so far only compared a GM line to a non-GM line (4, 8, 19, 20). However, potential effects of GM plants on microbial communities need to be evaluated in light of natural variation among cultivars of the same plant species. Recently, a study on rhizosphere communities of fructan producing GM potatoes in comparison to isogenic controls and conventional cultivars failed to show plant genotype effects (2). However, this result was only based on the analysis of *Bacteria* and did not consider potential effects on different microbial groups.

The objective of this study was to assess effects of the growth of zeaxanthin potatoes on microbial communities in the rhizosphere and to relate putative effects to natural variation among potato cultivars. Effects were ascertained on two different sites and in several years. In comparison to previous studies, this study provides a comprehensive in depth analysis on the response of various bacterial and fungal groups to potential effects of the two GM lines. We investigated the hypothesis that effects of the genetic modification on rhizosphere

communities were less pronounced than effects of genotype differences among cultivars resulting from conventional breeding.

MATERIALS AND METHODS

Potato cultivars and GM lines. Two GM potato lines (*Solanum tuberosum* L.) with altered zeaxanthin levels, their parental cultivar 'Baltica', as well as four additional commercial potato cultivars 'Selma', 'Désirée', 'Ditta' and 'Sibu' were planted. The GM lines SR47 ('Baltica' co-suppression) and SR48 ('Baltica' antisense) accumulate the carotenoid zeaxanthin in their tubers. The tubers of GM lines SR47 and SR48 (referred to by Römer et al. (34) as SR47-18 and SR48-17, respectively) contained up to 40 µg/g dry weight (dw) and 17 µg/g dw of zeaxanthin compared to 0.2 µg/g dw of 'Baltica'.

Field design. The potato cultivars and GM lines were grown at two different field sites in Southern Germany, Roggenstein (2005 and 2007) and Oberviehhausen (2006). Both sites differed considerably in their soil characteristics (Table 1). The potato plants were grown in a randomized block consisting of six replicated plots per cultivar/GM line with 40 plants each (see Fig. S1). In addition, only the commercial cultivars 'Baltica', 'Désirée' and 'Sibu' were grown in Roggenstein 2006 because the GM lines were not permitted at this site in 2006.

Sampling and sample processing. Sampling was carried out at three developmental stages of the plants: young plants (EC30), flowering plants (EC60) and senescent plants (EC90), according to Hack et al. (15) at the site in Roggenstein in 2005, 2006 and in Oberviehhausen in 2006. Because of delayed emergence of the GM lines due to very dry weather conditions, leading to strong differences in plant developmental stages mainly at EC30, only EC60 and EC90 plant stages were sampled in Roggenstein in 2007. Five plants per plot were carefully removed from the soil. After shaking the plants, the roots with adhering soil were combined, cut into pieces and treated as a composite sample. Four

composite samples of each cultivar and GM line were further processed. For isolation of the root-associated microbes, 10 g of root material was transferred into a sterile Stomacher bag and homogenized with 30 ml Milli-Q water for 60 s in a Stomacher laboratory blender (Seward, West Sussex, UK) at high speed. This homogenization step was repeated three times and the combined suspensions were collected in two 50 ml tubes. The first tube was centrifuged for 15 min at 4°C and 10,000 × *g*, the supernatant was discarded and the tube was filled with the content of the second tube prior to another centrifugation step. The resulting pellets containing the root-associated bacteria were frozen at -80°C until further extraction of DNA.

Extraction of DNA from rhizosphere samples. For extraction of the DNA, 0.5 g of the pellets obtained from 10 g root material were used. The bacterial cells were lyzed mechanically twice in a FastPrep FP120 bead beating system (Q-Biogene, Carlsbad, CA, USA) for 30 s at high speed. Thereafter, the DNA was extracted using the BIO-101 DNA spin kit for soil (Q-Biogene, Carlsbad, CA, USA) according to the manufacturer. The extracted DNA was further purified with the Geneclean Spin Kit (Q-Biogene, Carlsbad, CA, USA) as described by the manufacturer, except that the DNA was eluted in the same amount that was used in the beginning for purification. Yields of genomic DNA were checked on a 0.8% agarose gel and photographed under UV after ethidium bromide staining. The DNA yield was estimated using the 1Kb plus DNA ladder (Invitrogen, Karlsruhe, Germany) and diluted 1:5 with the elution buffer from the purification kit.

Development of a *Streptomycetaceae*-specific primer system. Primers specific for the *Streptomycetaceae* family were designed using the PROBE DESIGN and MATCH PROBE subroutines in the ARB software (http://www.arb-home.de). The Probe Match function of the Ribosomal Database Project II (http://rdp.cme.msu.edu/) was used for *in silico* analysis of the primer specificity based on the last ten 3`-end nucleotides. The primer pair

was highly specific for the *Streptomycetaceae* family, showing perfect matches with only five non *Streptomycetaceae* sequences related to the genera *Microbacterium* and *Spirillospora*.

Amplification of bacterial 16S rRNA and *Pseudomonas gacA* gene fragments. Amplification of the bacterial 16S rRNA gene from TC DNA for DGGE analysis was carried out using the primer pair F984GC/R1378 as described by Heuer et al. (17). For the amplification of group-specific 16S rRNA and *gacA* gene fragments a nested-PCR approach was applied. This consisted of a first specific amplification using group-specific primers (Table 2), followed by the amplification of the DGGE fragment. Amplification of the *Streptomycetaceae*-family was conducted with a reaction mixture consisting of 1 µl of template DNA (1-5 ng), 1 x Stoffel buffer II (Applied Biosystems), 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 5% (v/v) DMSO, 2.5 µg BSA, 0.1 µM of primers F126 and R1423 (Table 2) and 1.25 U AmpliTaq Gold (Applied Biosystems). An initial denaturation at 95°C for 10 min was followed by 30 cycles at 95°C for 1 min, 62°C for 1 min and 72°C for 1 min, and a final extension step at 72°C for 10 min. PCR-products were diluted 1:10 and used as templates for the F984GC/R1378 PCR with 20 cycles.

Amplification of fungal ITS-fragments. Amplification of the fungal ITS-fragment prior to DGGE analysis was performed with a nested-PCR approach using the primer pairs ITS1F/ITS4 and ITS1FGC/ITS2 (1) (Table 2). The reaction mixture for the first PCR (25 µl) was composed of 1 µl of template DNA (1-5 ng), 1 x Stoffel buffer II (Applied Biosystems), 0.2 mM dNTPs, 3.75 mM $MgCl_2$, 5% (v/v) DMSO, 0.1 µM of primers and 2.5 U AmpliTaq Gold (Applied Biosystems). Initial denaturation at 95°C for 5 min was followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min and a final extension step at 72°C for 10 min. Samples served as template for the second PCR. The reaction mixture was the same as described for the first PCR, except that 4% (v/v) DMSO and 0.2 µM of primers were used. PCR conditions were the same as described for the first PCR except for the number of cycles that were reduced to 25. *Ascomycetes*-specific amplification used the primer pairs

ITS1F/ITS4A (24) and ITS1FGC/ITS2 (Table 2). The reaction mixture (25 µl) contained 1 µl of template DNA (1-5 ng), 1 x Stoffel buffer II (Applied Biosystems), 0.2 mM dNTPs, 3.75 mM $MgCl_2$, 5% (v/v) DMSO, 0.1 µM of primers and 2.5 U AmpliTaq Gold (Applied Biosystems). Thermal cycling started with an initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 53°C for 35 s, and 72°C for 2 min. Final extension consisted of 72°C for 10 min. The samples were diluted 1:20 and served as template for the second PCR as described above.

Denaturing gradient gel electrophoresis (DGGE) of bacterial and fungal gene fragments. DGGE analysis was performed in a $PhorU_2$ apparatus (Ingeny, Goes, The Netherlands) with a double gradient for both community and group-specific 16S rRNA gene fragment separation. The gradient was composed of 46.5–65% denaturants (100% denaturants defined as 7 M urea and 40% formamide) and 6.2–9% acrylamide (12). Besides, a stacking gel with 15% acrylamide was pipetted on top. Approximately 3 µl aliquots of PCR-products were loaded side by side on the gel and run in 1 x Tris-acetate-EDTA buffer at a constant voltage of 140 V for 17 h at 58°C, and were silver-stained according to Heuer et al. (18). After the run the gels were air-dried and scanned transmissively (Epson 1680 Pro, Seiko-Epson, Japan). A marker composed of GC-clamped fragments (984-1378) of 11 bacterial strains with different electrophoretic mobilities (17) was loaded twice on each gel. For the analysis of fungal gene fragments the gradient consisted of 23–58% denaturants and 8% acrylamide. Gels were run in 1 x Tris-acetate-EDTA buffer at a constant voltage of 100 V for 18 h at 60°C. Subsequent processing of the gels was performed as described above for bacterial gene fragments.

Analysis of DGGE fingerprints and statistics. Analysis of DGGE fingerprints was performed using the GelCompar II program version 4.5 (Applied Maths, Ghent, Belgium) as described by Rademaker et al. (33) to convert and normalize the gel image. Modifications of settings were performed according to Smalla et al. (36). The pair-wise similarities of lanes

were calculated for each gel by Pearson correlation. The resulting similarity matrices were used for cluster analysis by the unweighted-pair group method using average linkages (UPGMA), and to test for significant treatment effects (23). The similarities of microbial fingerprints of 'Baltica' to those of each other potato cultivar were analyzed by Box-Whisker plots, using a SAS-macro written by Michael Friendly (http://euclid.psych.yorku.ca/ftp/sas/sssg/macros/ boxplot.sas) which plots the median, quartiles, 95% confidence limits, and Whisker lines that extend to the most extreme similarity values being no more than 1.5 times the interquartile range beyond the quartiles.

RESULTS

Effect of the genetic modification. PCR-DGGE fingerprints of bacterial rhizosphere communities of the parental 'Baltica' and the two GM lines were compared for both bacterial (*Bacteria*, *Actinobacteria*, *Alpha-* and *Betaproteobacteria*, *Streptomycetaceae, Bacillus, Pseudomonas*, *gac*A gene) as well as fungal (*Fungi* and *Ascomycetes*) fingerprints (Table 3). For bacteria, the differences between 'Baltica' and the GM lines ranged from non-detectable to maximally 4.5%. Significant differences could only be detected for Roggenstein 2005 samples. *Actinobacteria* fingerprints displayed significant differences at all three growth stages, but most pronounced at EC90, and *Betaproteobacteria* and *Streptomycetaceae* differed only at EC90. All other bacterial or group-specific fingerprints could not be distinguished between the GM lines and 'Baltica'. Similarly, the comparison of 'Baltica' and GM line fingerprints from Oberviehhausen (2006) and Roggenstein (2007) samples did not reveal significant differences for any of the bacterial groups analyzed at any plant growth stage. For fungi, the differences between the fingerprints of 'Baltica' and the GM lines were in general more pronounced than for bacteria, with a maximal deviation of 6.6%. In particular, effects of the plant genetic modification became apparent in the *Ascomycetes* fingerprints. All but two samplings revealed significant differences. The *Fungi* fingerprints showed a significant effect of the genetic modification at three of eight samplings.

Effect of plant genotype. The effect of the plant genotype on rhizosphere microbial communities was analyzed by comparing DGGE fingerprints of the five cultivars and the two GM lines (a total of seven plant genotypes). For bacteria, genotype-specific effects were strongly dependent on the site and the year of sampling (Table 3). Most pronounced differences among plant genotypes were found for Roggenstein 2005 samples. Significant differences were observed for *Bacteria* and *Actinobacteria* at all plant growth stages. In addition, effects on *Pseudomonas*, *Alphaproteobacteria* and *Bacillus* were revealed at several growth stages. The effect of the plant genotype became especially apparent at plant growth stage EC90 when significant differences were found for all bacterial groups except for *Streptomycetaceae*. Fingerprints for total *Bacteria* differed as much as 13% between cultivars and about 10% for the Gram-positive groups *Actinobacteria* and *Bacillus*.

The fingerprints of samples from Oberviehhausen 2006 and Roggenstein 2007 revealed much less evidence for plant genotype-specific effects. Nevertheless, significant differences were detected for *Bacteria*, *Pseudomonas*, *Actinobacteria* and *Betaproteobacteria* mainly at EC90. The maximal deviation between cultivars of 5.7% was observed for *Pseudomonas*. When the *Fungi* and *Ascomycetes* fingerprints of all potato genotypes were compared, significant differences were found for both sites at all plant growth stages. Differences among the cultivars were as high as 14% at EC30. In general, the comparison of all seven plant genotypes revealed higher differences than the comparison of 'Baltica' with the two GM lines for both bacteria and fungi.

Similarity of GM lines and cultivars to 'Baltica'. To test the hypothesis that the effect of the genetic modification of the GM lines on rhizosphere communities was less pronounced than the effect of genotype differences among cultivars, the similarity of DGGE fingerprints of all plant genotypes to 'Baltica' were compared. Analysis of median similarities of all bacterial fingerprints of both Roggenstein (2005 and 2007) and Oberviehhausen (2006) revealed that the GM lines were on average more similar to the parental 'Baltica' than all other cultivars (Fig. 1A). This suggests that the genetic modification of the GM lines had a

less pronounced effect on the associated bacterial communities than genetic differences among the potato cultivars. The GM line SR47 was slightly more similar to 'Baltica' than SR48, especially in Oberviehhausen 2006. For the Roggenstein site the confidence intervals of the cultivars 'Désirée' and 'Sibu' did not overlap with the confidence interval of the two GM lines. This indicated a significantly higher difference of these two cultivars to 'Baltica' compared to the differences between the two GM lines and 'Baltica'. In contrast, for Oberviehhausen samples, the confidence intervals of all cultivars and the two GM lines did overlap. When each bacterial group was analyzed separately, the general trend of a higher similarity of the GM lines to 'Baltica' was observed for all groups, except for *Streptomycetaceae* (data not shown). In particular *Actinobacteria* and *Betaproteobacteria* contributed to this trend. The analysis for the different plant growth stages revealed more pronounced cultivar-specific differences at EC90 than at EC30 and EC60 (data not shown). At EC90 the commercial cultivars 'Selma', 'Désirée' and 'Ditta' were significantly more different to 'Baltica' than both GM lines.

For fungi, it was even more clearly observed that the genetic modification had a less pronounced effect than genetic differences among the cultivars. Analysis of all fungal DGGE fingerprints of Roggenstein and Oberviehhausen revealed that median similarities to 'Baltica' were higher for the two GM lines than for all other cultivars (Fig. 1B). In Oberviehhausen all four cultivars were significantly less similar to 'Baltica' than the GM lines. In Roggenstein the same trend was found, with significant differences for the cultivars 'Désirée' and 'Ditta'. Analysis for each plant developmental stage showed that at both EC30 and EC90 all commercial cultivars were significantly more different to 'Baltica' than the two GM lines, while at EC60 the confidence intervals of all cultivars and GM lines overlapped (data not shown).

Effect of environmental factors on bacterial and fungal community fingerprints. Environmental factors differing between field sites and years had a much stronger impact on microbial rhizosphere communities than plant genotypes. For example, the DGGE fingerprints of *Fungi, Pseudomonas*, *Streptomycetaceae* and *Bacillus* of Roggenstein and

Oberviehhausen in 2006 at plant growth stage EC60 differed by 23%, 40%, 39%, and 15%, respectively. These differences were statistically highly significant (P<0.0001). Also comparisons of fingerprints of *Streptomycetaceae* and *Bacillus* from Roggenstein 2005 and Oberviehhausen 2006 at EC60 showed similar effects (supplemental Fig. S2b and c). These fingerprints differed by 31% and 34%, respectively. However, *Pseudomonas*-specific fingerprints for this comparison differed only by 6% (supplemental Fig. S2a).

DISCUSSION

Effect of the genetic modification. The present study provided evidence for an effect of GM potato plants with increased zeaxanthin contents on both bacterial and fungal rhizosphere communities (Table 3). Effects were especially apparent for *Ascomycetes* at both sites and in all years. *Actinobacteria* were affected at all three samplings in Roggenstein in 2005. Similarly, GM potatoes with an altered starch composition were shown to affect rhizosphere communities at the site in Oberviehhausen (29). In that study responses to the genetic modification were found only for *Pseudomonas* but not for *Fungi*. Effects on *Fungi* might have not been detected due to the lower resolution of fungal 18S rRNA gene profiles applied in that study compared to ITS profiles applied in the present study. In accordance to our study, effects on *Actinobacteria* were not observed at the Oberviehhausen site. The application of multiple analyses targeting different microbial groups on different field sites and in different years allowed for the detection of small differences between GM plants and their parental cultivar while other studies failed to detect such minor effects (19, 26, 32). Changes of soil microbial community composition might occur directly through transgene products or indirectly, e. g. via altered composition of root exudates (reviewed in 22). Due to the use of a tuber-specific promoter a direct effect of zeaxanthin was likely to occur when the tubers are fully developed. Indeed, the impact of GM potatoes on both bacterial and fungal communities became most apparent at growth stage EC90 (Table 3).

Effect of the genetic modification in relation to the effect of the plant genotype.

In this study rhizosphere microbial communities were more dissimilar among the investigated cultivars than between GM lines and the parental cultivar. This confirmed the hypothesis that effects of the genetic modification on rhizosphere microbial communities were less pronounced than effects of genotype differences among cultivars resulting from conventional breeding. In another study a large variation in the tuber metabolome among cultivars was observed, while metabolomes of GM lines and the parental cultivar were substantially identical (5).

The study by Becker et al. (2) compared the effect of fructan producing GM potatoes with isogenic controls and conventional cultivars grown at one field site in three consecutive years by terminal restriction fragment length polymorphism of bacterial 16S rRNA genes. They could neither detect GM effects nor cultivar effects on rhizosphere bacterial communities, probably due to insufficient resolution of bacterial fingerprints. In accordance with our results, several studies revealed cultivar effects on rhizosphere communities using taxa-specific DGGE fingerprinting (29, 31; 32).

More drastic effects on rhizosphere microbial communities resulting from genetic modification of an already existing pathway like zeaxanthin transformation are expected from transgene expression of antimicrobial compounds. For two of three GM potato lines expressing the antimicrobial peptide magainin II significant differences in bacterial rhizosphere communities were found in comparison to the parental line than among cultivars at the first sampling time (31). However, at senescence of the plants, all plant lines showed similar communities. This is in contrast to the present study where differences for *Bacteria* became most pronounced for the comparison of all seven plant genotypes at EC90. Similarly, Heuer et al. (19) and Lottmann et al. (26) detected differentiating ribotypes between the parental and the transgenic line only at EC90.

In the present study, the effect of the plant genotype on *Fungi*, especially on *Ascomycetes* was more pronounced (Table 3), and the higher similarity of the two GM lines to ‘Baltica’ was more obvious than for bacteria (Fig. 1B). Only few studies evaluated the effects of GM plants on fungal rhizosphere communities (13, 29). The study by Götz et al. (13) reported on

differences between T4-lysozyme producing potato plants and the parental cultivar based on differentiating bands. However, that study focused exclusively on endophytic fungi. In contrast to the present study DGGE fingerprinting based on 18S fragments could neither detect effects of potatoes with modified starch content nor cultivar effects on fungal communities in the rhizosphere (29). In the present study significant differences of the fungal community fingerprints among the plant genotypes were detected at all stages of plant development. This is even more surprising considering the findings by Costa et al. (6) reporting that the effect of the plant species (strawberry, oilseed rape) was less pronounced for fungi than for bacteria. The contrasting findings might either result from the different plant species analyzed, or from the lower resolution of the 18S rRNA based fingerprints.

In conclusion, effects through the growth of zeaxanthin potatoes on rhizosphere microbial communities can be detected by highly sensitive fingerprinting techniques and multiple analyses targeting different microbial groups and extensive sampling. Evaluating these effects in the context of natural cultivar variability showed that changes in the composition of bacterial and fungal communities associated with these GM plants did not exceed differences among cultivars. Moreover, the GM effects were negligible relative to environmental factors such as field site or year which by far exceeded effects of genetic modification or plant genotype (2, 19, 29, 32).

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TABLE 1: Characteristics of soils used in this study

	clay (%)	sand (%)	silt (%)	C_{org}	N_t	pH
Roggenstein	28.1	26.1	44.0	1.1	0.1	6.6
Oberviehhausen	14.1	54.6	31.3	1.9	0.2	6.5

TABLE 2: Primers used in this study targeting bacteria and fungi

Primer pairs	**Sequence 5'-3'**	**taxonomic group**	**annealing temp.**	**Reference**
F984GC/R1378	F984 AACGCGAAGAACCTTAC	*Bacteria*	53°C	17
	GC-clamp CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGG			30
	R1378 CGGTGTGTACAAGGCCCGGGAACG			
F243/R1378	F243 GGATGAGCCCGCGGCCTA	*Actinobacteria*	63°C	17
F203α/R1492	F203α CCGCATACGCCCTACGGGGGAAAGATTTAT	*Alphaproteobacteria*	56°C	11
	R1492 TACGG(C/T)TACCTTGTTACGACTT			
F948β/R1492	F948β CGCACAAGCGGTGGATGA	*Betaproteobacteria*	64°C	11
F311Ps/R1459Ps	F311Ps CTGGTCTGAGAGGATGATCAGT	*Pseudomonas*	63°C	29
	R1459Ps AATCACTCCGTGGTAACCGT			
BacF/R1378	BacF GGGAAACCGGGGCTAATACCGGAT	*Bacillus*	65°C	10
F126/R1423	F126 GCCCTGCACTCTGGGACAAGC	*Streptomycetaceae*	62°C	This study
	R1423 GTTAGGCCACCGGCTTCG			
gacA-1F/gacA2	gacA-1F TGATTAGGGTGYTAGTDGTCGA	*Pseudomonas gac*A gene	57°C	7
	gacA2 MGYCARYTCVACRTCRCTGSTGAT			38
gacA-1FGC/gacA2R	gacA-1FGC GC-clamp + GATTAGGGTGCTAGTGGTCGA	*Pseudomonas gac*A gene	52°C	7
	gacA2R GGTTTTCGGTGACAGGCA			
ITS1F/ITS4	ITS1F CTTGGTCATTTAGAGGAAGTAA	*Fungi*	55°C	1
	ITS4 TCCTCCGCTTATTGATATGC			
ITS1FGC/ITS2	ITS2 GCTGCGTTCTTCATCGATGC	*Fungi*	55°C	1
ITS1F/ITS4A	ITS4A CGCCGTTACTGGGGCAATCCCTG	*Ascomycetes*	53°C	24

TABLE 3: Percent difference between microbial fingerprints of 'Baltica' and GM lines, and among all potato genotypes.

		Roggenstein 2005		Oberviehhausen 2006		Roggenstein 2007	
	plant growth	Comparison of		Comparison of		Comparison of	
DGGE gel	stage	'Baltica'-GM lines	all genotypes	'Baltica'-GM lines	all genotypes	'Baltica'-GM lines	all genotypes
Bacteria	EC30	0.4	**4.4****	1.8	1.4	-	-
	EC60	1.7	**4.0****	0.0	1.2	0.0	0.8
	EC90	0.6	**13.1****	0.5	**4.5****	0.0	0.9
Pseudomonas	EC30	0.0	**3.1****	0.0	0.2	-	-
	EC60	0.1	0.3	0.0	0.1	0.0	0.0
	EC90	0.2	**2.4****	0.0	**5.7****	0.0	**1.4****
gacA	EC30	0.0	0.0	0.0	1.0	-	-
	EC60	0.2	0.0	0.1	0.3	0.0	**3.8****
	EC90	0.0	**5.1****	0.0	**3.3***	0.0	0.0
Actinobacteria	EC30	**2.6****	**6.4****	0.0	0.6	-	-
	EC60	**3.0****	**3.7****	0.0	0.0	0.0	**1.4***
	EC90	**4.5****	**9.7****	0.9	0.0	0.0	0.0
Streptomycetaceae	EC30	0.0	0.0	0.0	0.0	-	-
	EC60	0.5	0.1	0.0	1.2	0.0	0.0
	EC90	**2.8***	1.4	0.0	0.3	0.1	0.0
Alphaproteobacteria	EC30	0.0	**1.1***	1.8	0.0	-	-
	EC60	0.0	0.7	1.7	0.0	0.0	0.0
	EC90	0.0	**1.4***	0.0	0.2	0.0	0.0
Betaproteobacteria	EC30	0.0	1.1	1.1	**5.1****	-	-
	EC60	4.6	1.7	0.0	0.0	0.0	0.0
	EC90	**2.8***	**3.1****	0.0	**2.7****	0.0	0.0
Bacillus	EC30	3.2	0.2	0.0	0.0	-	-
	EC60	0.0	**1.8***	0.0	0.0	0.0	0.4
	EC90	0.0	**9.8****	0.0	0.0	0.0	0.0
Fungi	EC30	**5.4***	**14.3****	0.8	**3.0****	-	-
	EC60	1.2	**3.1****	**2.3****	**3.9****	1.5	**3.0****
	EC90	**1.9***	**6.9****	1.7	**4.2****	1.3	**5.8****
Ascomycetes	EC30	**4.8****	**13.6****	0.0	**3.5****	-	**-**
	EC60	3.2	**6.2****	**1.4****	**2.6****	**2.2****	**5.1****

	EC90	**6.6****	**9.0****	**1.8***	**3.9****	**3.5****	**7.9****

* Significant difference ($P \leq 0.05$), **highly significant difference ($P \leq 0.01$);

[a]EC30: young plants, EC60: flowering plants, EC90: senescent plants

Figure legends

FIG. 1. BOX-Whisker plots showing the similarity of microbial fingerprints of the two GM lines and the commercial cultivars to the parental 'Baltica' based on the global comparison of bacterial (*Bacteria*, *Actinobacteria, Alpha-* and *Betaproteobacteria*, *Bacillus*, *Streptomycetaceae*, *Pseudomonas, gacA*) (A) and fungal (*Fungi* and *Ascomycetes*) (B) fingerprints for the Roggenstein site (2005 and 2007) and the Oberviehhausen site (2006). The SAS-macro plots the median, quartiles, 95% confidence intervals (grey bars) and Whisker lines extending to the most extreme similarity values. The dashed lines comprise the confidence interval of both GM lines.

FIG. S1. Randomized field trial at the sampling sites in Roggenstein and Oberviehhausen.

FIG. S2. DGGE fingerprints of the bacterial taxa *Pseudomonas* (a), *Streptomycetaceae* (b) and *Bacillus* (c) at plant growth stage EC60 generated with four independent repetitions of the commercial cultivars 'Baltica' and 'Sibu' and the two GM lines SR47 and SR48 grown at the field trials in Roggenstein (R) in 2005 and Oberviehhausen (O) in 2006.
S: Bacterial standard comprised of 11 bacterial strains with different electrophoretic mobilities; AS: Antagonist standard composed of the most frequently identified *in vitro* antagonists towards the potato pathogens *Rhizoctonia solani* Kühn AG-3, *Verticillium dahliae* ELV25 and *Phytophthora infestans* [Mont.] De Bary, which have previously been isolated during this study and assigned by 16S rRNA gene sequencing to the genera *Pseudomonas*, *Streptomyces* and *Bacillus*. These standards were run in parallel to the respective group-specific fingerprints to assess the effect of plant genotype and site on putative antagonists in the community profiles.