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REGULAR ARTICLE

Effects of genetically modified amylopectin-accumulating potato plants on the abundance of beneficial and pathogenic microorganisms in the rhizosphere

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Abstract In this study, the potential effects of a genetically modified (GM) amylopectin-accumulating potato line (*Solanum tuberosum* L.) on plant beneficial bacteria and fungi as well as on phytopathogens in the rhizosphere were investigated in a greenhouse experiment and a field trial. For comparison, the non-transgenic parental cultivar of the GM line and a second non-transgenic cultivar were included in the study. Rhizospheres were sampled during young leaf development (EC30) and at florescence (EC60). The microbial community composition was analysed by real-time PCR to quantify the abundances of *Pseudomonas* spp., *Clavibacter michiganensis*, *Trichoderma* spp. and *Phytophthora infestans*. Additionally, total bacterial and fungal abundances were measured. None of the examined gene abundance patterns were affected by the genetic modification when wild type and GM line were compared. However, significant differences were observed between the two natural potato cultivars, especially during the early leaf development of the plants. Furthermore, gene abundance patterns were also influenced by the plant developmental stage. Interestingly, the impact of the cultivar and the plant vegetation stage on the microbial community structure was more pronounced in field than in greenhouse. Overall, field-grown plants showed a higher abundance of microorganisms in the rhizosphere than plants grown under greenhouse conditions.

Keywords Genetically modified potato plants · Real-time PCR · Gene abundance · Plant beneficial microorganisms · Phytopathogens · Rhizosphere

Introduction

Interactions between plants and microorganisms in the rhizosphere are of central importance for plant performance (Soerensen 1997). Rhizosphere microorganisms strongly influence plant growth and health via nutrient mobilization, production of plant growth hormones, induction of systemic resistance in the host plant and play a role as biological control agents (Raaijmakers et al. 2009). In turn, they benefit from nutrients provided

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by the root exudates and consequently, their abundance and community composition is highly affected by the plant species, different plant genotypes and plant vegetation stage (Grayston et al. 1998; Gyamfi et al. 2002; Kowalchuk et al. 2002; Marschner et al. 2006; Smalla et al. 2001). Contrary to plant beneficial microorganisms, soil-borne pathogens can severely impact plant growth and health and cause great economic losses (Raaijmakers et al. 2009). Despite the importance of plant–microbe interactions in the rhizosphere for crop quality and yield, there is still a lack of knowledge which factors drive abundance and activity pattern of selected functional groups of microorganisms in this compartment.

The discussion how genetically modified (GM) plants influence microbial rhizosphere communities in comparison to the wild type and other non-transgenic cultivars of the same species is still continuing and considerably affects the acceptance of these plants for commercial field release. Among other concerns, this is related to previous studies revealing shifts in diversity patterns of the rhizosphere microflora associated with GM plants, although the modification-dependent effects were described as minor compared to shifts caused by the soil type, plant genotype and plant developmental stage (Gyamfi et al. 2002; Heuer et al. 2002; Liu et al. 2005; Lottmann et al. 1999; Milling et al. 2004; Rasche et al. 2006; Weinert et al. 2009).

However, mainly in the field of renewable resources, GM plants may carry a huge potential for sustainable development, considering not only economical but also ecological aspects. For industrial processing of selected GM crops, e.g. less fossil energy is necessary compared to conventional cultivars. The most discussed GM constructs in this area are potatoes with modified starch composition. Starch consists of the two polysaccharides, amylose and amylopectin. Because of their different physico-chemical properties, amylose and amylopectin have to be separated for industrial use (Kraak 1993). To avoid the high production and energy costs of this separation process (Visser et al. 1991; Young 1984), the production of a potato tuber (*Solanum tuberosum* L.) containing starch composed purely from amylopectin is an important aim in plant breeding, mainly for the paper and textile industries.

Since a modified starch metabolism may also result in altered root exudates and thus influence microbial rhizosphere community (Milling et al. 2004), the aim of this study was to investigate the effect of a GM amylopectin-accumulating starch potato line on representative beneficial and pathogenic microorganisms in the rhizosphere. *Pseudomonas* spp. and *Trichoderma* spp. were selected as bacterial and fungal plant beneficial model microorganisms, respectively. They were chosen because both genera are ubiquitous in soil, are known to be excellent root colonisers (Cordier and Alabouvette 2009; Lugtenberg et al. 2001) and are most widely studied biocontrol agents (Alabouvette et al. 2009). Moreover, they can inhibit soil-borne pathogens by a variety of mechanisms, such as competition for resources, the synthesis of antibiotics and biosurfactants, the production of cell wall-degrading enzymes and the induction of systemic resistance in the host plants (De Meyer et al. 1998; Ghisalberti and Rowland 1993; Haran et al. 1996; Walsh et al. 2001; Winding et al. 2004). Furthermore, *Pseudomonas* spp. and *Trichoderma* spp. are commercially available bioinoculants and are used in agriculture to substitute fungicide application (Berg 2009).

The most important potato pathogen in temperate zones is the oomycete *Phytophthora infestans*, the causal agent of late blight disease (Tooley et al. 1997). Nevertheless, among all potato diseases, the bacterial ring rot of potatoes, caused by the actinomycete *Clavibacter michiganensis* subsp. *sepedonicus*, is probably the most regulated potato disease worldwide with a strict zero-tolerance quarantine of the pathogen in Canada, the USA and Europe (Bach et al. 2003; De Boer and Slack 1984). Due to the high economic losses caused by these two microorganisms, they were selected as representatives for potato pathogens.

To measure the abundance patterns of the plant beneficial and pathogenic indicator organisms mentioned above, real-time PCR assays were performed. The obtained data were linked to total bacterial and fungal abundance quantified via 16S rRNA gene respectively ITS rRNA gene copy numbers. The potential impact of genetic modification on microbial gene abundance was related to natural variation among potato cultivars and the effect of the plant vegetation stage. Furthermore, data from greenhouse and field studies were compared to visualise the importance of field experiments for genetically modified plants (Griffiths et al. 2000a; Kowalchuk et al. 2003; Liu et al. 2005). We postulated that the effects of the genetic modification were less pronounced than those of genotype differences among cultivars resulting from conventional breeding or of plant vegetation stage.

Materials and methods

Potato cultivars and the GM line

The GM potato line #1332 with increased amylopectin levels in its tubers, its parental cultivar 'Walli' and one additional potato cultivar used for industrial starch production, 'Jumbo', were used in this study. Both natural cultivars were provided by the Bavarian State Research Center for Agriculture (LfL), where also the GM line was developed. The GM line was genetically modified by the insertion of a gene fragment of the granule-bound starch synthase gene (*gbss*), which encodes one of the key enzymes for amylose formation, in antisense direction. Consequently, transcription leads to the formation of *gbss* dsRNA and thus inhibition of *gbss* expression and amylose production (Kuipers et al. 1994; Visser et al. 1991). Hence, the tuber starch of the GM line consists of more than 99% amylopectin, while the starch granules of 'Walli' and 'Jumbo' contain only 75–80% amylopectin (Reichmann, personal communication). A new method without using antibiotic- or herbicide-resistance marker genes for selection was used for transformation (De Vetten et al. 2003), resulting in marker-free GM line #1332.

Field experiment

The experimental field site was located in Freising, approximately 30 km north of Munich, Germany (48°24′N/11°43′E) and had been cultivated under conventional farming management with crop rotation (oilseed rape—winter barley—maize) before the experiment started in April 2007. The soil was characterised as follows: 24% clay, 61% silt, 15% sand, pH 6.6 (measured in 0.01 M $CaCl_2$), 0.6% total nitrogen content, 5.1% total carbon content and a maximum water-holding capacity of 38%. At the beginning of April, one week before potatoes were planted, NPK fertiliser (850 kg ha^{-1}, which was equivalent to 100 kg N ha^{-1}) was applied. The experiment was conducted in a randomised block design consisting of five replicate plots (1.5×3 m) per block with 20 plants per plot. During the vegetation period, the herbicide Artist® (2.5 kg ha^{-1}) and the fungicide 'Epok' (0.5 l ha^{-1} combined with 'Dantop', 35 g ha^{-1}) were applied once in April and May 2007, respectively.

Rhizosphere samples were collected at early leaf development (EC30; May) and the flowering stage (EC60; July) by mixing the roots of four plants per plot to form one composite sample. Individual plots were sampled separately and used as true replicates in this study. The roots were shaken vigorously to seperate loosely adhering soil. The soil layer still attaching to the roots can be defined as rhizosphere soil (Yanai et al. 2003). To standardize the amount of root relative to soil mass, the rhizospheric soil layer was reduced to 1 mm by careful brushing. Afterwards, the rhizosphere soil/root samples were immediately frozen on dry ice and stored at −80°C for nucleic acid extraction.

Greenhouse experiment

The greenhouse experiment started in April 2007 and was performed with soil collected from the field site five weeks before the potatoes were planted. Soil was sieved through a 4 mm mesh, filled into plastic pots (20 cm×20 cm×20 cm) and adjusted to 60% of the maximum water-holding capacity one week before planting the potato tubers. As was done for the field site, the same amount of NPK fertiliser (850 kg ha^{-1}) was applied one week before planting. One plant was cultivated per pot. Overall, 24 pots of each cultivar were used. During potato growth, irrigation was performed by hand to keep the water content of the soil constant. No herbicides or pesticides were applied. Rhizosphere samples were collected at early leaf development (EC30) and the flowering stage (EC60) in four independent replicates by mixing the roots of three plants to form one composite sample. Afterwards the samples were treated as described above.

Nucleic acid extraction

DNA was extracted from 0.5 g of rhizosphere soil/root complex according to the protocol described by Griffiths et al. (2000b). The extraction was performed using Precellys-Keramik kit lysing tubes

(PeqLab Biotechnology GmbH, Germany) in combination with the Precellys 24 bead-beating system (Bertin Technologies, France). The DNA yield and purity were checked with a spectrophotometer (Nanodrop, PeqLab, Germany). The extracts were stored at −80°C until use.

Real-time PCR assay

Quantitative real-time PCR was performed using an ABI 7300 Cycler (Applied Biosystems, Germany) with the following assay reagents: dimethyl sulfoxide (DMSO) and betaine (Sigma, Germany), primers (Metabion, Germany) and 2× Power SYBR Green PCR master mix (Applied Biosystems, Germany). The respective reaction mixtures (25 µl volume) for quantification of the genes given in Table 1 were composed as follows: 12.5 µl master mix, 10 pmol of each primer and 2 µl DNA template. For the amplification of *Pseudomonas* specific 16S rRNA, 2.5 µl betaine and 0.5 µl DMSO were added. Because of the small size of the amplicon (see Table 1), the quantification of *P. infestans* was conducted with a target-specific probe (Metabion, Germany) combined with the TaqMan Universal PCR master mix (Applied Biosystems, Germany) instead of SYBR Green. Therefore, the PCR reaction mixture contained 12.5 µl master mix, 8 pmol of each primer, 5 pmol probe and 2 µl DNA template. For the quantification, serial dilutions of plasmid DNA containing the PCR products of the respective genes listed in Table 1 were used to calculate standard curves. Data showing the quality of the standard curves are presented in Table 2. The PCR detection limit was assessed to 10 gene copies according to manufacturer's instruction. To avoid the inhibition of PCR caused by co-extracted humic substances, the optimal uniform dilution for each amplification assay was determined by a pre-experimental dilution series of randomly chosen DNA extracts (data not shown). Amplifications were performed in 96-well plates (Applied Biosystems, Germany) for all target genes as described in Table 1, conducting in triplicates for all standards, non-template controls and samples. To confirm the specificity of the SYBR Green-quantified amplicons, a melting curve analysis and a 1.5% agarose gel were performed after each PCR run. The amplification efficiency was calculated from the formula $\mathrm{Eff} = \left[10^{(-1/\mathrm{slope})} - 1\right]$ and resulted in the following average efficiencies (standard deviation less than 2% of mean): bacterial 16S rRNA, 86.7%, *Pseudomonas* specific 16S rRNA, 92.3%, intergenic spacer (IGS) rRNA of *C. michiganensis* ssp., 83.6%, fungal internal transcribed spacer (ITS) rRNA, 85.4%, *Trichoderma* specific ITS rRNA, 85.5%, and nuclear satellite DNA of *P. infestans*, 95.6%.

Table 1 Primer sets and thermal profiles used for the absolute quantification of the respective genes

Target gene	Primer set	Amplicon size	Reference	Thermal cycling profile	No. of cycles
Bacterial 16S rRNA	FP 16S	264 bp	Bach et al. (2002)	95°C–10 min	1
	RP 16S			95°C–20 s/62°C–60 s	40
16S rRNA of *Pseudomonas* spp.	9-27F	441 bp	Johnsen et al. (1999)	94°C–6 min	1
	PSM$_G$			92°C–30 s/52.5°C–30 s/68°C–60 s	40
IGS rRNA[a] of *C. michiganensis* ssp.	FP Cm	222 bp	Bach et al. (2003)	95°C–10 min	1
	RP Cm			95°C–30 s/64°C–30 s/72°C–30 s	40
Fungal ITS rRNA[b]	ITS1	545 bp	White et al. (1990)	95°C–10 min	1
	ITS4			94°C–30 s/50°C–30 s/72°C–60 s	40
ITS rRNA[b] of *Trichoderma* spp.	uTf	525 bp	Hagn et al. (2007)	95°C–5 min	1
	uTr			95°C–30 s/55.5°C–30 s/72°C–60 s	40
Nuclear satellite DNA of *P. infestans*	P3f	73 bp	Boehm et al. (1999)	50°C–2 min/ 95°C–10 min	1
	P4r FAM-labelled probe F2			95°C–30 s/56°C–30 s/72°C–30 s	45

[a] IGS rRNA: intergenic spacer rRNA

[b] ITS rRNA: internal transcribed spacer rRNA

Table 2 Quality of standard curves (n=4 ± standard deviations)

Target gene	Slope[a]	Intercept[c]	$R^{2,\ d}$	Copy no.[e]
Bacterial 16S rRNA	−3.69±0.07	39.20±0.76	0.997±0.002	10^3–10^8
16S rRNA of *Pseudomonas* spp.	−3.52±0.05	41.55±0.63	0.998±0.002	10^2–10^7
IGS rRNA of *C. michiganensis* ssp.	−3.79±0.04	40.01±0.26	0.998±0.002	10^1–10^6
Fungal ITS rRNA	−3.73±0.06	38.35±1.39	0.997±0.004	10^2–10^8
ITS rRNA of *Trichoderma* spp.	−3.73±0.06	42.56±1.10	0.996±0.004	10^1–10^6
Nuclear satellite DNA of *P. infestans*	−3.43±0.03	38.92±0.23	0.997±0.003	10^1–10^6

[a] Slope obtained for the standard curve

[b] Intercept obtained for the standard curve

[c] Correlation coefficient obtained for the standard curve

[d] The copy number represents the number of target genes added to the PCR reaction mix

Statistical analyses

The data were analysed by analysis of variance (ANOVA) at the significance level $p<0.05$ using SPSS 11.5 (SPSS, Inc.). The normal distribution of the data was checked by the Kolmogorov-Smirnov test and histograms. If necessary, the data were log-transformed prior to analysis. The homogeneity of the variances was checked by the Levene test. For the pairwise comparison of means with the ANOVA, either the Tukey test or, if the homogeneity of the variances was not given, the Games-Howell test was used.

Results

To contribute to the problem of different DNA extraction efficiencies among environmental samples, the quantified gene copy numbers were normalised to one gram dry weight (dw) of the rhizosphere soil/root complex as well as to nanogram extracted DNA (data not shown). Because both give comparable abundance patterns, similar extraction efficiencies for all samples could be assumed.

Abundance pattern of the indicator microorganisms in the greenhouse experiment

Neither the cultivar nor plant developmental stage influenced the abundance pattern of total 16S rRNA genes significantly (average 3.5×10^{10} copies g^{-1} dw); the *Pseudomonas*-specific 16S rRNA gene copy numbers also did not vary between the treatments analysed (average 2.3×10^{7} copies g^{-1} dw). In contrast, the IGS rRNA gene of *C. michiganensis* showed at the young leaf developmental stage a significantly higher gene copy number for 'Jumbo' (1.3×10^{6} copies g^{-1} dw) compared to 'Walli' (7.5×10^{5} copies g^{-1} dw) and #1332 (8.3×10^{5} copies g^{-1} dw) at the same plant age. As the abundance of *C. michiganensis* for 'Jumbo' decreased during plant development, no cultivar effect was observed at the flowering stage (average 8.9×10^{5} copies g^{-1} dw).

The abundances of fungal ITS rRNA genes were highest at EC30 for 'Jumbo' (7.0×10^{11} copies g^{-1} dw) and differed clearly from 'Walli' (2.1×10^{11} copies g^{-1} dw) and #1332 (2.6×10^{11} copies g^{-1} dw). As the ITS rRNA gene copy number of 'Walli' increased with increasing plant age, again no differences for the ITS rRNA genes were found among the cultivars at the flowering stage (average 4.3×10^{11} copies g^{-1} dw). In contrast, for the abundance pattern of the *Trichoderma*-specific ITS rRNA genes, no cultivar effect at early leaf development was observed and similar copy numbers were found among all cultivars within one plant development stage. However, the abundance pattern of the ITS rRNA genes of *Trichoderma* spp. showed a clear dependence on plant age and increased significantly with increasing plant age from 1.3×10^{6} copies g^{-1} dw at EC30 up to average 6.2×10^{6} copies g^{-1} dw at EC60. Interestingly, the abundance of the nuclear satellite DNA of *P. infestans* was influenced by both the plant developmental stage and cultivar, as gene copies were only detected at the flowering stage and only for 'Walli' and #1332 but not for 'Jumbo'. However, no significant differences for abundance of

P. infestans DNA were observed between 'Walli' and #1332 at EC60, revealing an average of 2.0×10^5 copies g^{-1} dw. All of the data from the greenhouse study are summarised in Fig. 1.

Abundance pattern of the indicator microorganisms in the field experiment

In the rhizosphere of 'Jumbo', a significantly lower total 16S rRNA gene copy number was detected (7.0×10^{10} copies g^{-1} dw) compared to 'Walli' (8.2×10^{10} copies g^{-1} dw) or #1332 (8.4×10^{10} copies g^{-1} dw) at young leaf development. In contrast, the abundance of the *Pseudomonas*-specific 16S rRNA genes was significantly higher for 'Jumbo' (1.9×10^8 copies g^{-1} dw) than for 'Walli' (1.4×10^7 copies g^{-1} dw) or #1332 (1.6×10^7 copies g^{-1} dw) at EC30. At the flowering stage, differences in abundance pattern among the cultivars were not observed for either the total 16S rRNA genes or for the *Pseudomonas*-specific 16S rRNA genes. Overall, universal 16S rRNA gene copy numbers decreased with increasing plant age to 5.5×10^{10} copies g^{-1} dw, whereas the abundance of *Pseudomonas*-specific 16S rRNA genes increased with increasing plant age to 3.5×10^8 copies g^{-1} dw. The abundance pattern of *C. michiganensis* was influenced neither by the cultivars under investigation nor by the plant developmental stage in the field trial (3.4×10^6 copies g^{-1} dw).

For fungal ITS rRNA genes, the highest gene copy numbers were detected in the rhizosphere samples of 'Jumbo' at both sampling time points (2.2×10^{12} g^{-1} dw and 1.5×10^{12} copies g^{-1} dw, respectively), while the gene abundances of 'Walli' and #1332 amounted to an average of 9.0×10^{11} copies g^{-1} dw and remained constant during plant development. In contrast to the overall fungal ITS rRNA genes, no cultivar effect was observed for the abundance of *Trichoderma*-specific ITS rRNA genes as similar copy numbers were found among all cultivars at one plant developmental stage (average 2.1×10^6 copies g^{-1} dw at EC30 and 1.2×10^6 copies g^{-1} dw at EC60). Similarly to the greenhouse study, the nuclear satellite DNA of *P. infestans* was only detected at the flowering stage. In contrast, the gene abundances were not affected by cultivar as no significant difference was observed among 'Jumbo', Walli' and #1332 (average of 1.1×10^5 copies g^{-1} dw). All of the data from the field study are summarised in Fig. 2.

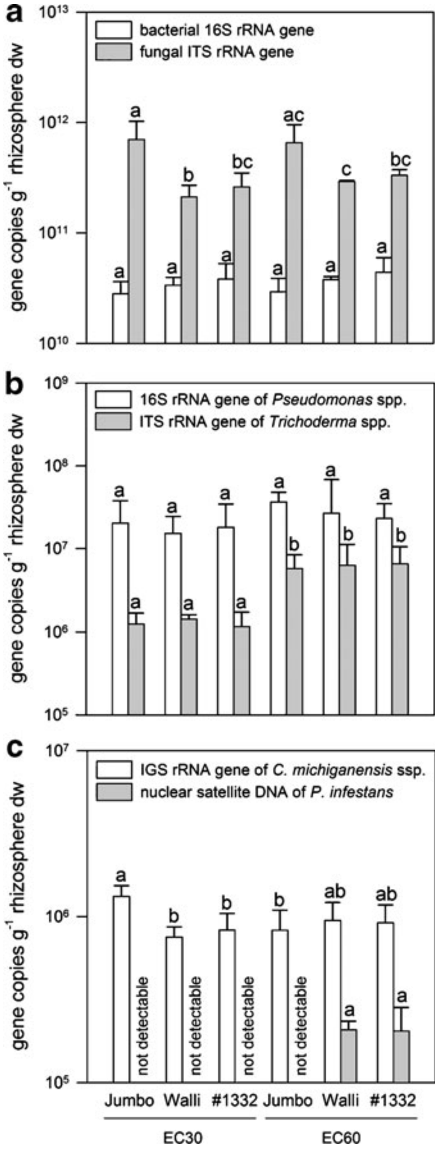

Fig. 1 Copy numbers of (**a**) bacterial 16S rRNA gene and fungal ITS rRNA gene, (**b**) 16S rRNA gene of *Pseudomonas* spp. and ITS rRNA gene of *Trichoderma* spp. and (**c**) IGS rRNA gene of *C. michiganensis* ssp. and nuclear satellite DNA of *P. infestans* per gram of rhizosphere soil/root complex dry weight (rhizosphere dw) for different cultivars ('Jumbo', 'Walli' and #1332) at two plant developmental stages (EC30: early leaf development and EC60: florescence) for greenhouse-grown potato plants ($n=4$); error bars represent standard deviations. For each panel different letters indicate significance at $p<0.05$

Discussion

Because the plant rhizosphere is a dynamic environment, the activity and composition of microbial

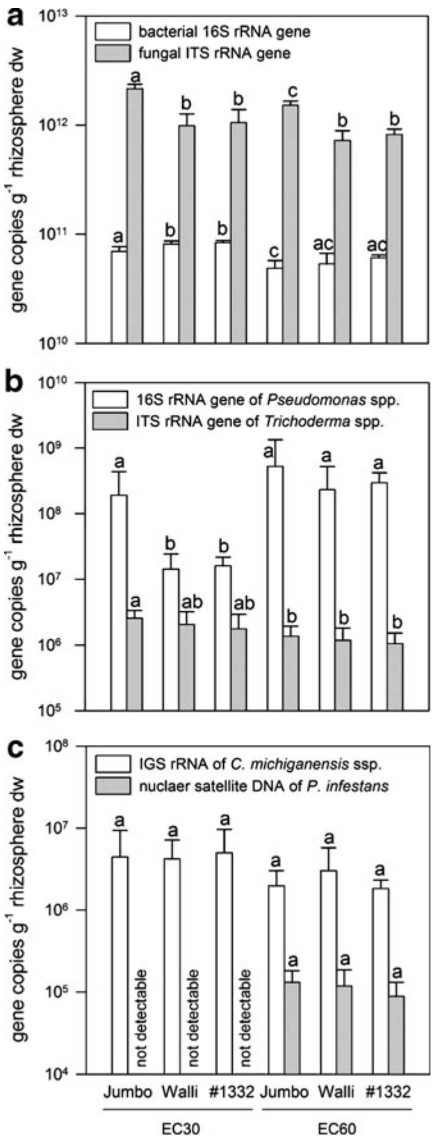

Fig. 2 Copy numbers of (**a**) bacterial 16S rRNA gene and fungal ITS rRNA gene, (**b**) 16S rRNA gene of *Pseudomonas* spp. and ITS rRNA gene of *Trichoderma* spp. and (**c**) IGS rRNA gene of *C. michiganensis* ssp. and nuclear satellite DNA of *P. infestans* per gram of rhizosphere soil/root complex dry weight (rhizosphere dw) for different cultivars ('Jumbo', 'Walli' and #1332) at two plant developmental stages (EC30: early leaf development and EC60: florescence) for field-grown potato plants ($n=5$); error bars represent standard deviations. For each panel different letters indicate significance at $p<0.05$

communities could be influenced by many parameters. The aim of this study was to investigate the potential effects of a GM marker-free amylopectin-accumulating potato line on the abundance of plant beneficial microbes as well as phytopathogens and to compare the modification-dependent impact with variations caused by plant cultivars obtained by classical breeding and plant developmental stage.

Our results revealed that the examined gene abundance patterns in the rhizosphere of the potato plants were not affected by the genetic modification in both the greenhouse and field study, as the data did not vary between the GM line and the non-transgenic parental cultivar. This confirms the findings of other studies, where GM plant-related effects on the microbial community structure were also not found (Heuer et al. 2002; Saxena and Stotzky 2001; Schmalenberger and Tebbe 2002). Although other reports showed significant changes in the rhizosphere microbial community caused by GM plants, the modification-dependent effects were described as minor compared to shifts caused by the soil type, plant genotype and plant developmental stage during the monitoring (Gyamfi et al. 2002; Lottmann et al. 1999; Milling et al. 2004; Rasche et al. 2006; Weinert et al. 2009). However, these differences in the data clearly indicate the need for a case-by-case study of GM plant-related effects because a generalisation of effects is not possible.

Although in this study the gene abundances of rhizosphere microorganisms of the GM potato line #1332 did not vary from those of the non-transgenic parental cultivar 'Walli', significant differences to the second non-transgenic cultivar 'Jumbo' were observed for most of the investigated genes. The abundance of bacteria and *Pseudomonas* spp. in the microbial rhizosphere community differed between 'Jumbo' and 'Walli' or GM line #1332, but only in the field study at the young leaf developmental stage. Interestingly, 'Jumbo' showed a lower copy number for total 16S rRNA genes but a higher abundance of *Pseudomonas* specific 16S rRNA genes compared to the other two potato cultivars. This might be related to the genotype-specific root exudation patterns (Grayston et al. 1998; Marschner et al. 2001; Söderberg et al. 2002), As many *Pseudomonas* strains produce antimicrobial compounds (Walsh et al. 2001; Winding et al. 2004), we suppose this may be the reason why the abundance of bacteria is reduced in the rhizosphere of 'Jumbo'.

The effects of the plant cultivar on fungi were more pronounced than on bacteria, as the abundance of ITS rRNA genes in the rhizosphere of 'Jumbo' was significantly higher compared to the other potato lines in both the greenhouse and field-grown plants. This finding was surprising because a previous study by Costa et al. (2006) reported that bacteria were more influenced by different plant species (strawberry and oilseed rape) than fungi. The contrary observations might result from the different plant species examined. Furthermore, Costa et al. (2006) looked more for changes in fungal diversity whereas the fungal abundance patterns were compared in this study. There are only few studies that investigated the potential impact of GM plants on fungal rhizosphere communities. Similarly to our results, Weinert et al. (2009) obtained significant differences in the fungal communities associated with different plant cultivars. In contrast, Milling et al. (2004) found no cultivar-dependent alteration in the fungal communities, but in that study, the NS1/NS2 primer system was used to amplify the fungal community, which may be biased as it lacks the amplification of major fungal groups (Hagn et al. 2003). However, the need for case-by-case studies is also clear for fungal communities.

Jones et al. (2004) stated that the amount and chemical composition of root exudates change during plant development, depending on the different plant stages. This may explain the clear influence of plant age on the microbial gene abundance patterns in the plant rhizospheres monitored in this study. The abundances of total 16S rRNA genes in the rhizosphere of all three field-grown potato lines decreased from EC30 to EC60. This finding was surprising and in contrast to other studies, where the strongest enrichment of bacterial populations in the rhizosphere of potatoes was found at flowering stage (Lottmann et al. 2000; Smalla et al. 2001). We suggest that the reduced abundance of total bacteria at EC60 may be due to the increased *Pseudomonas* abundance because many *Pseudomonas* strains are known to produce antimicrobial compounds (Walsh et al. 2001; Winding et al. 2004). This assumption is confirmed by the fact that in the greenhouse no alteration in the abundance of total 16S rRNA genes or 16S rRNA genes of *Pseudomonas* spp. during plant growth was observed. The abundance of both fungal ITS rRNA genes and *Trichoderma* spp. in the rhizosphere of field-grown plants also decreased from EC30 to EC60, while in the greenhouse the abundances remained stable or increased. This was not surprising because the fungicide ‘Epok’ was only applied in the field before the flowering stage. Although ‘Epok’ was developed for plant protection against *P. infestans*, the effects on non-target fungi cannot be excluded. Such unintended negative influences on fungi, especially biocontrol fungi like *Trichoderma* strains, were demonstrated for different fungicides (De Schutter et al. 2002; Dłużniewska 2003). Nevertheless, *P. infestans* was detected in the rhizosphere of both the greenhouse and field plants at the flowering stage, but due to the application of ‘Epok’ with lower abundance in field-grown plants. The observation that variations in the functional and structural characteristics of the associated microbial rhizosphere community during plant growing occurred was obtained in several other reports, also (Gyamfi et al. 2002; Heuer et al. 2002; Milling et al. 2004; Rasche et al. 2006; Schmalenberger and Tebbe 2002; Sessitsch et al. 2003).

By comparing the results of the greenhouse and field studies, it became obvious that the microbial community structure was affected in the greenhouse and field with very different intensities. This was particularly visible for the abundance pattern of bacteria, *Pseudomonas* spp. and fungi, which were strongly influenced by the cultivar and plant vegetation stage in the field but not in the greenhouse study. Similarly, Griffiths et al. (2000a) compared the effect of GM potatoes that produce lectins on non-target soil organisms in the greenhouse and field. No significant differences in the microbial rhizosphere population due to the genetic modification were observed in the greenhouse, but the field experiment showed alterations in microbial communities, although these did not persist to the next growing season. In our study, besides climatic conditions, the main difference between greenhouse study and field trial is related to the fungicide application in the field, which may influence microbial communties and thus explain at least a part of the differences observed in both studies.

In conclusion, no effects caused by the genetic modification of the marker-free amylopectin-accumulating potatoes on the investigated gene abundance patterns of plant beneficial microbes and phytopathogens were measured in this study. Because cultivar- and plant developmental stage-dependent effects were observed, this indicates the sensitivity of the used methods. However, these results cannot be generalised to other GM plants with different events, and the transfer of the results to other soil types or climatic regions must also be made with care.

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References

Alabouvette C, Olivain C, Migheli Q, Steinberg C (2009) Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. New Phytol 184:529–544

Bach HJ, Tomanova J, Schloter M, Munch JC (2002) Enumeration of total bacteria and bacteria with genes for proteolytic activity in pure cultures and in environmental samples by quantitative PCR mediated amplification. J Microbiol Meth 49:235–245

Bach HJ, Jessen I, Schloter M, Munch JC (2003) A TaqMan-PCR protocol for quantification and differentiation of the phytopathogenic *Clavibacter michiganensis* subspecies. J Microbiol Meth 52:85–91

Berg G (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Boehm J, Hahn A, Schubert R, Bahnweg G, Adler N, Nechwatal J, Oehlmann R, Oiwald W (1999) Real-time quantitative PCR: DNA determination in isolated spores of the mycorrhizal fungus *Glomus mosseae* and monitoring of *Phytophthora infestans* and *Phytophthora citricola* in their respective host plants. J Phytopathol 147:409–416

Cordier C, Alabouvette C (2009) Effects of the introduction of a biocontrol strain of *Trichoderma atroviride* on non target soil micro-organisms. Eur J Soil Biol 45:267–274

Costa R, Götz M, Mrotzek N, Lottmann J, Berg G, Smalla K (2006) Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiol Ecol 56:236–249

De Boer SH, Slack SA (1984) Current status and prospects for detecting and controlling bacterial ring rot of potatoes in North America. Plant Dis 68:841–844

De Meyer G, Bigirimana J, Elad Y, Höfte M (1998) Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. Eur J Plant Pathol 104:279–286

De Schutter B, Aerts R, Rombouts L (2002) The influence of fungicides on the growth of *Trichoderma asperellum*. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet 67:291–298

De Vetten N, Wolters A, Raemakers K, van der Meer I, ter Stege R, Heeres E, Heeres P, Visser R (2003) A transformation method for obtaining marker-free plants of a cross-pollinating and vegetatively propagated crop. Nat Biotechnol 21:439–442

Dłużniewska J (2003) Reaction of fungi of *Trichoderma* genus to selected abiotic factors. In: Electronic Journal of Polish Agricultural Universities. Available via http://www.ejpau.media.pl/volume6/issue2/agronomy/art-04.html. Accessed 01 Sept 2003

Ghisalberti EL, Rowland CY (1993) Antifungal metabolites from *Trichoderma harzianum*. J Nat Prod 56:1799–1804

Grayston SJ, Wang S, Campbell CD, Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30:369–378

Griffiths BS, Geoghegan IE, Robertson WM (2000a) Testing genetically engineered potato, producing the lectins GNA and Con A, on non-target soil organisms and processes. J Appl Ecol 37:159–170

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ (2000b) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl Environ Microbiol 66:5488–5491

Gyamfi S, Pfeifer U, Stierschneider M, Sessitsch A (2002) Effects of transgenic glufosinate-tolerant oilseed rape (*Brassica napus*) and the associated herbicide application on eubacterial and *Pseudomonas* communities in the rhizosphere. FEMS Microbiol Ecol 41:181–190

Hagn A, Pritsch K, Ludwig W, Schloter M (2003) Theoretical and practical approaches to evaluate suitable primer sets for the analysis of soil fungal communities. Acta Biotechnol 23:373–381

Hagn A, Wallisch S, Radl V, Charles Munch J, Schloter M (2007) A new cultivation independent approach to detect and monitor common *Trichoderma* species in soils. J Microbiol Meth 69:86–92

Haran S, Schickler H, Chet I (1996) Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. Microbiol 142:2321–2331

Heuer H, Kroppenstedt RM, Lottmann J, Berg G, Smalla K (2002) Effects of T4 lysozyme release from transgenic potato roots on bacterial rhizosphere communities are negligible relative to natural factors. Appl Environ Microbiol 68:1325–1335

Johnsen K, Enger O, Jacobsen CS, Thirup L, Torsvik V (1999) Quantitative selective PCR of 16S ribosomal DNA correlates well with selective agar plating in describing population dynamics of indigenous *Pseudomonas* spp. in soil hot spots. Appl Environ Microbiol 65:1786–1788

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480

Kowalchuk G, Buma D, de Boer W, Klinkhamer P, van Veen J (2002) Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie Leeuwenhoek 81:509–520

Kowalchuk GA, Bruinsma M, van Veen JA (2003) Assessing responses of soil microorganisms to GM plants. Trends Ecol Evol 18:403–410

Kraak A (1993) Industrial applications of potato starch products. Ind Crops Prod 1:107–112

Kuipers A, Jacobsen E, Visser R (1994) Formation and deposition of amylose in the potato tuber starch granule are affected by the reduction of granule-bound starch synthase gene expression. Plant Cell 6:43–52

Liu B, Zeng Q, Yan F, Xu H, Xu C (2005) Effects of transgenic plants on soil microorganisms. Plant Soil 271:1–13

Lottmann J, Heuer H, Smalla K, Berg G (1999) Influence of transgenic T4-lysozyme-producing potato plants on potentially beneficial plant-associated bacteria. FEMS Microbiol Ecol 29:365–377

Lottmann J, Heuer H, Vries J, Mahn A, During K, Wackernagel W, Smalla K, Berg G (2000) Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. FEMS Microbiol Ecol 33:41–49

Lugtenberg BJJ, Dekkers L, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by *Pseudomonas*. Ann Rev Phytopathol 39:461–490

Marschner P, Yang CH, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33:1437–1445

Marschner P, Solaiman Z, Rengel Z (2006) Rhizosphere properties of *Poaceae* genotypes under P-limiting conditions. Plant Soil 283:11–24

Milling A, Smalla K, Maidl F, Schloter M, Munch J (2004) Effects of transgenic potatoes with an altered starch composition on the diversity of soil and rhizosphere bacteria and fungi. Plant Soil 266:23–39

Raaijmakers J, Paulitz T, Steinberg C, Alabouvette C, Moënne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321:341–361

Rasche F, Hodl V, Poll C, Kandeler E, Gerzabek MH, van Elsas JD, Sessitsch A (2006) Rhizosphere bacteria affected by transgenic potatoes with antibacterial activities compared with the effects of soil, wild-type potatoes, vegetation stage and pathogen exposure. FEMS Microbiol Ecol 56:219–235

Saxena D, Stotzky G (2001) *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biol Biochem 33:1225–1230

Schmalenberger A, Tebbe CC (2002) Bacterial community composition in the rhizosphere of a transgenic, herbicide-resistant maize (*Zea mays*) and comparison to its non-transgenic cultivar *Bosphore*. FEMS Microbiol Ecol 40:29–37

Sessitsch A, Kan FY, Pfeifer U (2003) Diversity and community structure of culturable *Bacillus* spp. populations in the rhizospheres of transgenic potatoes expressing the lytic peptide cecropin B. Appl Soil Ecol 22:149–158

Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67:4742–4751

Söderberg KH, Olsson PA, Bååth E (2002) Structure and activity of the bacterial community in the rhizosphere of different plant species and the effect of arbuscular mycorrhizal colonisation. FEMS Microbiol Ecol 40:223–231

Soerensen J (1997) The rhizosphere as a habitat for soil microorganisms. Marcel Dekker, New York

Tooley PW, Bunyard BA, Carras MM, Hatziloukas E (1997) Development of PCR primers from internal transcribed spacer region 2 for detection of *Phytophthora* species infecting potatoes. Appl Environ Microbiol 63:1467–1475

Visser RGF, Somhorst I, Kuipers GJ, Ruys NJ, Feenstra WJ, Jacobsen E (1991) Inhibition of the expression of the gene for granule-bound starch synthase in potato by antisense constructs. Mol Gen Genet 225:289–296

Walsh UF, Morrissey JP, O'Gara F (2001) *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. Curr Opin Biotechnol 12:289–295

Weinert N, Meincke R, Gottwald C, Heuer H, Gomes NCM, Schloter M, Berg G, Smalla K (2009) Rhizosphere communities of genetically modified zeaxanthin-accumulating potato plants and their parent cultivar differ less than those of different potato cultivars. Appl Environ Microbiol 75:3859–3865

White T, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic, New York

Winding A, Binnerup SJ, Pritchard H (2004) Non-target effects of bacterial biological control agents suppressing root pathogenic fungi. FEMS Microbiol Ecol 47:129–141

Yanai RD, Majdi H, Park BB (2003) Measured and modelled differences in nutrient concentrations between rhizosphere and bulk soil in a Norway spruce stand. Plant Soil 257:133–142

Young AH (1984) Fractionation of starch. Academic, New York