BioControl (2006) 51:533–546
DOI 10.1007/s10526-005-4240-z

Effect of chitosan and a biocontrol streptomycete on field and potato tuber bacterial communities

Karine PRÉVOST, Geneviève COUTURE, Bill SHIPLEY, Ryszard BRZEZINSKI and Carole BEAULIEU*
Département de biologie, Université de Sherbrooke, Sherbrooke, Québec, J1K 2R1, Canada
**Author for correspondence; e-mail: carole.beaulieu@usherbrooke.ca*

Received 15 November 2004; accepted in revised form 19 October 2005

Abstract. Four treatments applied on potato seed tubers i.e. talc only, chitosan only, *Streptomyces melanosporofaciens* strain EF-76 in talc and *S. melanosporofaciens* strain EF-76 in chitosan were compared for their efficiency to reduce common scab incidence. As previously reported the joint application of the geldanamycin-producing *S. melanosporofaciens* strain EF-76 and chitosan was effective to control common scab of potato. Nevertheless, field application of *S. melanosporofaciens* EF-76 did not allow the selection of geldanamycin-resistant actinomycetes (GRA) in the bulk soil during the potato growing season. The number of GRA on harvested potato tubers was, however, significantly higher in treatments that contained chitosan than in other treatments suggesting that chitosan might promote the establishment of the antagonistic actinomycete on progeny tubers. Biolog EcoPlates were used to determine the metabolic profiles of the bacterial soil communities. A permutation MANOVA analysis detected significant differences within the metabolic profiles of the bacterial communities at the potato flowering period but not at the beginning of the season or a week before harvest. A combination of *S. melanosporofaciens* EF-76 and chitosan thus represents a promising tool against common scab, with low short-term impact on soil bacterial communities.

Key words: biocontrol, Biolog, chitosan, geldanamycin

Introduction

Streptomyces scabiei (Thaxter) Lambert and Loria (syn. *Streptomyces scabies*) is the main causal agent of common scab, a widely-distributed disease that causes important economic losses in potato production (Lambert and Loria, 1989; Goyer et al., 1996). Common scab symptoms consist of superficial to deep corky lesions on the tubers. Methods used to control common scab include chemical treatments of seed potato tubers (Davis et al., 1976), irrigation (Adams and Lapwood,

1978), rotation strategies (Li et al., 1999), use of a tolerant cultivar (Bouchek-Mechiche et al., 2000), soil amendment with chitinous compounds (Vruggink, 1970; Beauséjour et al., 2003) and biological control with non-pathogenic streptomycetes (Liu et al., 1995; Ryan and Kinkel, 1997; Doumbou et al., 1998; Agbessi et al., 2003; Beauséjour et al., 2003). Recently, Beauséjour et al. (2003) reported the use of a biological agent (*Streptomyces melanosporofaciens* Arcamone et al. strain EF-76) in combination with chitosan to control common scab.

Chitosan, or poly-$\beta(1 \rightarrow 4)$-2-amino-2-deoxy-β-D-glucan, is a natural polymer that exhibits interesting properties for the biocontrol of plant diseases. Chitosan not only inhibits the growth of several plant pathogens (Benhamou and Thériault, 1992; Helander et al., 2001) but is also known as a potent elicitor of plant defense mechanisms (Kauss et al., 1989; Benhamou et al., 1994).

Streptomyces melanosporofaciens strain EF-76 (Doumbou et al., 2001) is a chitosanolytic bacterium (Beauséjour et al., 2003) that was isolated from potato tuber tissue (Faucher et al., 1992). This strain synthesizes geldanamycin (Toussaint et al., 1997), an ansamycin-type antibiotic exhibiting antimicrobial activity (DeBoer et al., 1970) against diverse phytopathogenic fungi and gram-positive bacteria including *S. scabiei* (Agbessi et al., 2003).

Potential environmental risks associated with the use of biocontrol agents in the field are still poorly documented (Lottmann et al., 2000; Schwieger and Tebbe, 2000). For instance, the introduction of *S. melanosporofaciens*, as well as application of chitosan, might affect some indigenous microorganisms in addition to the target pathogen and consequently might disturb soil communities. Therefore, the effect of biological control on soil communities has to be studied since such indigenous soil microorganisms play central roles in the cycling of elements that are essential for soil fertility and plant productivity.

Because one must consider entire communities of soil microorganisms, several approaches for the assessment of the diversity in soil microbial communities have been developed. One of these methods is the analysis of the carbon source utilization pattern of the environmental microbial community using the Biolog system (Garland and Mills, 1991). Metabolic fingerprints obtained with this method can be used to assess differences between the microbial communities of soils that were treated or not with a biocontrol agent (Ellis et al., 1995; Vahjen et al., 1995; Natsch et al., 1998; Bjorklof et al., 2003). The purpose of this study was to use the Biolog system to determine if the

addition of *S. melanosporofaciens* EF-76 and of chitosan has an effect on the bacterial communities of soil and potato tubers.

Materials and methods

Streptomyces melanosporofaciens *EF-76 and culture conditions*

Streptomyces melanosporofaciens was maintained on yeast malt extract agar (YME) medium (Pridham et al., 1956-57). EF-76 biomass used for field inoculation was prepared as described by Beauséjour et al. (2003).

Field experiment and sampling

A field assay was performed in a naturally infested field at l'Assomption (Quebec, Canada) in 2003. At planting, a formulation powder (0.5 g) was placed on each tuber (*Solanum tuberosum* cv. Shepody); this powder was composed of one carrier [talc (Sigma-Aldrich Canada, Oakville, ON) or chitosan (Marinard Biotech, Rivière-au-Renard, QC)] with or without dried biomass of *S. melanosporofaciens* strain EF-76 (1/300 w:w inoculum/carrier). The inoculum thus consists of about 10^5 colony forming units per tuber. Experimental plots, consisting of four rows made up of 26 seed tubers, were arranged as randomized complete blocks with four replicates (Beauséjour et al., 2003). Soil was sampled at four different periods: before treatment application, 12 days after treatment application, at the time of flowering (67 days after treatment application) and one week before harvest (117 days after treatment application). Six soil cores (20 cm deep and 2 cm diameter) were randomly sampled from each plot, to enumerate geldanamycin-resistant actinomycetes (GRA), and to assess metabolic diversity of bacterial communities. Progeny tubers from each plot were subsequently harvested and weighed (kg) to determine the yield. Common scab symptoms were estimated visually on 100 tubers harvested from each plot according to Beauséjour et al. (2003). These tubers were then kept to enumerate GRA and to determine the metabolic profiles of the bacterial tuber communities.

Microbial cell extraction from soil and potato tubers

Ten tubers were randomly taken from each plot. Two groups of five tubers from each plot were used for microbial cell extraction. Tubers were washed to remove surplus soil and peeled. Bacterial suspensions

were obtained by shaking the potato skin (15 g) in 100 ml of sterile sodium pyrophosphate 0.1% (w:v) for 30 min at room temperature. The mixture was filtered through cheese cloth and then centrifuged at 55× *g* for 10 min. The supernatants were then centrifuged at 3500× *g* for 15 min. The bacterial pellets were suspended in NaCl 0.85% (w:v). The same protocol was used to extract microbial cells from the soil, but the bacterial suspensions were obtained from 3 g of soil and were not filtered before centrifugation.

Enumeration of GRA in soil and on potato tubers

Enumeration of GRA was carried out as previously described (Jobin et al., 2005). Briefly, bacterial pellets recovered from soil samples and from potato tubers were resuspended in 1 ml NaCl solution (0.85%). Serial dilutions of these suspension were plated on a semi-selective medium. The semi-selective culture medium consisted of actinomycete isolation agar (Difco, Detroit, MI) supplemented with cycloheximide (200 μg/ml), nystatin (5.6 μg/ml), nalidixic acid (15 μg/ml) and geldanamycin (25 μg/ml). The bacteria were enumerated after 8 days of incubation at 30 °C.

Soil and potato tuber bacterial communities

The metabolic diversity of bacterial communities from the field trial was analyzed using the Biolog EcoPlate™ system (Biolog Inc., Hayward, CA). All samples at each sampling time were analyzed the same day. Bacteria were recovered from soil samples and from potato tubers as described before. Bacterial pellets were dispersed in 30 ml of sterile saline solution (NaCl 0.85%). These cell suspensions were then diluted with sterile saline solution to obtain a cell suspension of defined optical density ($OD_{600} = 0.2$) (Zak et al., 1994; Garland, 1997; Staddon et al., 1997). These were used to inoculate the Biolog EcoPlate (150 μl per wells). The plates were incubated at 25 °C in the dark for 96 h and the optical densities were measured at 24, 48, 72, 96 h with Fluorescence plate reader (BIO-TEK® FL600™, Vermont, USA) at 595 nm. The OD of the control well was subtracted from the OD of the other wells to correct for background activity.

Statistical analyses

The statistical tests used to assess community differences are based on the OD values corrected after 72 h of incubation. Each observational

unit consisted of 31 correlated continuous response variables (the OD values of each of the 31 sources of carbon, representing the microbial community response) and categorical factors classifying the treatment, block (row) and plot from which the observational unit came. The two largest trends in the microbial community responses to the treatments were quantified by the first two axes of a principal component analysis (PCA). PCA was done by SAS System Windows (Version 6.12) statistical package. Although the first two PCA axes allow one to plot the two largest trends in the data and thus visualize these trends, the first two axes never capture all of the variation in the 31 response variables (i.e. community responses); usually less than 50% of the total variation is captured in the first two axes.

Since there were 31 dependent variables with possible dependencies between them, and with both a blocking and a nesting structure, the proper parametric model is a nested one-way multivariate ANOVA with blocking (MANOVA). The proper error term relative to the treatment effect is the variability between plots within treatments. The statistical significance of a given single carbon source can be tested by comparing the *F*-ratio of the mean square of the treatment term to the mean square for the plots nested within treatments.

However, because the number of dependent variables exceeded the number of plots, a parametric MANOVA is not possible. Also, since a parametric MANOVA is sensitive to deviations from multivariate normality, we chose instead to use a more robust non-parametric permutation MANOVA (PMANOVA) (Pesarin, 2001). This method consists of calculating, for each carbon source, the *F*-statistic for the treatment effect, randomly re-assigning the treatment allocation to plots within blocks consistent with the null hypothesis, re-calculating the *F*-statistic for this permuted data set, and repeating the permutation procedure 1000 times in order to define a permutation sampling distribution for the *F*-statistic. The estimated probability is the proportion of times the observed *F*-value exceeds the 1000 permutated values. The 95% confidence interval (Manly, 1997) for an estimated probability p_i with this number of permuted data sets is $p_i \pm 1.96\sqrt{0.001p_i\ (1-p_i)}$; for example, an estimated probability of 0.05 would have a 95% confidence interval of (0.043, 0.057). The joint probability for the entire set of 31 dependent variables was obtained using Fisher's omnibus combining function, which is distributed as a chi-squared with 62 degrees of freedom (from the 31 carbon sources), given the null hypothesis that the multivariate distribution of the 31 carbon sources is equivalent across treatments; i.e. that the treatments

had no effect on any of the 31 dependent variables. The PMANOVA program is available from the authors.

Results

Field trial

Four treatments were compared in the field assay: talc (control treatment), chitosan, talc supplemented with *S. melanosporofaciens* EF-76 and chitosan supplemented with *S. melanosporofaciens* EF-76. Talc only was considered as the control treatment since application of talc on seed potato pieces is a current agricultural practice. The treatment chitosan supplemented with *S. melanosporofaciens* EF-76 was the only treatment that reduced common scab incidence. This treatment doubles the percentage of marketable tubers from 9.5% to 21% (Table 1). The yield values of the four treatments did not significantly differ ($p > 0.05$, ANOVA test) (Table 1).

Enumeration of GRA

The soil GRA population was the same before and after treatment application. Introduction of *S. melanosporofaciens* EF-76, a geldanamycin producer, did not change the population of GRA in soil. The GRA number stayed approximately the same throughout the season,

Table 1. Marketable tubers and potato yield from field plots amended or not with chitosan and *S. melanosporofaciens* EF-76

	Treatment			
	Talc (control)	Chitosan	EF-76 in talc	EF-76 in chitosan
Marketable tubers[1] (%)	9.50 b[2]	8.75 b	9.75 b	21.00 a
Yield (kg/plot)	48.4 a[3]	37.0 a	37.6 a	40.3 a

[1]Tubers are considered marketable when less then 5% of their surface was covered by scab lesions.
[2]Data within a line accompanied by a same letter did not significantly differ ($p > 0.05$, χ^2 test).
[3]Data within a line accompanied by a same letter did not significantly differ ($p > 0.05$, ANOVA test).

Table 2. Effect of chitosan and *S. melanosporofaciens* EF-76 application on the GRA population of both field soil and potato tubers

Sample	Geldanamycin-resistant actinomycetes (CFU/g of soil or potato skin)[1]			
	Talc (control)	Chitosan	EF-76 in talc	EF-76 in chitosan
Soil[2]				
Before treatment application	3.37×10^5	2.95×10^5	3.60×10^5	3.59×10^5
Twelve days after treatment application	3.17×10^5	4.21×10^5	2.88×10^5	4.18×10^5
Flowering	4.60×10^5	5.55×10^5	5.43×10^5	4.21×10^5
One week before harvest	3.91×10^4	5.63×10^4	5.61×10^4	4.33×10^4
Potato tuber				
Tubers at harvest[3]	7.25×10^4 b	1.51×10^5 a	1.26×10^5 ab	1.44×10^5 a

[1]CFU: colony forming unit.
[2]Population of soil samples at each sampling period did not significantly differ ($p > 0.05$, ANOVA test).
[3]Data within a line accompanied by the same letter did not significantly differ ($p > 0.05$, ANOVA test).

but diminished from about 3–5$\times$ 10^5 to 4–5$\times$ 10^4 colony forming units (CFU)/g of soil one week before harvest (Table 2). However, the quantity of GRA on harvested tubers differed between treatments. The GRA population was 7.25×10^4 CFU/g of tuber skin in the control treatment (talc). GRA number in the strain EF-76 in talc treatment did not differ significantly ($p > 0.05$) from the control. The GRA population on tubers was higher in the chitosan only and in the strain EF-76 in chitosan treatments with 1.44 and 1.51×10^5 CFU/g of tuber skin ($p > 0.05$), respectively (Table 2).

Soil and potato tuber bacterial communities

PCA visually represents the carbon source utilization patterns of each microbial community. Figure 1 shows the results for the first two principle components of the overall analysis. Analysis for soil microbial communities was done before treatment application (Figure 1a), 12 days after treatment application (Figure 1b), at flowering (Figure 1c) and one week before harvest (Figure 1d). PCA axes 1 and 2 accounted for 35.1% to 50.1% of the total variance, depending on

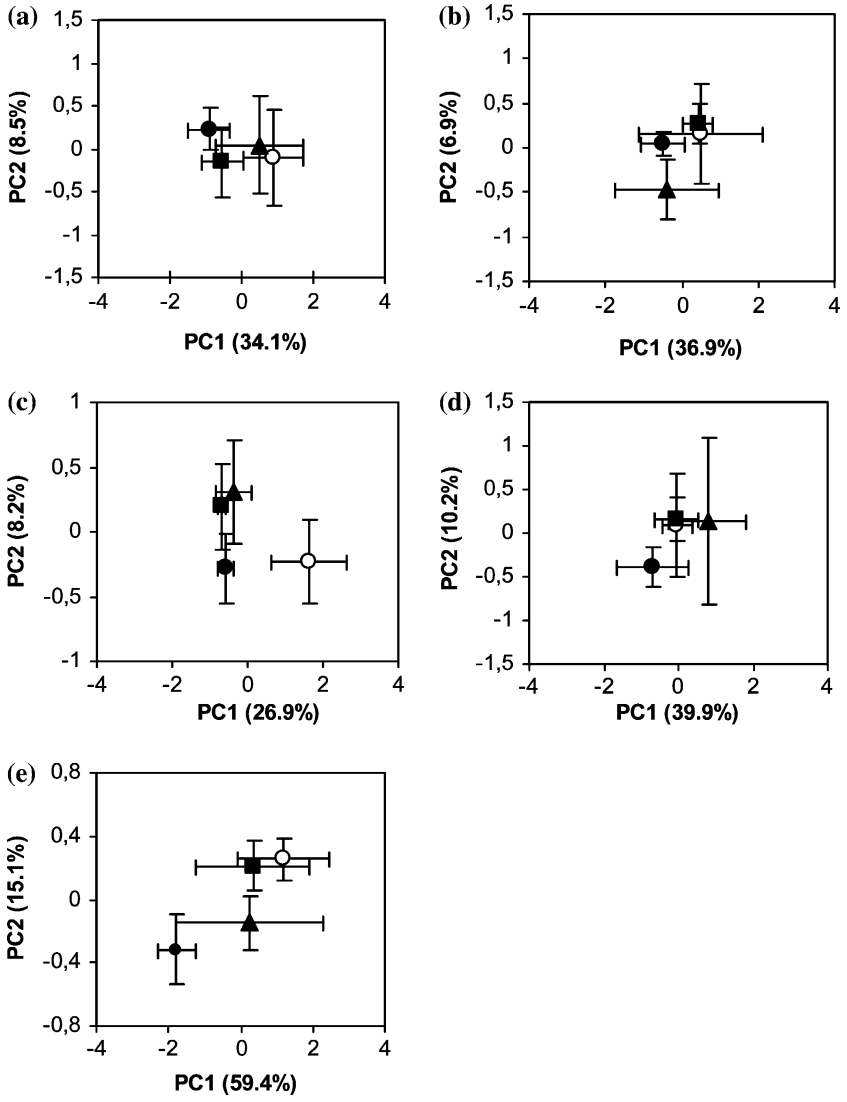

Figure 1. Principal component analysis (PCA) of carbon source utilization patterns of the microbial communities generated with Biolog EcoPlate. PC1, principal component 1; PC2, principal component 2. (a) Soil microbial communities before treatment application. (b) Soil microbial communities 12 days after treatment application. (c) Soil microbial communities at the flowering period. (d) Soil microbial communities one week before harvest. (e) Microbial communities on potato tubers at harvest. Vertical and horizontal lines represent SE (Standard Error) of the mean ($n = 4$); Data points represent treatments: talc (empty circle), strain EF-76 in talc (filled triangle), chitosan (filled square) and strain EF-76 in chitosan (filled circle).

Table 3. PMANOVA analysis of carbon source utilization pattern by microbial communities of both soil and potato tubers

	Chi-square	Degrees of freedom	Probability	
Soil communities				
Before treatment	77.55	62	0.088	NS[1]
Twelve days	34.5	62	0.998	NS
Flowering	105.95	62	0.001	S[2]
One week before harvest	33.46	62	0.999	NS
Potato tuber communities				
Harvest	47.94	62	0.905	NS

[1]NS: no significant difference.
[2]S indicates that the metabolic profiles of the communities analyzed significantly differs ($p < 0.05$).

the treatment. PMANOVA analysis did not show significant differences ($p > 0.05$) between treatments, before treatment application, 12 days after treatment or one week before harvest (Table 3). PCA ordination of soil samples for carbon utilization patterns by microbial communities at flowering shows two clusters. The control treatment represents one of the clusters while all other treatments form the second cluster. A PMANOVA analysis did, however, show a significant difference ($p < 0.05$) between treatments at flowering (Table 4). Metabolic patterns of the chitosan treatment and the strain EF-76 in chitosan treatment significantly differed from talc (control) and from each other.

The microbial communities on harvested potato tubers, as opposed to those in the soil, were also analyzed. Figure 1e represents the carbon source utilization patterns of microbial communities on these tubers. PCA axes 1 and 2 accounted for 74.5% of the variance in the data set (Figure 1e). PCA analysis seems to show differences between talc (control) and strain EF-76 in chitosan treatment (Figure 1e), but a PMANOVA analysis, that takes into account 100% of the variance in data set, did not show a significant difference ($p > 0.05$) among treatments (Table 3).

Discussion

A previous study has shown that simultaneous application of chitosan flakes and lyophilized *S. melanosporofaciens* EF-76 biomass protected

Table 4. PMANOVA analysis of carbon source utilization pattern by soil microbial communities at the flowering period

Treatments compared		Chi-square	Degrees of freedom	Probability	
Talc (control)	Chitosan	168.43	62	0.000	S[1]
Talc (control)	Strain EF-76 in talc	80.27	62	0.059	NS[2]
Talc (control)	Strain EF-76 in chitosan	123.27	62	0.000	S
Chitosan	Strain EF-76 in talc	75.45	62	0.117	NS
Chitosan	Strain EF-76 in chitosan	100.93	62	0.001	S
Strain EF-76 in talc	Strain EF-76 in chitosan	69.87	62	0.230	NS

[1]S indicates that the metabolic profiles of the communities analyzed significantly differ ($p < 0.05$).
[2]NS: No significant difference.

progeny tubers against common scab of potato (Beauséjour et al., 2003). Here, we confirmed that the chitosan supplemented with *S. melanosporofaciens* EF-76 spores treatment reduced common scab incidence. Although this study and the work of Beauséjour et al. (2003) were carried in a similar manner (same field, same potato cultivar and same inoculum applied on tubers), the effectiveness of the combination of chitosan and EF-76 spores to control common scab differed between studies. While Beauséjour et al. (2003) observed that the disease incidence associated with the control plots in 2000 was comparable to the one reported in this study for 2003 (90.5 and 91.0%, respectively), the disease incidence associated with the EF-76 in chitosan treatment was reduced to 56% in 2000 (Beauséjour et al., 2003) compared to 79% in 2003 (this study). In another study, Ryan et al. (2004) also reported that the relative effectiveness of different antagonists of *S. scabiei* varied among growing seasons.

In this study, we also analyzed the possible effects of chitosan and EF-76 on the soil and tuber bacterial communities. Application of chitosan induced variations within the metabolic profiles of the soil bacterial communities at the flowering period. However, differences in the bacterial communities that were observed at flowering were only transient since no such difference was found one week before harvest. Transient effects on the microflora functionality were also observed in microcosms following the inoculation with the biocontrol agents *Pseudomonas fluorescens* (Natsch et al., 1998) or *Corynebacterium glutamicum* (Vahjen et al., 1995).

The shift induced by chitosan in the soil microbial community occurred between 13 and 67 days after chitosan application. This delay could be attributed to both the kinetics of chitosan degradation (Jobin et al., 2005) and the fact that chitosan was not uniformly distributed in soil. Disturbance of microflora would possibly have been detected sooner at proximity to the seed tubers than in the bulk soil. Since no effect on the number of GRA in the bulk soil was observed within treatments, differences within the metabolic profiles at the flowering period are unlikely to be due to an increase of EF-76 population in the bulk soil. Furthermore, the introduction of EF-76 into potato field did not result in the selection of GRA in the bulk soil.

Our data suggest that chitosan may contribute to the establishment and survival of GRA population on harvested tubers. The GRA number was effectively higher for the chitosan only and the EF-76 in chitosan treatment than for control treatment. Several studies have shown that a chitinous amendment stimulated the growth of Gram-positive bacteria and especially of actinomycetes (Labrie et al., 2001; Vruggink, 1970). Strain like *S. melanosporofaciens* EF-76 can benefit from chitosan as a source of nutrients, but not the pathogen *S. scabiei* which lacks this chitosanolytic behavior (Beauséjour et al., 2003). While chitosan application on seed tubers appeared to improve the colonization of progeny tubers by GRA, not all GRA were effective against the common scab-inducing actinomycetes as illustrated by the fact that the potato tubers from the chitosan only treatment harbored as much GRA as those from the EF-76 in chitosan treatment but did not increase the percentage of marketable tubers.

Despite changes in the GRA population on potato tubers, no differences in the carbon sources utilization patterns of the tuber communities were detected among treatments. The use of more than one method to evaluate differences in bacterial communities is thus a practice to encourage. Miethling et al. (2000) found differences in the rhizosphere microbial community of alfalfa after the application of the biocontrol agent *Sinorhizobium meliloti* L33 using TGGE but not using the Biolog system. The Biolog system remains a valuable and rapid tool for microbial community analysis but has some limitations (Van Elsas et al., 1998). For example, no metabolic activity was detected when spores or cells of *S. melanosporofaciens* EF-76 and of other actinomycete strains were inoculated in Ecoplates (data not shown) suggesting that the Biolog Ecoplates are not suitable to detect changes in the actinomycete population.

While the chitosan only and the EF-76 only treatments were inefficient to control common scab in this study, the same treatments protected potato crop against the disease in years 2000 and 2001, respectively (Beauséjour et al., 2003). In the absence of chitosan, specific environmental conditions might thus be necessary for the colonization of EF-76 on progeny tubers. On the other hand, the selection by chitosan of a microbial population antagonistic to *S. scabiei* might depend on the initial soil and seed tuber microflora. Nevertheless, the combined application of chitosan and EF-76 regularly ensured a level of protection against common scab (Beauséjour et al., 2003; this study). This might be due to the fact that chitosan contributed to the establishment of actinomycetes such as *S. melanosporofaciens* on the progeny tubers. The impact of a combined application of chitosan and EF-76 on microbial communities appears to be low in the field while an increase of GRA has been observed on progeny tubers. Further studies have to be carried out to evaluate the effect on potato innocuity of an increase of the tuber GRA population.

Acknowledgements

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Conseil de recherches en pêches et en agroalimentaire du Québec (CORPAQ). Chitosan was kindly donated by Marinard Biotech Inc. (Rivière-au-Renard, Canada). We thank N. Clermont and M. Blain-Fortin for their technical assistance.

References

Adams, M.J. and D.H. Lapwood, 1978. Studies on the lenticel development, surface microflora and infections by common scab (*Streptomyces scabies*) of potato tubers growing in wet and dry soils. *Ann. Appl. Biol.* 90: 335–343.

Agbessi, S., J. Beauséjour, C. Déry and C. Beaulieu, 2003. Antagonistic properties of two recombinant strains of *Streptomyces melanosporofaciens* obtained by intraspecific protoplast fusion. *Appl. Microbiol. Biotechnol.* 62: 233–238.

Beauséjour, J., N. Clermont and C. Beaulieu, 2003. Effect of *Streptomyces melanosporofaciens* strain EF-76 and of chitosan on common scab of potato *Plant Soil* 256: 463–468.

Benhamou, N., P.J. Lafontaine and M. Nicole, 1994. Induction of systemic resistance to *Fusarium* Crown and root rot in tomato plants by seed treatment with chitosan. *Phytopathology* 84: 1432–1444.

Benhamou, N. and G. Thériault, 1992. Treatment with chitosan enhances resistance of tomato plants to the crown and root rot pathogen, *Fusarium* oxysporum f. sp. *radicis-lycopersici*. *Physiol. Mol. Plant. Pathol.* 41: 33–52.

Bjorklof, K., R. Sen and K.S. Jorgensen, 2003. Maintenance and impacts of an inoculated mer/luc-tagged *Pseudomonas fluorescens* on microbial communities in birch rhizospheres developed on humus and peat. *Microb. Ecol.* 45: 39–52.

Bouchek-Mechiche, K., C. Pasco, D. Andrivon and B. Jouan, 2000. Differences in host range, pathogenicity to potato cultivars and response to soil temperature among *Streptomyces* species causing common and netted scab in France. *Plant Pathol.* 49: 3–10.

Davis, J.R., G.M. McMaster, R.H. Callihan, J.G. Garner and R.E. McDole, 1976. The relationship of irrigation timing and soil treatments to control potato scab. *Phytopathology* 64: 1404–1410.

DeBoer, C., P.A. Meulman, R.J. Wnuk and D.H. Peterson, 1970. Geldanamycin, a new antibiotic. *J. Antibiotics* 23: 442–447.

Doumbou, C.L., V. Akimov and C. Beaulieu, 1998. Selection and characterization of microorganisms utilizing thaxtomin A, a phytotoxin produced by *Streptomyces scabies*. *Appl. Environ. Microbiol.* 64: 4313–4316.

Doumbou, C.L., V. Akimov, M. Côté, P.-M. Charest and C. Beaulieu, 2001. Taxonomic study on nonpathogenic streptomycetes isolated from common scab lesions on potato tubers. *Syst. Appl. Microbiol.* 24: 451–456.

Ellis, R.J., I.P. Thompson and M.J. Bailey, 1995. Metabolic profiling as a means of characterizing plant-associated microbial communities. *FEMS Microbiol. Ecol.* 16: 9–18.

Faucher, E., T. Savard and C. Beaulieu, 1992. Characterization of actinomycetes isolated from common scab lesions on potato tubers. *Can. J. Plant Pathol.* 14: 197–202.

Garland, J.L., 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiol. Ecol.* 24: 289–300.

Garland, J.L. and A.L. Mills, 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Appl. Environ. Microbiol.* 57: 2351–2359.

Goyer, C., B. Otrysko and C. Beaulieu, 1996. Taxonomic studies on streptomycetes causing potato common scab: a review. *Can. J. Plant Pathol.* 18: 107–113.

Helander, I.M., E.-L. Nurmiaho-Lassila, R. Ahvenainen, J. Rhoades and S. Roller, 2001. Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *Int. J. Food Microbiol.* 71: 235–244.

Jobin, G., G. Couture, C. Goyer, R. Brzezinski and C. Beaulieu, 2005. Streptomyces spores entrapped in chitosan beads as novel biocontrol tool against common scab. *Appl. Microbiol. Biotechnol.* 68: 104–108.

Kauss, H., W. Jenlick and A. Domard, 1989. The degrees of polymerization and N-acetylation of chitosan determine its ability to elicit callose formation in suspension cells and protoplasts of *Catharanthus roseus*. *Planta* 178: 385–392.

Labrie, C., P. Leclerc, N. Côté, S. Roy, R. Brzezinski, R. Hogue and C. Beaulieu, 2001. Effect of chitin waste-based composts produced by two-phase composting on two oomycete plant pathogens. *Plant Soil* 235: 27–34.

Lambert, D.H. and R. Loria, 1989. *Streptomyces scabies* sp. nov., nom. rev. *Int. J. Syst. Bacteriol.* 39: 387–392.

Li, H., L.É. Parent, C. Tremblay and A. Karam, 1999. Potato response to crop sequence and nitrogen fertilization sod breakup in a Gleyed Humo-Ferric Podzol. *Can. J. Plant. Sci.* 79: 439–446.

Liu, D., N.A. Anderson and L.L. Kinkel, 1995. Biological control of potato scab in the field with antagonistic *Streptomyces scabies*. *Phytopathology* 85: 827–831.

Lottmann, J., H.J. Heuer, J. De Vries, A. Mahn, K. Düring, W. Wackernagel, K. Smalla and G. Berg, 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.

Manly, B.F.J., 1997. *Randomization, Bootstrap and Monte Carlo Methods in Biology*. 2nd edition. Chapman & Hall, London, England 399.

Miethling, R., G. Wieland, H. Backhaus and C.C. Tebbe, 2000. Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. *Microb. Ecol.* 40: 43–56.

Natsch, A., C. Keel, N. Hebecker, E. Laasik and G. Défago, 1998. Impact of *Pseudomonas fluorescens* strain CHA0 and a derivative with improved biocontrol activity on the culturable resident bacterial community on cucumber roots. *FEMS Microbiol. Ecol.* 27: 365–380.

Pesarin, F., 2001. *Multivariate Permutation Tests with Applications in Biostatistics*. John Wiley & Sons, New York, USA 408.

Pridham, T.G., P. Anderson, C. Foley, L.A. Lindenfelser, C.W. Hessetine and R.G. Benedict, 1956–57. A selection of media for maintenance and taxonomic study of *Streptomyces*. *Antibiotics Annu.* 1956-57: 947–953.

Ryan, A.D. and L.L. Kinkel, 1997. Inoculum density and population dynamics of suppressive and pathogenic *Streptomyces*strains and their relationship to bacterial control of potato scab. *Biol. Control* 10: 180–186.

Ryan, A.D., L.L. Kinkel and J.C. Schottel, 2004. Effect of pathogen isolate, potato cultivar, and antagonist strain on potato scab severity and biological control. *Biocontrol Sci. Technol.* 14: 301–311.

Schwieger, F. and C.C. Tebbe, 2000. Effect of field inoculation with *Sinorhizobium meliloti* L33 on the composition of bacterial communities in rhizospheres of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*)-linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. *Appl. Environ. Microbiol.* 66: 3556–3565.

Staddon, W.J., L.C. Duchesne and J.T. Trevors, 1997. Microbial diversity and community structure of postdisturbance forest soils as determined by sole-carbon-source utilization patterns. *Microb. Ecol.* 34: 125–130.

Toussaint, V., D. Valois, M. Dodier, E. Faucher, C. Déry, R. Brzezinski, L. Ruest and C. Beaulieu, 1997. Characterization of actinomycetes antagonistic to *Phytophtora fragariae* var. *rubi*, the causal agent of raspberry root rot. *Phytoprotection* 78: 43–51.

Van Elsas, J.D., G.F. Duarte, A.S. Rosado and K. Smalla, 1998. Microbiological and molecular biological methods for monitoring microbial inoculants and their effects in the soil environment. *J. Microbiol. Methods* 32: 133–154.

Vahjen, W., J.C. Munch and C.C. Tebbe, 1995. Carbon source utilization of soil extracted microorganisms as a tool to detect the effect of soil supplemented with genetically engineered and non-engineered *Corynebacterium glutamicum* and a recombinant peptide at the community level. *FEMS Microbiol. Ecol.* 18: 317–328.

Vruggink, H., 1970. The effect of chitin amendment on actinomycetes in soil and on the infection of potato tubers by *Streptomyces scabies*. *Neth. J. Plant Pathol.* 76: 293–295.

Zak, J.C., M.R. Willig, D.L. Moorhead and H.G. Wildman, 1994. Functional diversity of microbial communities: a quantitative approach. *Soil Biol. Biochem.* 26: 1102–1108.