Phytoprotection

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Volume 90, numéro 3, 2009

URI : https://id.erudit.org/iderudit/045779ar
DOI : https://doi.org/10.7202/045779ar

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Éditeur(s)

Société de protection des plantes du Québec (SPPQ)

ISSN

0031-9511 (imprimé)
1710-1603 (numérique)

Découvrir la revue

Citer cet article

Roy, J., Lafontaine, P. J., Chabot, R. & Beaulieu, C. (2009). Dehydrated pork manure by-product: effect of a chitosan amendment on bacterial community and common scab incidence. *Phytoprotection*, *90*(3), 107–115. https://doi.org/10.7202/045779ar

Résumé de l'article

Un amendement en chitosane a modifié la composition de la communauté microbienne associée à un sous-produit déshydraté de fumier de porc. Le produit amendé (biosolide PC) contenait un nombre de bactéries anaérobies inférieur à celui du produit non amendé (biosolide P). Le chitosane a aussi réduit de façon significative la population fongique. Une banque de gènes de l'ARNr 16S construite à partir de l'ADN extrait de la communauté bactérienne associée aux biosolides P et PC a révélé que les ordres bactériens *Xanthomonodales*, *Pseudomonadales*, *Enterobacteriales*, *Burkholderiales*, *Actinomycetales*, *Bacillales*, *Clostridiales* et *Lactobacillales* se trouvaient dans les deux types de biosolides. Les bactéries du genre *Stenotrophomonas* étaient les plus abondantes dans les deux types de biosolides. L'addition de chitosane a toutefois induit des changements dans la population de quelques genres de bactéries. Par exemple, les clones transportant un gène d'ARNr 16S correspondant au genre *Bacillus* doublaient dans le biosolide PC. Dans des essais en champs entrepris dans le but de tester leur effet sur l'incidence de la gale commune, les biosolides P et PC ont été appliqués comme traitement des semences de pomme de terre. Le biosolide P a augmenté l'incidence de la maladie par un facteur de 1,33 et de 2,85 dans deux expériences indépendantes. Toutefois, quand le chitosane était ajouté au traitement de semences, l'effet stimulant du biosolide P sur la gale commune était aboli.

Dehydrated pork manure by-product: effect of a chitosan amendment on bacterial community and common scab incidence

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Received 2010-08-06; accepted 2010-12-14

PHYTOPROTECTION 90 : 107-115

Chitosan amendment modified the composition of a microbial community associated with dehydrated pork manure by-product. The amended product (biosolid PC) contained a lower number of anaerobic bacteria than the non-amended product (biosolid P). Chitosan also significantly reduced the fungal population. A 16S rRNA gene bank constructed from DNA extracted from the bacterial community associated with both P and PC biosolids revealed that bacterial orders *Xanthomonodales*, *Pseudomonadales*, *Enterobacteriales*, *Burkholderiales*, *Actinomycetales*, *Bacillales*, *Clostridiales* and *Lactobacillales* were found in both biosolids. Bacteria from the *Stenotrophomonas* genus were abundant in both biosolids. However, the addition of chitosan appeared to induce changes in the population of some bacterial genera. For example, clones carrying a 16S rRNA gene corresponding to the *Bacillus* genus were doubled in biosolid PC. In field trials carried out to test their effect on common scab incidence, biosolids P and PC were applied as potato seed treatment. Biosolid P increased disease incidence by a factor of 1.33 and 2.85 in two independent experiments. However, when chitosan was added to the seed treatment, the stimulating effect of biosolid P on common scab was cancelled out.

Keywords: 16S rRNA gene, liquid manure, potato, *Streptomyces scabiei*, *Streptomyces scabies*.

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Un amendement en chitosane a modifié la composition de la communauté microbienne associée à un sous-produit déshydraté de fumier de porc. Le produit amendé (biosolide PC) contenait un nombre de bactéries anaérobies inférieur à celui du produit non amendé (biosolide P). Le chitosane a aussi réduit de façon significative la population fongique. Une banque de gènes de l'ARNr 16S construite à partir de l'ADN extrait de la communauté bactérienne associée aux biosolides P et PC a révélé que les ordres bactériens *Xanthomonodales*, *Pseudomonadales*, *Enterobacteriales*, *Burkholderiales*, *Actinomycetales*, *Bacillales*, *Clostridiales* et *Lactobacillales* se trouvaient dans les deux types de biosolides. Les bactéries du genre *Stenotrophomonas* étaient les plus abondantes dans les deux types de biosolides. L'addition de chitosane a toutefois induit des changements dans la population de quelques genres de bactéries. Par exemple, les clones transportant un gène d'ARNr 16S correspondant au genre *Bacillus* doublaient dans le biosolide PC. Dans des essais en champs entrepris dans le but de tester leur effet sur l'incidence de la gale commune, les biosolides P et PC ont été appliqués comme traitement des semences de pomme de terre. Le biosolide P a augmenté l'incidence de la maladie par un facteur de 1,33 et de 2,85 dans deux expériences indépendantes. Toutefois, quand le chitosane était ajouté au traitement de semences, l'effet stimulant du biosolide P sur la gale commune était aboli.

Mots clés : gène d'ARNr 16S, lisier, pomme de terre, *Streptomyces scabiei*, *Streptomyces scabies*.

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INTRODUCTION

Animal manure is commonly used in agriculture as a fertilizer to improve soil fertility and plant productivity (Schröder 2005). Some studies have shown that manure as well as some other types of organic matter may reduce the severity and incidence of some plant diseases (Hadar *et al.* 1992; Huber and Watson 1970). Although the mechanisms of disease suppression associated with organic matter are not fully understood, it has been postulated that such material could affect both the viability and survival of plant pathogens through the restriction of available nutrients, the release of antimicrobial substances (Bailey and Lazarovits 2003), or the modification of soil properties (Keinath and Loria 1989; Pennypacker 1989). In addition, organic amendments might stimulate the growth of microbial populations antagonistic to pathogens (Vruggink 1970). Several organisms antagonistic to soilborne pathogens have also been isolated from suppressive composts (Kwok *et al.* 1987; Labrie *et al.* 2001), suggesting that microorganisms colonizing organic matter might be at least partly responsible for the disease suppression effect attributed to those products.

Although the suppressive effect of animal manure has been demonstrated for several plant diseases (Gorissen *et al.* 2004; Osunlaja 1990), manure amendment is usually not recommended in potato fields. Some authors have reported that soil amendment with animal manure induced an increase in common scab incidence (Blodgett 1940), a disease caused by *Streptomyces scabies* (Lambert and Loria 1989) that results in important economic losses for the potato industry in Canada (Hill and Lazarovits 2005). However, the relationship between common scab incidence and manure application is still controversial. While some studies have established a correlation between manure application and common scab symptoms (Hooker 1981), other works have suggested that soil amendment with cattle and chicken manure reduced the incidence of this disease (Conn and Lazarovits 1999).

Other organic materials have proven to be efficient in reducing common scab of patato. For example, soil amendment with shrimp shells (Vruggink 1970) or with composts containing chitinous material (Côté *et al.* 2001) reduced common scab incidence. Beauséjour *et al.* (2003) also showed that a potato seed treatment with chitosan, a natural polymer known to inhibit the growth of several bacteria (Kurita 2006; Liu *et al.* 2001), could decrease the incidence of common scab of potato.

Here, we study a pork manure by-product. The technology used to obtain this product was developed by Envirogain (Saint-Romuald, QC, Canada). The aims of this study were to determine the effect of a chitosan amendment on the microbial community associated with the pork manure by-product and the effect of both amended and non-amended pork manure by-products on common scab of potato.

MATERIALS AND METHODS

Preparation of biosolid P and biosolid PC

Biosolid P was obtained as followed. Swine manure (Ferme CEPP, Saint-Anselme, QC, Canada) was first collected and homogenized. The first step in the manure treatment consisted of a physical separation of the liquid and solid components of pork manure. Liquid manure was detoxified in an aerobic-anoxic bioreactor that produces alternatively nitrification-denitrification conditions for a hydrolic retention time of more than 30 d at 35-40°C. Polyacrylamide (LPM Technologies Inc., Saint-Nicolas, QC, Canada), a flocculating agent, was added and mixed with the treated liquid manure at a concentration of 20 g m^{-3}. The mixture was allowed to settle during 60 min. The biological mud was then filtered and mixed with the solid components recovered in the first step of the biological treatment. This mixture was dried at 50°C for 24 h (93% of dry matter) to obtain biosolid P. Biosolid PC was obtained by adding non-sterile chitosan (Marinard Biotech, Rivière-au-Renard, QC, Canada) to biosolid P (1 g 100 g^{-1} of biosolid) after the drying step. Following this addition, biosolid PC was stored at room temperature for 10 d.

Microbial cell extraction

Microbial cells were extracted from 3 g of biosolid as previously described (Prévost *et al.* 2006). Briefly, a biosolid sample was added to 100 mL of sterile sodium pyrophosphate 0.1% (w:v) for 30 min at room temperature. This suspension was centrifuged at 55 g for 10 min. The resulting supernatant was centrifuged again at 3500 g for 15 min to recover microbial cells.

Enumeration of total bacteria, actinomycetes and fungi in biosolids

Microbial cells were extracted from 3 g of biosolid as described above and were dispersed in 10 mL of 0.85% NaCl. Bacteria, actinomycetes and fungi were enumerated by dilution plating on Nutrient Agar (NA; Difco Laboratories, Detroit, MI, USA) supplemented with 0.3 µg mL^{-1} cycloheximide and 0.05 µg mL^{-1} nystatin, Actinomycetes Isolation Agar (AIA; Difco Laboratories, Detroit, MI, USA) supplemented with the same antibiotics, and Potato Dextrose Agar (PDA; Difco Laboratories, Detroit, MI, USA) supplemented with 10 µg mL^{-1} penicillin G, 0.1 µg mL^{-1} ampicilin and 0.1 µg mL^{-1} kanamycin. NA, PDA and AIA plates were incubated at 28°C for 3, 7 and 8 d, respectively. Anaerobic bacteria count was also determined on NA plates supplemented with 0.3 µg mL^{-1} cycloheximide and 0.05 µg mL^{-1} nystatin, but the incubation was carried out in anaerobic jars for 3 d. All counts were carried out on four biosolid samples and were expressed as colony forming-unit (CFU) per g of dried biosolid.

DNA extraction from biosolid microorganisms

Microbial cells were recovered as described above and their DNA was extracted using the Kutchma *et al.* (1998) protocol modified as follows. Microbial cells (50 mg) were washed in 1 mL of TE (50 mM Tris-HCl, 20 mM EDTA, pH 8.0) and the suspension was centrifuged for 4 min at 16 000 *g*. The microbial pellet was resuspended in 1 mL of ice-cold acetone and

incubated on ice for 5 min. The acetone was then carefully removed by aspiration and the cells were lyzed with 500 µL of TE containing 1 mg mL^{-1} lysozyme (Sigma-Aldrich, Oakville, ON, Canada). SDS (sodium dodecyl sulphate) and NaCl were added to the lysozyme solution to a final concentration of 1.5% (v:v) and 37 mg $µL^{-1}$, respectively. The samples were submitted to a freeze-thaw cycle (-70°C/65°C) and then they were centrifuged at 16 000 *g* for 5 min to pellet cell debris. DNAse-free RNase (Ribonuclease A; Sigma-Aldrich, Oakville, ON, Canada) was added to the supernatant at a final concentration of 200 µg mL^{-1}. The extracted DNA was purified by a phenol:chloroform:isoamyl alcohol (25:24:1) extraction, precipitated with ethanol 95% and washed with ethanol 70%. DNA was finally dissolved in 300 µl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at -20°C.

PCR amplification of the 16s rRNA gene, cloning and sequencing

PCR amplification was carried out by using the universal primers for the 16s rRNA gene: BSF8/20 (5'-AGAGTTTGATCCTGGCTCAG-3') and BSR1541/20 (5-'AAGGA GGTGATCCAGCCGCA-3') (Whitehead and Cotta 2004). Amplification was performed in 50 µL total volume of a reaction mixture containing ~20 ng of biosolid DNA, 0.5 µL of *Taq* DNA polymerase (Amersham Biosciences, Piscataway, NJ, USA), an appropriate dilution of the manufacturer's buffer, each deoxynucleoside triphosphate at a concentration of 250 µM, each primer at a concentration of 0.2 pmol $µL^{-1}$, and 5 µL of DMSO. DNA amplification was performed with a T-personal thermocycler (Whatman Biometra, Goettingen, Germany) and the following program was used: one step of initial denaturation at 95°C for 10 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 90 s, and extension at 72°C for 90 s. This was followed by a final step of extention consisting of incubation at 72°C for 10 min. Amplicons were migrated in a 1.0% agarose gel and electropheresis was carried out in Tris-acetate-EDTA buffer (Sambrook and Russel 2001). Bands of about 1500 pb were excised from the gel and DNA was recovered using GFX PCR DNA and Gel Band Purification kit (Amersham Biosciences, Piscataway, NJ, USA). The 16s rDNA library was constructed by cloning the purified amplification products into vector pCR 2.1 (Invitrogen Corporation, Carlsbad, CA, USA) using a vector/insert ratio of 1:1, and the resulting plasmids were transformed into OneShot®INVαF' chemically competent *Escherichia coli* (Migula) Castellani & Chalmers cells (Invitrogen Corporation, Carlsbad, CA, USA). The transformants were plated onto Luria-Bertani (LB) plates containing 100 µg mL^{-1} ampicilin and 1 mg mL^{-1} X-gal (5-bromo-α-chloro-3-indol-β-D-galactopyraniside). White colonies were randomly picked up, grown in LB medium containing 100 µg mL^{-1} ampicilin, and their plasmids were isolated by using the Gene Elute™ Plasmid Miniprep Kit (Sigma-Aldrich, Oakville, ON, Canada). Plasmids carrying inserts were digested with *Eco*RI. One hundred clones from each biosolid were conserved and their inserts were sequenced at the Quebec Innovation Centre (Montréal, QC, Canada).

Phylogenetic analysis

The 16S rRNA gene sequences of our clones were compared with those from the RDP database using the BLAST program (Madden *et al.* 1996) from NCBI. The chimers check program from the Ribosome Database Project II site (Maidak *et al.* 2000) was used to detect inserts consisting of chimera. The partial 16S rDNA sequences obtained in this study were deposited in the GenBank database under accession numbers DQ374451 through DQ374468.

Field experiments

Trials were conducted in fields naturally infested with common scab-inducing pathogens as previously described (Beauséjour *et al.* 2003). The first site was located in L'Assomption (QC, Canada) and consisted of loamy soil. The second was located in Lavaltrie (QC, Canada) and consisted of sandy soil. Potatoes (*Solanum tuberosum* L. cv. Shepody) were grown in the L'Assomption and Lavaltrie sites in 2004 and 2005, respectively.

Band fertilisation with NH_4NO_3, P_2O_5 and K_2O (N-P-K) in 2004 at L'Assomption was 160, 120 and 190 kg ha^{-1}, respectively. Split application of NH_4NO_3 at 100 kg ha^{-1} and 60 kg ha^{-1} was carried out at planting and 7 wk later, respectively. Band N-P-K fertilisation in 2005 at Lavaltrie was 180, 100 and 200 kg ha^{-1}. Application of NH_4NO_3 and P_2O_5 was split. NH_4NO_3 was applied at planting and 7 wk later at 120 and 60 kg ha^{-1}, respectively, and P_2O_5 was applied at 120 kg ha^{-1} and 80 kg ha^{-1}, respectively. An experimental plot (7.5 m x 4.0 m) consisted of four rows planted with 26 seed tubers. Plots were set up as randomized complete blocks with four replicates, and were sepa-

Table 1. Counts of bacteria, actinomycetes and fungi from biosolids P and PC

Manure by-product	Counts (CFU g^{-1} of biosolid)[1]			
	Bacteria[2]		Actinomycetes	Fungi
	Aerobic	Anaerobic		
Biosolid P	4.1×10^5a	2.0×10^4a	7.5×10^2a	1.0×10^3a
Biosolid PC	3.5×10^5a	1.1×10^4b	7.0×10^2a	7.5×10^2b

[1] CFU: colony-forming unit.
[2] Within a column, data followed by the same letter do not differ significantly ($P > 0.05$, LSD test).

rated by two flanking rows of potatoes. A potato seed treatment was carried out at plantation. Each potato seed was covered with 2 g of biosolid P, biosolid PC or talc. At harvest, all potato tubers were harvested and potato yield was measured for each plot. One hundred tubers from each plot were used to evaluate disease incidence, which was determined as the number of infected tubers covered by scab lesions on more than 5% of their surface over the total number of tubers.

Statistical analyses

Statistical analyses were carried out using the GLM procedure of the SAS 9.1 statistical package. Most sets of data requiring statistical analyses were performed with one-way ANOVAs followed by LSD tests. A χ^2 test was carried out to compare disease incidence between treatments.

RESULTS

Bacteria, actinomycetes and fungi counts in biosolids P and PC

The main microbial groups found in the biosolids were enumerated on culture media. Chitosan amendment brought slight changes in the microflora composition of biosolid P. Chitosan-amended biosolid PC contained significantly lower numbers of anaerobic bacteria (1.1×10^4 CFU g^{-1}) than the non-amended product (2.0×10^4 CFU g^{-1}) (Table 1). Chitosan also significantly reduced the number of fungi from 1.0×10^3 CFU g^{-1} in biosolid P to 7.5×10^2 CFU g^{-1} in biosolid PC. Counts of total aerobic bacteria and actinomycetes did not significantly differ within biosolids (Table 1).

Sequencing of library clones

Partial sequences of the 16S rDNA gene were obtained for 100 clones associated with biosolids P and PC. Sequences associated with clones 20B and 70B were considered as putative chimeras (Maidak *et al.* 2000) and were not further analyzed. BLAST algorithms from NCBI were used to align clone sequences with previously characterized genes from

Table 2. Phylogenetic groups associated with biosolids P and PC

		Proportion of clones (%)	
Order	**Genus**	**Biosolid P**	**Biosolid PC**
Xanthomonodales	*Stenotrophomonas*	26	28
	Xanthomonas	3	4
	Thermomonas	0	1
Pseudomonadales	*Acinetobacter*	9	6
	Pseudomonas	0	2
	Psychrobacter	1	0
Enterobacteriales	*Shigella*	1	0
	Photorabdus	0	1
	Salmonella	1	0
Burkholderiales	*Comamonas*	2	7
	Bordetella	1	0
	Acidovorax	2	1
	Alcaligenes	1	0
Actinomycetales	*Brevibacterium*	3	4
	Arthrobacter	4	4
	Terrabacter	1	0
	Corynebacterium	2	0
	Nocardiodes	1	0
Bacillales	*Ureibacillus*	4	4
	Bacillus	5	10
	Caryophanon	1	4
	Sporosarcina	0	1
	Staphylococcus	4	2
	Turicibacteraceae	2	0
Clostridiales	*Clostridium*	1	1
Lactobacillales	*Globicatella*	0	2
	Tricochococcus	1	0
	Enterococcus	0	2

the RDP database. A number of clones (21 in biosolid P and 16 in biosolid PC) were associated with bacteria of unknown taxonomic identity, while respectively 77% and 84% of the clones from biosolid P and biosolid PC shared between 95 and 99% identity with the 16S rDNA gene of an identified bacterial strain.

Phylogenetic analysis

Table 2 presents the list of clones found in biosolids P and PC that shared at least 95% similarity with a 16S rDNA sequence from a strain belonging to a characterized bacterial genus. In both biosolids, clones were associated with the following bacterial orders: *Xanthomonodales*, *Pseudomonadales, Enterobacteriales*, *Burkholderiales*, *Actinomycetales, Bacillales, Clostridiales* and *Lactobacillales*. Bacteria from the *Xanthomonodales* order represented the main phylogenetic group in both biosolids, and bacteria from the *Stenotrophomonas* genus were the most abundant. They accounted for 26 and 28% of P and PC biosolid clones, respectively. Some genera were similarly represented within both biosolids. This is the case for *Arthrobacter*, *Ureibacillus* and *Clostridium*.

However, chitosan appeared to induce change in the population of some bacterial genera (Table 2). For example, clones associated with the genus *Bacillus* doubled to reach 10% of the PC clones. *Comamonas*, which represented 2% of the biosolid P clones, accounted for 7% of the biosolid PC clones. *Caryophanon* represented 1 and 4% of the P and PC biosolid clones, respectively. *Pseudomonas*, *Globicatella*, *Enterococcus*, *Thermomonas*, *Photorabdus* and *Sporsarcina*, which were not detected in biosolid P, were found in a proportion of 1 to 2% in biosolid PC. On the other hand, the addition of chitosan appeared to reduce the population of some other bacterial genera. For instance, the proportion of clones associated with the genera *Acinetobacter* and *Staphyloccocus* was reduced by about 50% in biosolid PC. *Corynebacterium*, which represented 2% of the biosolid P clone library, was not detected in biosolid PC.

The 16S rDNA sequences of 21 and 16% of the clones from biosolids P and PC, respectively, showed homology with uncultured bacteria for which a 16S rRNA gene sequence was deposited in the database. Table 3 presents the origin of the uncultured bacteria associated with the biosolid clones. Most of the uncultured bacteria originated from a farm environment, i.e. animal, manure or agricultural soil. The majority of the unidentified clones shared similarity with bacteria isolated from swine manure biofilms or from pig gastrointestinal tracts.

Table 3. Proportion of biosolid clones showing 16S rDNA sequence homology with sequences from uncultured bacteria

Uncultured bacterium	Accession number[1]	Origin	Proportion of clones (%)	
			Biosolid P	Biosolid PC
Actinobacterium EB1077	DQ374451	Pasture soil	2	0
Bacterium SM-2	DQ374452	Swine manure biofilm	6	6
β-proteobacterium AKYG862	DQ374453	Farm soil adjacent to a silage storage bunker	1	0
Clone p-406-o3	DQ374454	Pig gastrointestinal tract	1	0
Clone THM-10	DQ374455	Pig gastrointestinal tract	2	1
Clone p-248-o5	DQ374456	Pig gastrointestinal tract	2	1
Clone p-4936-6wbz	DQ374467	Pig gastrointestinal tract	0	1
Clone 2	DQ374457	Piglet gastrointestinal tract	1	0
Clone D-66	DQ374458	Teat canals of healthy lactating bovines	1	0
Clone B-85	DQ374465	Teat canals of healthy lactating bovines	0	1
Clone GZKB112	DQ374459	Municipal solid waste landfill	2	1
Clone TSBw02	DQ374460	Polychlorinated dioxin dechlorinating microcosm	1	0
Bacterium DSSD95	DQ374461	Drinking water in a distribution system	1	0
Uncultured Acinetobacter	DQ374462	Ectoparasitic chewing lice of pocket gophers	2	0
Bacterium B24	DQ374463	Feedlot manure	0	2
Clone sl2_505	DQ374464	Grassland soil	0	1
α-proteobacterium Bigi7	DQ374466	Industrial waste gas biofilter	0	1
Clone A-3L	DQ374468	Anaerobic swine lagoon	0	1

[1]These sequences exhibit more than 95% homology with the 16S rDNA sequences of biosolid clones.

Table 4. Effect of potato seed treatments consisting of dehydrated pork manure by-product amended or not with chitosan on common scab of potato

Potato seed treatment	L'Assomption (2004)		Lavaltrie (2005)	
	Disease incidence (%)[1]	Yield (kg/plot)[2]	Disease incidence (%)[1]	Yield (kg/plot)[2]
Talc (control)	57 b	50.4 a	20 a	24.8 a
Biosolid P	76 c	48.7 a	57 b	26.4 a
Biosolid PC	49 a	58.6 a	22 a	23.6 a

[1] Within a column, data followed by the same letter do not differ significantly ($P < 0.05$; χ^2 test).
[2] Within a column, data followed by the same letter do not differ significantly ($P < 0.05$; LSD test).

Effect of pork manure biosolid on common scab of potato

Field assays were carried out over 2 consecutive yr and the effect of biosolids on common scab of potato was analyzed. As shown in Table 4, common scab was more severe in 2004 than in 2005 as indicated by the higher level of disease incidence (57.0 *versus* 19.5%) in the control treatment. A seed treatment with biosolid P increased the amount of diseased tubers by a factor of 1.33 and 2.85 in 2004 and 2005, respectively. The addition of chitosan in biosolid P suppressed the stimulating effect of this seed treatment on common scab disease. Biosolid PC used as seed treatment reduced by approximately 15% the level of common scab in 2004 compared with the control treatment. Such a reduction was not observed in 2005, but common scab incidence was brought back to a level comparable to that of the control treatment ($P > 0.05$, χ^2 test) (Table 4). Application of biosolids P and PC as seed treatments did not significantly affect potato yield.

DISCUSSION

Microbial analysis of the biosolids used in this study revealed the origin of this organic material. Indeed, the 16Sr DNA sequences identified from the biosolid microbial community were often associated with those of microorganisms previously isolated from manure (*Stenotrophomonas*, *Bacillus* and *Acinetobacter*) (Laukovà 2001; Lueng and Topp 2001; Sasaki *et al.* 2005; Snell-Castro *et al.* 2005) or with uncultured bacteria from manure or pig microflora. The fact that a considerable proportion of clones in the biosolids (21 and 16% in biosolids P and PC, respectively) could not be associated with a bacterium of known taxonomic identity shows the need for further research on the microbiology of manure and manure by-products. Marti *et al.* (2009) also reported that a high proportion of 16S rRNA gene sequences found in manure presented a low level of similarity to sequences in databases.

Previous studies using culture-dependent methods established the fact that the main taxonomic groups found in swine manure are Gram-positive anaerobic bacteria, such as *Clostridium*, *Lactobacillus* and *Streptoccoccus* (Whitehead and Cotta 2001). Although bacteria belonging to the *Clostridiales* and *Lactobacillales* orders were also found in biosolids, they did not represent a high proportion of the clones. The bacterial composition of the biosolids and the raw manure appeared to differ considerably, suggesting that the biological treatment of pork manure modified considerably the microflora of the product. However, data obtained from culture-dependent and culture-independent methods are not always congruent (Dobrovol'skaya *et al.* 2001; Zhang *et al.* 2009). One could therefore not exclude the possibility that differences between the microbial composition of swine manure (Whitehead and Cotta 2001) and biosolids (this work) are a consequence of the experimental procedure used in both studies.

The data presented here suggest that Gram-negative bacteria belonging to the *Stenotrophomonas* genus prevailed in the biosolids. *Stenotrophomonas* is a widespread bacterium in the environment. It was found in the soil, on plant surfaces as well as in aquatic environments (Denton and Kerr 1998). *Stenotrophomonas* strains also represent a bacterial group of agronomic importance, and they were found to be associated with the rhizosphere of numerous plant species (Berg 1996; Berg *et al.* 1996; Lottmann *et al.* 1999).

Members of the genera *Acinetobacter* and *Bacillus* also appeared to be predominant bacteria in biosolids. Bacteria of the *Acinetobacter* genus are widely distributed in nature and could be isolated from soil, water and human skin (Jawad *et al.* 1998). In activated sludge systems, they participated in the removal of phosphorus (Beachman *et al.* 1990; Mino *et al.* 1998). In these applications, some authors have even established that *Acinetobacter* was the primary biological agent responsible for phosphate removal (Auling *et al.* 1991; Wagner *et al.* 1994). The presence of an important amount of phosphate in manure could thus explain the presence of these bacteria in the by-products of the manure transformation process. The fact that anaerobic and aerobic conditions alternated during the biological treatment of swine manure could also have favoured *Acinetobacter*. These conditions have been reported to promote the growth of microorganisms accumulating phosphates (Grady *et al.* 1999).

Although *Bacillus* strains have frequently been isolated from manure (Lueng and Topp 2001; Ouwerkerk and Klieve 2001; Snell-Castro *et al.* 2005), they did

represent a low proportion of the manure microbial colonizers. The transformation process used to obtain the biosolids may have exposed manure microorganisms to dryness and high temperatures. These conditions could have introduced a positive selective bias for bacteria such as *Bacillus* species that produce heat-resistant spores.

Biosolids P and PC were compared for their microbial composition. The number of bacteria and actinomycetes colonizing the biosolids did not appear to be affected by the addition of chitosan, while the populations of both fungal organisms and anaerobic bacteria only slightly decreased in the presence of chitosan. This slight decrease might be attributed to the antimicrobial activity of chitosan. This polymer has been shown to be generally more toxic on fungi than on bacteria (Savard *et al.* 2002). The decrease in anaerobic bacteria population might confer an advantage to biosolid PC in agricultural practices. Anaerobic bacteria such as *Eubacterium* and *Clostridium* are the main contributors of volatile fatty acids associated with manure odor (Rappert and Müller 2005; Zhu 2000), while others are potential pathogens (Nervig *et al.* 1981). Few studies have reported the effect of chitosan on anaerobic bacteria and most of these focused on human pathogen or food spoilage bacteria. Inatsu *et al.* (2005) established that chitosan exhibited a bactericidal activity against *E. coli*, *Salmonella enteritidis* (Gaertner) Castellani & Chalmers, *Staphylococcus aureus* Rosenbach and *Listeria monocytogenes* (Murray *et al.*) Pirie in fermented chinese cabbage. The 16S rDNA analysis carried out in this study suggest that the potential pathogenic bacteria usually found in manure, such as *E. coli*, *Campylobacter* and *Salmonella* (Sobsey *et al.* 2001), did not account for a high proportion of the biosolid microflora. Nevertheless, other potential human pathogens were detected in biosolid P (7% of the clones were associated with *Samonella*, *Staphylococcus*, *Shigella* and *Bordetella*), while no clone related to these genera was identified in biosolid PC. The growth of *Shigella dysenteriae* (Shiga) Castellani & Chalmers, *Salmonella typhimurium* (Loeffer) Castellani & Chalmers, and *Staphylococcus aureus* has previously been found to be inhibited in the presence of chitosan (Helander *et al.* 2001; Liu *et al.* 2001; Tsai *et al.* 2000). However, since the antimicrobial action of chitosan is influenced by intrinsic factors such as the degree of chitosan polymerization, the proportion of acetylated residues in the polymer and environmental conditions (Rabea *et al.* 2003), further analyses would be required to determine the antimicrobial activity of chitosan on potential pathogens present in both manure and biosolids.

While chitosan has a negative effect on some microbial groups, *Bacillus* and *Comamonas* prevailed in biosolid PC. The ability of several *Bacillus* and *Comamonas* strains to produce chitosanases (Choong Soo *et al.* 2005; Kurakake *et al.* 2000; Omumasaba *et al.* 2000) could explain their prevalence since chitosan would represent an additional carbon and nitrogen source for such chitosanolytic microorganisms.

The use of animal manure for potato crops is controversial since some authors have observed an increase in common scab incidence following soil amendment with manure (Blodgett 1940), while others have reported common scab suppression through manure amendment (Conn and Lazarovits 1999). These contradictory results might be explained by the complexity of the manure substrate (Conn and Lazarovits 1999). Even if biological treatments modifying the microbial composition of manure could also influence the properties of this organic material, our study reveals that manure solids could effectively contribute to common scab incidence.

When applied as seed treatment in 2004 and 2005, biosolid P significantly increased common scab incidence. The mechanisms linked to the stimulating effect of a local application of biosolid P (seed treatment) on disease symptom development have yet to be elucidated. Biosolid P possibly brought nutrients or physicochemical conditions that conferred an advantage to the *S. scabies* population or that might contribute to a better colonization of the potato seed tubers by the *S. scabies*. Han *et al.* (2008) suggested that the soil population of common scab-inducing pathogens colonized seed potatoes at planting and modified root development. Alternatively, the microbial community of biosolid P might contribute to the increased level of disease by competing with *S. scabies* antagonistic agents that colonize soil and potato organs (Liu *et al.* 1995) or by secreting compounds that stimulate toxin biosynthesis in *S. scabies* (Agbessi *et al.* 2003).

The addition of chitosan to biosolid P cancelled the stimulating effect of this product on common scab incidence. In 2005, biosolid PC even decreased common scab incidence by 14% when compared with the control treatment. One could postulate that chitosan may contribute to disease suppression by eliciting plant defence mechanisms (Benhamou *et al.* 1994) or by inhibiting the growth of the pathogen. This is unlikely the case since Jobin *et al.* (2005) showed that chitosan beads were degraded within 1 wk after their addition to a soil substrate. The manure by-product used in this study was incubated in the presence of chitosan for over 1 wk before application on potato tubers.

In other studies, a soil amendment with chitinous material (Vruggink 1970) or shrimp waste-based composts was shown to both stimulate the growth of Gram-positive bacteria and to reduce common scab incidence (Côté *et al.* 2001). Interestingly, some microorganisms antagonistic to *S. scabies* were able to grow on chitosan as carbon and nitrogen sources (Beauséjour *et al.* 2003). Chitosan amendment could therefore have modified the microflora of biosolid P to promote bacterial populations antagonistic to *S. scabies*. The increase in *Bacillus* population, a genus known to comprise numerous chitosanase producers (Kurakake *et al.* 2000; Omumasaba *et al.* 2000) as well as biocontrol agents of common scab (Han *et al.* 2005; Sturz *et al.* 2004), could explain why biosolid PC annihilated the stimulating effect of the non-amended manure by-product on common scab.

Several studies have demonstrated that chitosan contributes to plant protection (Bautista-Banos *et al.* 2006) by stimulating plant defence mechanisms (Benhamou *et al.* 1994) and by inhibiting the growth

of several plant pathogens (Rabea *et al.* 2003), but as showed here, chitosan amendment could also modify the microbial communities of an agricultural product. Such an amendment could promote the innocuity or the biocontrol efficiency of organic fertilizers, plant growth substrates and seed treatments.

ACKNOWLEDGEMENTS

This work was supported by grants from the Conseil de recherches en pêche et en agroalimentaire du Québec (CORPAQ) and the Natural Sciences and Engineering Research Council (NSERC) of Canada. We also acknowledge the contribution of Envirogain. We thank M. Lapointe for technical assistance and S. Roy for a critical review of the manuscript.

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