Pseudomonas Spp. can Inhibit Streptomyces scabiei Growth and Repress the Expression of Genes Involved in Pathogenesis

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Abstract

Common scab, caused by Streptomyces scabiei, is an economically important disease affecting potato crops worldwide. Confirmed and putative pathogenicity- and virulence-related factors, including the phytotoxic thaxtomins, the necrosis protein Nec1 and the tomatinase TomA, have been characterized in S. scabiei. Using plate inhibition assays, the ability of three antimicrobial metabolite-producing Pseudomonas strains (LBUM 223, LBUM 300 and LBUM 647) to inhibit the growth of S. scabiei was studied. Their capacity to alter the expression of thaxtomin biosynthesis genes (txtA and txtC), nec1 and tomA was also investigated using newly developed TaqMan probe-based quantitative reverse transcription-polymerase chain reaction assays. Pseudomonas sp. LBUM 223 significantly inhibited S. scabiei growth and repressed transcription of all targeted genes in the pathogen. S. scabiei growth was also significantly inhibited by Pseudomonas sp. LBUM 300; however, this strain failed to alter the expression of any of the targeted genes. Finally, Pseudomonas sp. LBUM 647 was unsuccessful both at inhibiting pathogen growth and at repressing gene transcription in S. scabiei. To our knowledge, this is the first demonstration that an antagonistic organism can repress the expression of key genes involved in S. scabiei pathogenesis. This capacity is unlikely a trait common to all Pseudomonas spp.

Keywords: Common scab of potato; Streptomyces scabiei; Pseudomonas sp.; Growth inhibition; Gene expression

Abbreviations: ABI: Applied Biosystems; OBA: Oat Bran Agar; PCA: Phenazine-1-Carboxylic Acid; PCR: polymerase chain reaction; RT: Reverse Transcription; SAS: Statistical Analysis Software

Introduction

Common scab of potato, caused primarily by Streptomyces scabiei [1], prevails in many regions of the world cultivating potato [2], including Canada [3]. Severe symptoms of the disease, described as superficial, raised or sunken necrotic lesions on the tuber’s surface [4], render the diseased tubers unmarketable, resulting in important economic losses [3]. Scab-causing streptomycetes induce symptom development on potato tubers by producing thaxtomins [5,6], a family of phytotoxic cyclic dipeptides [7]. Biosynthesis of thaxtomin A, the most potent toxin of the thaxtomin family [8], is carried out by numerous enzymes, including the thaxtomin synthetase TxtA [5] and the mono oxygenase TxtC [9]. The necrogenic protein Nec1, which is involved in the colonization of the infection site, is another virulence factor [10] and the tomatinase TomA, which hydrolyzes the tomato phytotoxic lipopeptide α-tomatine [11], is suspected to be involved in pathogenesis [12].

Some Pseudomonas spp., which are omnipresent soil-inhabiting bacteria [13], are able to protect plants from diseases, such as take-all [14, 15] and black root rot [14], through their interaction with the causative plant pathogens. Production of antimicrobial secondary metabolites, such as phenazine-1-carboxylic acid (PCA) [15], 2,4-diacyltetloroglucinol [14] and hydrogen cyanide [16], enables some strains of Pseudomonas to inhibit the growth of plant pathogens. Other Pseudomonas strains can alter molecular processes leading to the production of pathogenicity- and virulence factors by the plant pathogen. For example, Pseudomonas strain G degrades a diffusible signal factor required for the expression of virulence factors in the black root pathogen Xanthomonas campestris pv. campestris [17,18]. However, only one study has described Pseudomonas spp.-mediated growth inhibition of plant-pathogenic S. scabiei [19] and none, to our knowledge, has ever reported on the ability of an antagonistic microorganism to alter the expression of pathogenicity- or virulence-associated genes in S. scabiei.

In this study, we sought to evaluate the ability of three Pseudomonas strains to (i) inhibit S. scabiei growth, using plate inhibition assays and (ii) alter the expression of four pathogenicity- and virulence-associated genes (txtA, txtC, nec1 and tomA) in the pathogen, using newly developed TaqMan probe-based quantitative reverse transcription (RT)-polymerase chain reaction (PCR) assays. Furthermore, we sought to describe the relationship between txtA and txtC gene expression in S. scabiei and thaxtomin A production by the pathogen using time-course assays. The Pseudomonas strains studied here (LBUM 223, LBUM 300 and LBUM 647) are capable of inhibiting the growth of many plant pathogens [20,21]. Furthermore, characterization of the Pseudomonas strains revealed that their respective genomes harbor antimicrobial metabolite biosynthesis genes. Pseudomonas sp. LBUM 223 and Pseudomonas sp. LBUM 647 possess the operons for phenazine and hydrogen cyanide biosynthesis, respectively, whereas Pseudomonas sp. LBUM 300 possesses both the 2,4-diacyltetloroglucinol and the hydrogen cyanide biosynthesis operons [20,21].

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Materials and Methods

Bacterial strains used in this study

*S. scabies* strain LBUM 848 [22] and *Pseudomonas* strains LBUM 223, LBUM 300 and LBUM 647 [20,21] are described elsewhere.

Plate inhibition assays

Four treatments were prepared in triplicate, for a total of 12 samples: (i) *S. scabies* only (no antagonist), (ii) *S. scabies* and LBUM 223, (iii) *S. scabies* and LBUM 300 and (iv) *S. scabies* and LBUM 647. *S. scabies* and each *Pseudomonas* strain were grown in 10 ml of oat bran broth (pH 7.2), prepared as described previously [23] and 10 ml of tryptic soy broth (BD, Mississauga, ON, Canada), respectively. Cultures were incubated with continuous shaking at 28°C for 6 days (*S. scabies*) or 24 h (*Pseudomonas* strains). A 100-μl aliquot of *S. scabies* culture was spread onto 20-ml oat bran agar (OBA) plates (100 x 15 mm). OBA medium (pH 7.2) was prepared as described previously [23]. After briefly air-drying plates, a 20-μl aliquot of *Pseudomonas* sp. culture, containing approximately 7.3 x 10^7 CFU, was spotted on the surface of the medium in the center of the plate. Plates were randomized (complete randomized bloc design) and incuated at 28°C for 6 days. The inhibition zone (distance between mycelia and spores established on the plate. Plates were randomized (complete randomized bloc design) and incubated at 28°C for 6 days. The inhibition zone (distance between the edge of the antagonist spot and that of the vegetative mycelium growth inhibition area) was measured and total RNA was extracted from all the *S. scabies* mycelia and spores established on the plate. The experiment was performed three times.

RNA extractions and DNase treatments

Total RNA was extracted from *S. scabies* cells using the Ultraclean Microbial RNA Isolation kit (MO BIO Laboratories, Carlsbad, CA, USA). Mycelia and spores of the pathogen were retrieved from OBA plates by gently scraping the surface of the medium with a sterile metal spatula. Cells were transferred into Micro RNA Bead Tubes, to which were then added 300 and 15 μl of solutions MR1 and MR2, respectively. The remaining extraction steps were performed essentially as described by the manufacturer; however, a supplementary cell homogenization step, using a Fast Prep FP120 (Qbiogene, Carlsbad, CA, USA), was added at this point in the process. The high-performance liquid chromatography system consisted of a Series 1100 quaternary pump (Agilent Technologies, Wilmington, DE, USA), an 1100 quaternary pump (Agilent Technologies, Wilmington, DE, USA), a Gilson 118 UV/Vis detector (Gilson Medical Electronics, Middleton, WI, USA) and Class VP chromatographic software (Shimadzu Scientific Instruments, Columbia, MD, USA). The mobile phase was water: acetonitrile (73:27), filtered through a 0.45-μm syringe filters and injected into a Supelco LC-18 column (4.6 x 150 mm) using a Rheodyne Model 7125 injector with a 20-μl sample loop. The high-performance liquid chromatography system consisted of a Series 1100 quaternary pump (Agilent Technologies, Wilmington, DE, USA), a Gilson 118 UV/Vis detector (Gilson Medical Electronics, Middleton, WI, USA) and Class VP chromatographic software (Shimadzu Scientific Instruments, Columbia, MD, USA). The mobile phase was water: acetonitrile (73:27), at a flow rate of 1.0 ml/min. Thaxtomin was eluted at a retention time of 6.3 min and was detected at 380 nm. Calibration standards were prepared by dissolving thaxtomin A (provided by R.R. King, Potato Research Center, Agriculture and Agri-

| Targeted gene | Name   | Sequence 5'→3' | Amplicon size (bp) |
|---------------|--------|----------------|--------------------|
| *rpoB*        | rpoBF  | CGT CGG CTC CAT CAA GGA | 68                 |
| *rpoB*        | rpoBr  | GGC GGT TGT TCT GGT CCA T | 69                 |
| *rpoB*        | rpoBprobe | CTT CGG CAC CAG CCA | 61                 |
| *txtA*        | txtAfor | TTG TCA ACT CCG TGA TCC AGT A | 54                 |
| *txtA*        | txtAr  | GGG ACA CCT CGC GCA GTA | 54                 |
| *txtC*        | txtCfor | ACC ATC TCG TCG TCG TTG GT | 67                 |
| *txtC*        | txtCrev | CGT GGA CGA CGG AGA ACT TC | 67                 |
| *txtC*        | txtCprobe | TTA TGC ACT GCA GCC GG | 67                 |
| *nec1*        | nec1for | GCT TGG GGC GGT ATG CT | 67                 |
| *nec1*        | nec1rev | TGC AGG CGA GGT TTA AA | 67                 |
| *tomA*        | tomAfor | CCA GAA GCT CGG ACT CGA AGT | 67                 |
| *tomA*        | tomArev | CGT CTG ATC CAC GTG GGA GGT | 67                 |

*F*, forward primer; *R*, reverse primer; *P*, TaqMan probe

Table 1: Sequences of primers and TaqMan probes used in this study.
Food Canada, Fredericton, NB, Canada) in the mobile phase. A three-point standard curve yielded a correlation coefficient of 0.9997. The concentration of stock solutions of thaxtomin A was confirmed using the Beer-Lambert Law and the molecular absorptivity of thaxtomin A in ethanol (4050 at 398 nm). An extraction efficiency of 86±4% (mean ± standard deviation, n = 2) was estimated by quantifying extracted thaxtomin A from OBA plates supplemented with 20 μg of purified thaxtomin A.

**Primer and probe design**

The following sequences were retrieved from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov); accession numbers FJ007430-FJ007480 (rpoB), FJ007753-FJ007759, AF255732 (txtA), FJ007580-FJ007629, AF393159 (txtC), AM293590, AM293591, AF385166-AF385180 (nec1), FJ007748-FJ007759 and AY707079 (tomA). Multiple sequence alignments were performed for each data set using the ClustalW function in the BioEdit Sequence Alignment Editor 7.0.4.1 software [24]. PCR primers and TaqMan probes targeting 100% conserved sequences were designed with Primer Express 3.0 (Applied Biosystems (ABI), Foster City, CA, USA). Probes were labeled with the reporter dye 6-carboxyfluorescein (5´end) and the minor groove binder non-fluorescent quencher (3´end). Primers and probes were synthesized by Integrated DNA Technologies (Coralville, IA and USA) and ABI, respectively.

**Quantitative RT-PCRs**

RNA transcripts of genes rpoB, txtA, txtC, nec1 and tomA were reversely transcribed using the TaqMan Reverse Transcription Reagents kit (ABI) and the reverse primers rpoBr, txtArev, txtCrev, nec1rev and tomArev, respectively (Table 1). Twenty-microliter RT reactions were prepared and carried out in a PTC-200 DNA Engine Thermal Cycler (MJ Research, Waltham, MA, USA), as described by the manufacturer. RT products were stored at -20°C. Products were reversely transcribed using the TaqMan Reverse Transcription Reagents kit (ABI) and the reverse primers rpoBr, txtArev, txtCrev, nec1rev and tomArev, respectively (Table 1). Twenty-microliter RT reactions were performed and carried out in a PTC-200 DNA Engine Thermal Cycler (MJ Research, Waltham, MA, USA), as described by the manufacturer. RT products were stored at -20°C. Products were

**Data processing**

Data from each experimental replicate were processed independently as follows. Raw fluorescence data were baseline-corrected using the default settings of the 7500 System SDS Software version 1.4 (ABI). Baseline-corrected [25]. For a given gene, calculated midpoints and amplification efficiencies of each individual reaction were averaged among all samples and used to calculate R0̄ values. Gene expression values of the target genes were normalized to those of the endogenous reference gene using the following formula: R0̄ = R0̄/R0̄. Fold changes correspond to the quotient of the normalized gene expression value in the treatment and the mean normalized gene expression value in the calibrator. The calibrator of the plate inhibition assays and the time-course studies consisted of the "Day 3" samples, respectively. Mean fold change values and standard errors were calculated using the Statistical Analysis Software (SAS) 9.1.3 (SAS Institute, Cary, NC, USA, 2002-2003). Fluorescence data (ΔRn) were exported from the SDS software. Amplification plots of the endogenous reference gene (rpoB) and each of the target genes (txtA, txtC, nec1 and tomA) were analyzed using the DART-PCR 1.0 workbook

**Statistical analyses**

**Plate inhibition assays:** Inhibition zones data were rank-transformed. Relative fold change values were power-transformed (x^1/0.20) to ensure normal distribution of residuals and homogeneity of variances. Using the MIXED procedure in SAS, univariate one-way mixed-model analyses of variance were carried out to investigate the effect of the antagonist treatment on (i) S. scabies growth in vitro and on (ii) txtA, (iii) txtC, (iv) nec1 and (v) tomA gene expression in the pathogen. The antagonist treatment and the experimental replicate were the fixed and random effects, respectively. A posteriori multiple comparisons of least squared means were performed using the Tukey-Kramer method. P values equal to or smaller than 0.05 were deemed significant. Using the GLM (general linear model) procedure in SAS, a multivariate analysis of variance was undertaken to correct for false-discoveries. The effect of the antagonist treatment on the overall expression of pathogenicity- and virulence-associated genes in the pathogen was investigated. Wilks’ lambda statistic was used to ascertain statistical significance.

**Time-course studies:** Cross-correlations between mean relative txtA and txtC fold change values and mean thaxtomin A production were evaluated using the cross-correlation function of the ARIMA (auto-regressive integrated moving average) procedure of SAS. Correlation coefficients greater than two standard errors were deemed statistically significant.

**Results**

**Growth inhibition of S. scabies**

The ability of *Pseudomonas* strains LBUM 223, LBUM 300 and LBUM 647 to inhibit *S. scabies* growth was examined. The antagonist treatment significantly affected pathogen growth in vitro (F1, 20 = 135.29, P < 0.01). Of the three *Pseudomonas* strains tested, only *Pseudomonas* strain LBUM 223 and LBUM 300 significantly inhibited pathogen growth on OBA medium. Furthermore, the inhibitory activity of *Pseudomonas* sp. LBUM 223 was significantly greater than that of *Pseudomonas* sp. LBUM 300 by approximately 30% (Figure 1).

**Figure 1: Growth inhibition of common scab-inducing *S. scabies* by various *Pseudomonas* strains.** In plate inhibition assays, *S. scabies* was either grown in the absence of an antagonist or was confronted with a *Pseudomonas* strain (LBUM 223, LBUM 300 or LBUM 647). Growth inhibition zones were measured after 6 days. Data are presented as mean ± standard error (n = 9). Means of groups annotated with different letters were significantly different (P < 0.05).
four pathogenicity- and virulence-associated genes in *S. scabies* confronted with either *Pseudomonas* sp. LBUM 300 or *Pseudomonas* sp. LBUM 647 did not significantly differ from those noted in the pathogen grown in the absence of an antagonist (Figure 2). The multivariate analysis further corroborated the above results (results not shown).

**txtA** and **txtC** gene expression and thaxtomin A production over time

A time-course study was undertaken to assess the correlation between **txtA** and **txtC** gene expression and thaxtomin A production (Figure 3). Thaxtomin A production was first detected at 2 days post-inoculation at the onset of aerial mycelium growth. It increased from day 2 to day 5 and then generally decreased. Thaxtomin A was detected at all sampling dates from day 2 to day 10. **txtA** and **txtC** gene transcripts were detected from day 3 to day 10. Gene expression was not investigated prior to day 3 as sufficient amounts of biological material were not available for total RNA extractions. Cross-correlations were used to determine the correlation between the trends in thaxtomin biosynthesis gene expression and those in thaxtomin A production. **txtA** and **txtC** gene expression were significantly cross-correlated (*R* = 0.81) and both **txtA** gene expression and **txtC** gene expression were significantly cross-correlated with thaxtomin A production (*R* = 0.97 and *R* = 0.79, respectively). In general, mean thaxtomin A production as well as mean **txtA** and **txtC** gene expression levels reached a maximum at day 5, then generally decreased. No time-delayed effects were noted, indicating that production of thaxtomin A closely followed **txtA** and **txtC** gene expression.

**Discussion**

In this study, three *Pseudomonas* strains were investigated for their ability to (i) inhibit the growth of common scab-inducing *S. scabies* and to (ii) alter the expression of four pathogenicity- and virulence-related genes in this pathogen. *Pseudomonas* strains LBUM 223 and LBUM 300 effectively inhibited the growth of *S. scabies* in vitro. As *Pseudomonas* sp. LBUM 647 failed to inhibit *S. scabies* growth, the ability to affect growth of the target organism is unlikely a trait common to all antagonistic *Pseudomonas* spp.

*Pseudomonas* sp. LBUM 223 was also able to repress the expression of the pathogenicity- and virulence-associated genes **txtA**, **txtC**, **nec1** and **tomA**, whereas *Pseudomonas* strains LBUM 300 and LBUM 647 were unable to significantly alter the expression of any of these investigated genes. To our knowledge, repression of genes involved in *S. scabies* pathogenesis by another antagonistic organism has never been demonstrated. Reactions catalyzed by the thaxtomin synthetase **TtxA** [5] and the mono oxygenase **TtxC** [9] are crucial to the synthesis of the plant toxin thaxtomin A by scab-causing streptomycetes. The reduction in thaxtomin A production by *S. scabies* resulting from a decrease in **txtA** and **txtC** gene expression (this study) would likely lessen pathogen virulence. It has been demonstrated previously that strain virulence on potato is positively correlated with the quantities of thaxtomin A produced [26].

Many bacteria can control plant diseases by altering molecular processes leading to the production of pathogenicity and/or virulence factors by the pathogen. For example, *Bacillus thuringiensis* subsp. *israelensis* B23 suppresses *Erwinia carotovora*-induced soft rot development on potato tuber slices in part by degrading N-acylhomoserine lactones [27] which are required for the expression of virulence factors in the pathogen [28]. Expression of the gene
aiIA (encoding the N-acylhomoserine lactonase responsible for the inactivation of N-acylhomoserine lactones in *E. carotovora* itself results in a decrease in the production of pectolytic enzymes, virulence factors of this pathogen [29, 30]. This renders the pathogen avirulent on numerous host plants [29]. In this study, the exact mechanism leading to the repression of pathogenicity- and virulence-associated genes in *S. scabies* remains unknown.

**Pseudomonas** sp. LBUM 223 harbors phenazine biosynthesis genes [20] and produces PCA (unpublished results). PCA production by **Pseudomonas** sp. LBUM 223 may be involved in *S. scabies* growth inhibition and repression of pathogenicity- and virulence-associated gene expression. The role of PCA in pathogen antagonism is well documented. For example, a **Pseudomonas fluorescens** strain inhibits the growth of the fungal pathogen *Gaumannomyces graminis* var. tritici, the causative agent of take-all of wheat, in part by producing PCA [15]. Although PCA-mediated growth inhibition of different plant pathogens in *vitro* has been demonstrated [15,31], the involvement of PCA in repression of the expression of pathogenicity- and virulence-related genes in a plant pathogen has, to our knowledge, yet to be demonstrated. Additional studies are required to better characterize the molecular processes underlying gene repression in *S. scabies* by **Pseudomonas** spp.

Our results indicate that thaxtomin A production appears to begin during vegetative mycelium growth at the onset of aerial mycelium growth, between 24 and 48 hours post-inoculation. This corroborates results obtained previously [32], which demonstrated that thaxtomin A production begins approximately 24 to 48 hours following the inoculation of oat bran broth with *S. scabies*. Thaxtomin A production peaked during sporulation and decreased thereafter. Interestingly, in this study, decreasing txtA and txtC expression appeared to mirror the decrease in thaxtomin A quantity. These results suggest that thaxtomin A is degraded or modified by the producing *S. scabies*. Several members of the *Streptomyces* genus, such as non-pathogenic *Streptomyces* strains EF-50 and EF-73, are known to be able to degrade thaxtomin A in *vitro* [33]. Also, it has been previously demonstrated that *S. scabies* isolates produce a de-12-N- methyl analogue of thaxtomin A in oatmeal broth after prolonged incubation, likely by modifying previously produced thaxtomin A [34]. To our knowledge, a relationship between txtA and txtC expression, thaxtomin A production and *S. scabies* morphological differentiation stages has not been described previously.

**Pseudomonas** sp. LBUM 223, which inhibited *S. scabies* growth and repressed txtA, txtC, nec1 and tomA transcription in the pathogen, showed potential as a biological control agent of common scab. It is impossible at this stage to clearly determine how many different determinants in *Pseudomonas* sp. LBUM 223 are involved in *S. scabies* growth inhibition and in txtA, txtC, nec1 and tomA gene repression. Thorough characterization of the bacterial interactions occurring between potato-pathogenic *S. scabies* and antagonistic *Pseudomonas* spp. under *in vitro* conditions constitutes a first step in determining the potential of these strains to control common scab of potato. Experiments performed under soil conditions represent the next logical step to validate the results obtained in this study. Development of a PCA-nonproducing mutant of *Pseudomonas* sp. LBUM 223 is currently underway to better address the implication of PCA production in this system.

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**References**

1. Lambert DH, Loria R (1989) *Pseudomonas* scabies spp. nov., nom. rev. Int J Syst Bacteriol 39: 387-392.
2. Locci R (1994) Actinomycetes as plant pathogens. Eur.J.PlantPathol 100: 179-200.
3. Hill J, Lazarovits G (2005) A mail survey of growers to estimate potato common scab prevalence and economic loss in Canada. Can J Plant Pathol 27: 46-52.
4. Loria R, Bukhaid RA, Fry BA, King RR (1997) Plant pathogenicity in the genus *Streptomyces*. Plant Dis 81: 836-846.
5. Healy FG, Wach M, Krasnoff SB, Gibson DM, Loria R (2000) The txtAB genes of the plant pathogen *Streptomyces* acidiscabies encode a peptide synthetase required for phytotoxin thaxtomin A production and pathogenicity. Mol Microbiol 38: 794-804.
6. Lawrence CH, Clark MC, King RR (1990) Induction of common scab symptoms in aseptically cultured potato tubers by the vitoxin, thaxtomin. Phytopathology 80: 606-608.
7. King RR, Lawrence CH, Clark MC, Calhoun LA (1989) Isolation and characterization of phytotoxins associated with *Streptomyces* scabies. J Chem Soc Chem Commun 13: 849-850.
8. Hiltunen LH, Laakso I, Chobot V, Hakala KS, Weckman A, et al. (2006) Influence of thaxtomin in different combinations and concentrations on growth of micropropagated potato shoot cultures. J Agric. Food Chem 54: 3372-3379.
9. Healy FG, Krasnoff SB, Wach M, Gibson DM, Loria R (2002) Involvement of a cytochrome P450 monooxygenase in thaxtomin A biosynthesis by *Streptomyces* acidiscabies. J Bacteriol 184: 2019-2029.
10. Joshi M, Rong X, Moll S, Kers J, Franco C, et al. (2007) *Streptomyces* turgidiscabies secretes a novel virulence protein, Nec1, which facilitates infection. Mol Plant Microbe Interact 20: 599-608.
11. Seipke RF, Loria R (2008) *Streptomyces* scabies 87-22 possesses a functional tomatinlase. J Bacteriol 190: 7684-7692.
12. Loria R, Kers J, Joshi M (2006) Evolution of plant pathogenicity in *Streptomyces*. Annu Rev Phytopathol 44: 469-487.
13. Weller DM (2007) *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. Phytopathology 97: 250-256.
14. Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, et al. (1992) Suppression of root diseases by *Pseudomonas* fluorescens CHA0: importance of the bacterial secondary metabolite 2,4-diacylthioglycolic acid. Mol Plant Microbe Interact 5: 4-13.
15. Thomashow LS, Weller DM (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaumannomyces graminis* var. *tritici*. J Bacteriol 170: 3499-3506.
16. Ahi P, Voisard C, Défago G (1986) Iron bound-siderophores, cyanic acid and antibiotics involved in suppression of *Thielaviopsis basicola* by a *Pseudomonas fluorescens* strain. J Phytopathol 116: 121-134.
17. Barber CE, Tang JL, Feng JX, Pan MQ, Wilson T JG, et al. (1997) A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. Mol Microbiol 24: 555-566.
18. Newman KL, Chatterjee S, Ho KA, Lindow SE (2008) Virulence of plant pathogenic bacteria attenuated by degradation of fatty acid cell-to-cell signaling factors. Mol Plant Microbe Interact 21: 326-334.
19. Sessitsch A, Reiter B, Berg G (2004) Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. Can. J.Microbiol 50: 239-249.
20. Paulin MM (2007) Étude moléculaire de l’antibiose et de son impact sur des agents phytopathogènes fongiques. M.Sc. Thesis, Université de Moncton, Moncton, NB, Canada.
21. Paulin MM, Novinscak A, St-Arnaud M, Goyer C, DeCoste NJ, et al. (2009) Transcriptional activity of antifungal metabolite-encoding genes *phdB* and *hcnBC* in *Pseudomonas* spp. using qRT-PCR. FEMS Microbiol Ecol 68: 212-222.
22. St-Onge R, Goyer C, Coflin R, Filion M (2008) Genetic diversity of Streptomyces spp. causing common scab of potato in eastern Canada. Syst. Appl. Microbiol 31: 474-484.

23. Goyer C, Vachon J, Beaulieu C (1998) Pathogenicity of Streptomyces scabies mutants altered in thaxtomin A production. Phytopathology 88: 442-445.

24. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95-98.

25. Peirson SN, Butler JN, Foster RG (2003) Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis. Nucleic Acids Res 31: e73.

26. Kinkel LL, Bowers JH, Shimizu K, Neeno-Eckwall EC, Schottel JL (1998) Quantitative relationships among thaxtomin A production, potato scab severity and fatty acid composition in Streptomyces. Can. J. Microbiol 44: 768-776.

27. Dong Y-H, Zhang X-F, Xu J-L, Zhang L-H (2004) Insecticidal Bacillus thuringiensis silences Erwinia carotovora virulence by a new form of microbial antagonism, signal interference. Appl Environ Microbiol 70: 954-960.

28. Newton JA, Fray RG (2004) Integration of environmental and host-derived signals with quorum sensing during plant-microbe interactions. Cell Microbiol 6: 213-224.

29. Dong Y-H, Xu J-L, Li X-Z, Zhang L-H (2000) AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora. Proc Natl Acad Sci USA 97: 3526-3531.

30. Dong Y-H, Wang L-H, Xu J-L, Zhang H-B, Zhang X-F, et al. (2001) Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. Nature 411: 813-817.

31. Brisbane PG, Janik LJ, Tate ME, Warren RFO (1987) Revised structure for the phenazine antibiotic from Pseudomonas fluorescens 2-79 (NRRL B-15132). Antimicrob Agents Chemother 31: 1967-1971.

32. Beauséjour J, Goyer C, Vachon J, Beaulieu C (1999) Production of thaxtomin A by Streptomyces scabies strains in plant extract containing media. Can J Microbiol 45: 764-768.

33. Doumbou CL, Akimov V, Beaulieu C (1998) Selection and characterization of microorganisms utilizing thaxtomin A, a phytoalexin produced by Streptomyces scabies. Appl Environ Microbiol 64: 4313-4318.

34. King RR, Lawrence CH (1996) Characterization of new thaxtomin A analogues generated in vitro by Streptomyces scabies. J Agric Food Chem 44:1108-1110.