Pseudomonas spp. can Inhibit Streptomyces scabies Growth and Repress the Expression of Genes Involved in Pathogenesis

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Abstract

Common scab, caused by Streptomyces scabies, is an economically important disease affecting potato crops worldwide. Confirmed and putative pathogenicity- and virulence-related factors, including the phytotoxic thaxtomins, the necrosis protein Nect, and the tomatinase TomA, have been characterized in S. scabies. 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. scabies 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. scabies growth and repressed transcription of all targeted genes in the pathogen. S. scabies 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. scabies. To our knowledge, this is the first demonstration that an antagonistic organism can repress the expression of key genes involved in S. scabies pathogenesis. This capacity is unlikely a trait common to all Pseudomonas spp.

Keywords: Common scab of potato; Streptomyces scabies; 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 scabies [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 streptomyces 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 phytotoxiccin 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-diacetylphloroglucinol [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- or 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. scabies [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. scabies.

In this study, we sought to evaluate the ability of three Pseudomonas strains to (i) inhibit S. scabies growth, using plate inhibition assays and to (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. scabies 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-diacylphloroglucinol and the hydrogen cyanide biosynthesis operons [20,21].

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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 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 l aliquot of solutions MR1 and MR2, respectively. Cells were transferred into Micro RNA Bead Tubes, to which was 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) at a speed of 5.5 for 45 s, was performed prior to the vortex homogenization step, using a Fast Prep FP120 (Qbiogene, Carlsbad, CA, USA) at a speed of 5.5 for 45 s, was performed prior to the vortex homogenization step. Co-extracted DNA was digested using the Turbo DNA-free kit (Ambion, Austin, TX, USA), as described previously [21]. Thorough removal of DNA was confirmed using real-time quantitative PCR (data not shown). RNA was stored at -80°C.

Thaxtomin a extraction and quantification

Following the retrieval of S. scabies cells for total RNA extraction, the remaining OBA medium was crushed in 40 ml of ethyl acetate for 1 min using a Polytron PT 10-35 homogenizer (Kinematica, Bohemia, NY, USA) at a speed of 2.5. The resulting slurry was centrifuged at 2,000 x g for 5 min (4°C). The supernatant was recovered and treated with anhydrous sodium sulphate in order to remove all traces of water. The ethyl acetate extract was then recovered by filtration through a Whatman filter paper and the ethyl acetate was allowed to evaporate completely under a closed fume hood. Extracts were stored at 4°C until processed. Dried extracts were re-dissolved in 1.0 ml of water: acetonitrile (73:27), filtered through 0.45-μm syringe filters and injected into a Supelco LC-18 column (4.6 x 150 mm) using a Rhosted 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 A 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-

<table>
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<td>CGA TCA CCG AAC TCG ACG T</td>
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</table>

*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), FJ007531-FJ007579, AF255732 (txtA), FJ007580-FJ007629, AF393159 (txtC), AM293590, AM293591, AF385166-AF385180 (nec1), FJ007481-FJ007529 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 products were stored at -20°C. RT products, was included in each quantitative PCR run. Fluorescence (50 amplification cycles). A negative control, containing no RT products, was included in each quantitative PCR run. Fluorescence was measured during the extension step.

**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 R0 values. Gene expression values of the target genes were normalized to those of the endogenous reference gene using the following formula: \[ R_{target} = \frac{R_{target}}{R_{reference}} \] 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 calibrators of the plate inhibition assays and the time-course studies consisted of the “S. scabies only” and 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^100) 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 (autoregressive 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 (F13, 30 = 135.29; P < 0.01). Of the three Pseudomonas strains tested, only Pseudomonas sp. 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).
The ability of the three *Pseudomonas* strains to alter the expression of four pathogenicity- and virulence-associated genes in *S. scabies* was also investigated. The antagonist treatment significantly altered the expression of *txtA* \((F_{2,29} = 3.74; P = 0.02)\), *txtC* \((F_{2,29} = 6.94; P < 0.01)\), *nec1* \((F_{2,29} = 8.50; P < 0.01)\) and *tomA* \((F_{2,29} = 7.42; P < 0.01)\) transcripts in *S. scabies* after 6 days using quantitative RT-PCR assays. Data are presented as mean ± standard error \((n = 9)\). Means of groups annotated with different letters were significantly different \((P < 0.05)\). The antagonist treatment significantly cross-correlated (R = 0.81) and both *txtA* gene expression and *txtC* gene expression were significantly cross-correlated \((R = 0.97\) 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 *S. scabies*. *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 monoxygenase 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* subspp. *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 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.

**txtA, txtC, nec1 and tomA gene expression in S. scabies**

The ability of the three *Pseudomonas* strains to alter the expression of four pathogenicity- and virulence-associated genes in the pathogen was also investigated. The antagonist treatment significantly altered the expression of *txtA* \((F_{3,10} = 3.74; P = 0.02)\), *txtC* \((F_{3,10} = 6.94; P < 0.01)\), *nec1* \((F_{3,10} = 8.50; P < 0.01)\) and *tomA* \((F_{3,10} = 7.42; P < 0.01)\) in the pathogen after 6 days using quantitative RT-PCR assays. Data are presented as mean ± standard error \((n = 9)\). Means of groups annotated with different letters were significantly different \((P < 0.05)\). Treatment levels of *txtA*, *txtC*, *nec1* and *tomA* in *S. scabies* confronted with *Pseudomonas* sp. LBUM 223 were significantly lower than those noted in the pathogen grown in the absence of an antagonist by approximately 46, 52, 64 and 54%, respectively (Figure 2). In contrast, transcription levels of the
ai/A (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 genes expression. The role of PCA in pathogen antagonism is well documented. For example, a *Pseudomonas fluorescens* strain inhibits the growth of the fungal pathogen *Gaeumannomyces 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 repressing 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*, *ncel* 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*, *ncel* 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**


