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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 Nec1 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 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 phytoanticipin 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 and/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 rot 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-diacetylphloroglucinol 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 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 scrapping 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) 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* and DNA-*free* kits (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.

Time-course assays

txtA and *txtC* gene expression levels in *S. scabies* and thaxtomin A production were assessed every 24 h over the course of 10 days. Studies were carried out in quadruplicates, for a total of 44 samples. A 100-µl aliquot of *S. scabies* culture, prepared as described above, was spread onto OBA plates. Plates were randomized (complete randomized bloc design) and incubated at 28°C. The "Day 0" plates were placed at 4°C immediately following plate inoculation until thaxtomin extractions (at approximately 4 h post-inoculation). At each sampling date, the morphological differentiation stage of *S. scabies* (vegetative and aerial mycelium growth, spore formation) was noted. Total RNA was extracted from *S. scabies* once mycelium growth had sufficiently progressed (starting at 3 days post-inoculation). At each time point, thaxtomins were extracted from the OBA medium following total RNA extraction (if any).

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 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	F[a]	CGT CGC CTC CAT CAA GGA	68
	rpoBr	R[a]	GCG GGT TGT TCT GGT CCA T	
	rpoBprobe	P[a]	CTT CGG CAC CAG CCA	
txtA	txtAfor	F[a]	TGC TCA ACT CCG TGA TCC AGT A	69
	txtArev	R[a]	GGG ACA CCT CGC GCA GTA	
	txtAprobe	P[a]	CCT CAG GCG ATT ACC TGT	
txtC	txtCfor	F[a]	ACC ATC TCG CTG TCC TTG GT	61
	txtCrev	R[a]	CGT GGA CGA CGG AGA ACT TC	
	txtCprobe	P[a]	TTA TGC ACT GCA GCC GG	
nec1	nec1for	F[a]	GCT TGG GCC GGT ATG CT	54
	nec1rev	R[a]	TGC AGG CGA GGT GTT TTA AA	
	nec1probe	P[a]	CTT CCT GAA AGC GCC	
tomA	tomAfor	F[a]	CCA GAA GCT CGG ACT CGA AGT	67
	tomArev	R[a]	CTG CTG ATC CAC GTC GTA GGT	
	tomAprobe	P[a]	CGA TCA CCG AAC TCG ACG T	

[a]**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 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 amplified by singleplex quantitative PCR. Twenty-five-microliter reactions, containing 0.2 µM of TaqMan probe (Table 1) and 0.5 μM each of forward and reverse primer (Table 1), were prepared using the TaqMan PCR Core Reagents kit (ABI) and carried out in triplicate in a 7500 Real Time PCR System (ABI), as described by the manufacturer (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 R_0 values. Gene expression values of the target genes were normalized to those of the endogenous reference gene using the following formula: $R_{0\ \text{Target gene}}/R_{0\ \text{Reference gene}}$. 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 ($x^{0.0001}$) 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* ($F_{3, 30} = 135.29$; $P < 0.01$). Of the three *Pseudomonas* strains tested, only *Pseudomonas* strains 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).

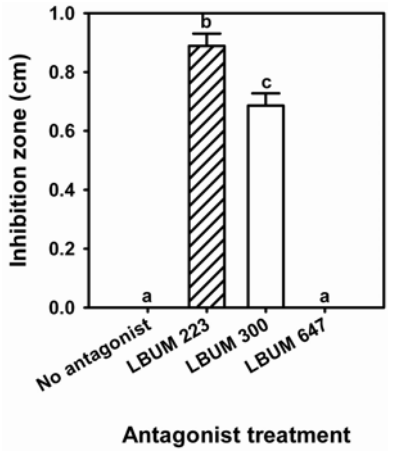

Figure 1: Growth inhibition of common scab-inducing *S. scabies* by various *Pseudomonas* strains. In plate inhibition assays, *S. scabies* was either grown in the absence of an antagonist or was confronted with a *Pseudomonas* strain (LBUM 223, LBUM 300 or LBUM 647). Growth inhibition zones were measured after 6 days. Data are presented as mean ± standard error ($n = 9$). Means of groups annotated with different letters were significantly different ($P < 0.05$).

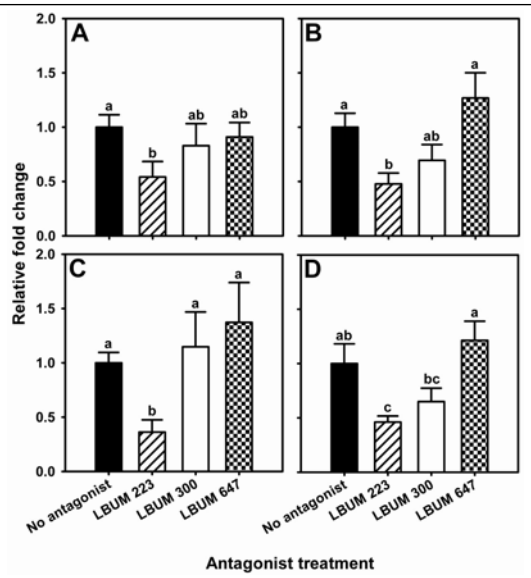

Figure 2: Repression by *Pseudomonas* strains of pathogenicity- and virulence-associated gene expression in *S. scabies*. In plate inhibition assays, *S. scabies* was either grown in the absence of an antagonist or was confronted with a *Pseudomonas* strain (LBUM 223, LBUM 300 or LBUM 647). Transcripts of genes *txtA* (**A**), *txtC* (**B**), *nec1* (**C**) and *tomA* (**D**) were quantified 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 ($P < 0.05$).

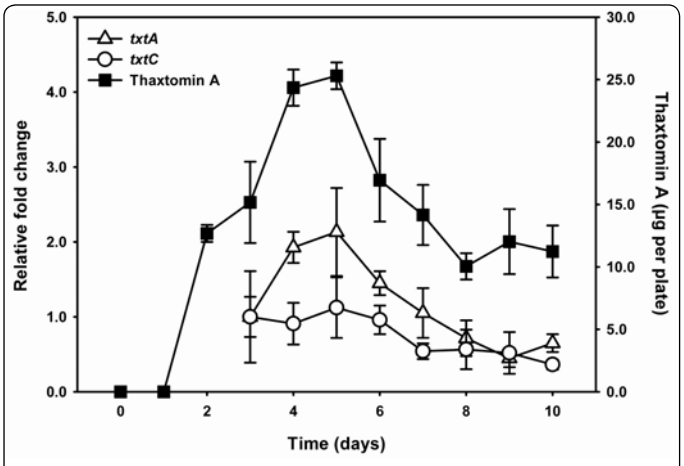

Figure 3: *txtA* and *txtC* gene expression and thaxtomin A production in *S. scabies* over time. *txtA* and *txtC* gene expression was monitored in *S. scabies* every 24 h over the course of 10 days using quantitative RT-PCR assays. Concurrently, thaxtomin A was quantified in the growth medium using high-performance liquid chromatography. All data are presented as mean ± standard error (n = 4).

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* ($F_{3, 29} = 3.74$; $P = 0.02$), *txtC* ($F_{3, 29} = 6.94$; $P < 0.01$), *nec1* ($F_{3, 29} = 8.50$; $P < 0.01$) and *tomA* ($F_{3, 29} = 7.42$; $P < 0.01$). Transcription 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 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 TxtA [5] and the mono oxygenase TxtC [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 *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*, *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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