J Ind Microbiol Biotechnol (2004) 31: 517–524
DOI 10.1007/s10295-004-0180-3

ORIGINAL PAPER

P. J. Slininger · K. D. Burkhead · D. A. Schisler

Antifungal and sprout regulatory bioactivities of phenylacetic acid, indole-3-acetic acid, and tyrosol isolated from the potato dry rot suppressive bacterium *Enterobacter cloacae* S11:T:07

Received: 29 December 2003 / Accepted: 1 October 2004 / Published online: 19 November 2004

Abstract *Enterobacter cloacae* S11: T:07 (NRRL B-21050) is a promising biological control agent that has significantly reduced both fungal dry rot disease and sprouting in laboratory and pilot potato storages. The metabolites phenylacetic acid (PAA), indole-3-acetic acid (IAA), and tyrosol (TSL) were isolated from S11:T:07 liquid cultures provided with three different growth media. The bioactivities of these metabolites were investigated via thin-layer chromatography bioautography of antifungal activity, wounded potato assays of dry rot suppressiveness, and cored potato eye assays of sprout inhibition. Relative accumulations of PAA, IAA, and TSL in cultures were nutrient dependent. For the first time, IAA, TSL, and PAA were shown to have antifungal activity against the dry rot causative pathogen *Gibberella pulicaris*, and to suppress dry rot infection of wounded potatoes. Disease suppression was optimal when all three metabolites were applied in combination. Dosages of IAA that resulted in disease suppression also resulted in sprout inhibition. These results suggest the potential for designing culture production and formulation conditions to achieve a dual purpose biological control agent able to suppress both dry rot and sprouting of stored potatoes.

Keywords Antibiotic · Sprout inhibitor · Biological control · Potato · Postharvest

Introduction

Gibberella pulicaris (Fr.:Fr.) Sacc. (anamorph: *Fusarium sambucinum* Fuckel) is a serious pathogen in potato tuber storages, where it is a primary causal agent of Fusarium dry rot [3]. Fusarium dry rot is one of the most important postharvest diseases of potato occurring worldwide. It affects tubers in storage and seed pieces after planting. Yield losses attributed to dry rot in storage range from 6 to 25% with up to 60% of tubers affected in some cases [40]. This disease causes greater losses in storage and transit of both seed and commercial potatoes than any other postharvest disease [34].

Measures for controlling Fusarium dry rot in storage are limited. In the 1970s and most of the 1980s, the fungicide thiabendazol (TBZ) was utilized to effectively control the disease [24, 30]. However, resistance to TBZ, the only chemical registered for postharvest use on tubers for human consumption, is now widespread among strains of *G. pulicaris* with reports of 60–95% of field strains of *G. pulicaris* showing TBZ-resistance [11, 16, 19, 41, 52]. High levels of resistance to Fusarium dry rot in potato cultivars and breeders' selections are not apparent [33] and all commonly grown potato cultivars are susceptible [25, 35, 54].

In addition to dry rot control measures, the potato industry is in search of alternative agents for sprout control. Because of processing demands, over 54% of the annual potato harvest must be stored at 7° to 13°C, a temperature range above that needed for ideal sprout control [1]. If storage temperatures exceeding 3°C are required, sprout inhibitors must be applied to control sprouting. The potato industry has become very dependent on CIPC (1-methylethyl-3-chlorophenylcarbamate) as the most efficient sprout inhibitor with fewest detrimental side-effects on process potato quality [26]. A tolerance of 50 mg/kg potato had been established for residues of CIPC [12], but more recently this tolerance has been reduced to 30 mg/kg [13] because of the persistence of CIPC in the environment and potato tissue, and concerns about its toxicity [29].

Enterobacter cloacae (Jordan) Hormaeche and Edwards S11:T:07 (= NRRL B-21050) is being developed as a biological control agent (BCA) to protect potatoes entering storage from Fusarium dry rot [37, 38, 43], and

P. J. Slininger (✉) · K. D. Burkhead · D. A. Schisler
Crop Bioprotection Research Unit,
United States Department of Agriculture,
Agricultural Research Service, 1815 N. University Street,
Peoria, IL 61604, USA
E-mail: slininpj@ncaur.usda.gov
Tel.: +1-309-6816596
Fax: +1-309-6816693

has significantly reduced the level of dry rot disease in pilot trials [39]. Strain S11:T:07 and other dry rot suppressive strains isolated in our laboratory have recently been shown to also possess the ability to suppress sprouting [46, 47]. Previous studies indicated that S11:T:07 produced several antifungal compounds [5] that could potentially play a role in dry rot disease suppression. Phenylacetic acid (PAA) was first isolated from strain S11:T:07 and shown to have antifungal activity against *G. pulicaris* [6]. This report describes the liquid culture production, isolation, and purification procedures used in the recovery and identification of the plant hormone indole-3-acetic acid (IAA), and tyrosol (TSL), from culture S11:T:07. In addition, the bioactivities of PAA, TSL, and IAA applied alone and in combination are investigated in wounded potato assays to assess dry rot suppression, and in cored potato eye assays to evaluate sprout inhibition. The results of these studies elucidate strategies for designing a novel dual purpose BCA that could be sprayed onto potatoes entering storage to both suppress fungal infection and inhibit sprouting.

Material and methods

Cultures

Stock cultures of the biocontrol strain *E. cloacae* S11:T:07 (deposited as NRRL B-21050 in the Agricultural Research Service Patent Culture Collection, National Center for Agricultural Utilization Research, USDA, Peoria, Ill.) were maintained at −80°C in 10% glycerol as previously described [37].

The dry rot causative pathogen *G. pulicaris* (accession number R-6380, Fusarium Research Center, Pennsylvania State University) was maintained as described elsewhere [10].

Thin-layer chromatography bioautography

The bioautography procedure of Burkhead et al. [4, 5] was modified as follows: *G. pulicaris* R-6380 was grown on the surface of clarified V-8 juice agar (CV8), which had been prepared in slanted 120-ml wide-mouth jars. CV8 contained, per liter, 175 ml V-8 juice stock (3% $CaCO_3$ in centrifuged V-8 juice), 825 ml distilled water, and 18 g Bacto agar. The conidia from each 1-week-old jar culture were suspended by gentle agitation with a sterile inoculating loop in 50 ml Sabouraud maltose broth (SMB). The use of jar cultures to grow the fungus allowed us to attach the plastic spray head of a spray unit (Sigma) directly onto the jar after the suspension was made. Spore suspensions (approximately 4×10^6 conidia/ml) were sprayed onto thin-layer chromatography (TLC) plates, which had been spotted with samples, developed, and dried. The inoculated TLC plates were kept in plastic bags at room temperature as described previously [4]. In areas of TLC plates containing antifungal compounds, salmon-colored fungal growth failed to develop, leaving an obvious white clearing zone.

Isolation and identification of IAA from strain S11:T:07

Strain S11:T:07 was grown in a two-stage liquid fermentation as previously described [5], except that two media in addition to SMB were employed for shake-flask cultures: minimal defined liquid (MDL) and semi-defined complete liquid (SDCL) culture media as previously reported [43]. Crude ethyl acetate extract containing the unknown antifungal compound was obtained from 3-l of 4th day second-stage SDCL culture (two 1.5-l cultures incubated in 2.8-l Fernbach flasks, pH adjusted from 8.2 to 3 at harvest). The presence of an unknown antifungal compound was indicated and followed by TLC-bioautography through multiple chromatographic steps [5]. Silica gel and acidified silica gel adsorbants were employed for column and preparative TLC [6], with mixtures of chloroform/methanol, or ethyl acetate as solvents. The purified antifungal unknown was obtained on acidified silica TLC developed in chloroform/methanol (95:5). Gas chromatography-electron impact mass spectroscopy (GC-EIMS) analysis of the purified extract was performed on a Varian Saturn GC/MS/MS 4D and a Varian Star 3600CX set to ramp from 100°C to 250°C over 50 min and then hold for 10 min. Proton nuclear magnetic resonance (^{1}H-NMR) analysis was performed using a Bruker model ARX-400 spectrometer and D_2O solvent. HPLC analysis was conducted on a C-18 column (Alltech Alltima C18 5 U, 4.6 mm ×250 mm, 5-Φ particles) eluted at 1 ml/min with 60:10:30 pH 2.5 phosphoric acidified water:methanol:tetrahydrofuran, and a TSP Spectromonitor 5000 photodiode array detector was used to provide retention time-dependent UV spectra.

Isolation and identification of TSL from strain S11:T:07

Strain S11:T:07 was grown in a two-stage liquid fermentation on SMB medium as previously described [5]. After 7 days of stage-two growth, 3-l culture (6 2.8-l Fernbachs of 500 ml SMB) was adjusted to pH 2 and extracted with ethyl acetate. The crude extract was applied to 200 g 40 mm silica gel for flash chromatography (Baker 7024-01). The extract was eluted with chloroform:acetone (9:1), and the fractions collected were evaluated for antifungal antibiotic content by TLC-bioautography. The unidentified antifungal compound eluted after PAA.

Further purification of the antibiotic was achieved using acidified TLC plates developed in chloroform:methanol (95:5) as described above for IAA isolation. Bands at R_f 0.13–0.22 were scraped off, the yellow residue eluted with chloroform and reapplied to another acidic plate developed as above. The band at R_f 0.13 was eluted and used for spectral analyses. EIMS of TSL was

obtained using a Varian 3400 gas chromatograph connected to a Finnigan MAT INCOS 50 mass spectrometer. Proton (^{1}H) and carbon (^{13}C) NMR spectra were obtained using a Bruker model ARX-400 spectrometer. IR spectra were obtained by using a Perkin-Elmer model 16 PC Fourier Transform infrared spectrometer.

Potato source

The potatoes (*Solanum tuberosum* L.) used in bioassays were size B Russet Burbank seed potatoes (averaging 96.5 g) obtained from Felix Zeloski Farms (Eagle River, Wis.). Upon receipt in mid-November, the potatoes were stored at 4°C until treatment.

Potato bioassay of the dose response of dry rot to IAA, TSL, and PAA concentrations

Methods for conducting bioassays on the biological control of dry rot have been reported previously [37, 38] and form the basis for our assay of IAA, TSL, and PAA antifungal activities upon application to wounded potatoes. Prior to tuber application, stock suspensions of conidia and stock solutions of IAA (Sigma I-2886), PAA (Sigma P-4514), and TSL (4-hyroxyphenethyl alcohol, Aldrich no.18,825-5) in diluent were mixed such that final concentrations applied to potato wounds were: 5×10^5 conidia/ml, 0–5 mg/ml PAA or IAA, and zero or 0.005 mg/ml TSL. Tubers were inoculated, incubated, harvested, and evaluated for disease symptoms according to previously described methods [37, 38]. Tubers were uniformly wounded at four sites spaced at equal distances around the circumference of tubers using a blunted steel nail. Wounds were then inoculated with 5 µl of each treatment, consisting of a mixed suspension of conidia and metabolites IAA, PAA, and/or TSL. For each treatment, four to nine wounds, each on different potatoes were inoculated. Three different sterile diluents were used to suspend IAA, PAA, TSL, and conidia in all treatments of each experiment: Milli-Q-purified water, pH 7.2 buffer (0.004% phosphate buffer with 0.019% $MgCl_2$; Aid-Pack, Gloucester, Mass.), and pH 4 buffer (0.0307 M citric acid and 0.0386 M dibasic potassium phosphate). Controls consisted of wounds inoculated with diluent only or suspensions of 5×10^5 macroconidia/ml in diluent. The effects of IAA, TSL, and PAA concentrations on disease severity were tested in eight different experiments. Data were expressed in terms of relative disease level (RD), where RD = 100% ×(disease rating of treatment)/(average disease rating of control wounds inoculated with the pathogen only).

Potato bioassay of the dose response of sprouting to PAA, TSL, and IAA

In this assay (adapted from [28]), treatments were applied to eyes that had been cored from whole, washed Russet Burbank potatoes according to the procedure of Slininger et al. [47]. Concentrated stocks of PAA, TSL, and IAA (Sigma) were prepared. Ten-milliliter stocks containing 5 g/l in pH 7 Fisher Aid-Pack phosphate buffer were adjusted with 4 *N* NaOH to maintain pH 7, upon dissolution of the compounds. All treatment solutions ranging from 0 to 5 g/l in concentration were prepared by dilution of the stocks with pH 7 buffer. After warming to room temperature, the eye area of each core (2 g average weight) was coated with 12 µl of a given treatment. Control treatments included water, pH 7 buffer, or 10 ppm CIPC (per weight potato) and were delivered in 12-µl volumes to be consistent with all other treatments. Ten replicates of each treatment were stored in the dark in cotton-plugged conical tubes, at 15°C, and 90–95% relative humidity. A nondestructive measurement of total sprout length/core length was taken at 2-week intervals. "Total sprout length" is the sum of the lengths of all sprouts forming on the core and accounts for the growth of multiple sprouts, as opposed to a single sprout. Standardization per core length was to reduce any bias of sprout growth due to core size. Tubes were not opened until the final monitoring after 4 weeks of storage, when sprout weight was measured and sprout weight percent calculated as 100×(sprout weight)/(core + sprout weight).

Results

Isolation and identification of IAA from strain S11:T:07

TLC-bioautography indicated that S11:T:07 produced a major antifungal compound in SDCL and that this compound was different from PAA, the major antifungal compound produced in SMB (Fig. 1). SMB supported a higher level of PAA accumulation than SDCL, but the reverse was true for the unknown accumulation. No active compounds were detected from S11:T:07 grown in MDL, nor was any activity detected in control extracts of uninoculated media. Consequently, for identification purposes, isolation and purification of the unknown antifungal compound was approached using a 96-h SDCL culture. TLC-bioautography analysis of extracts from cells and supernatant of S11:T:07 showed that both contained the unknown antibiotic. Chromatographic purification of the extract from 3 l S11:T:07 culture grown in SDCL yielded a 2.2 mg sample suitable for spectral analysis. Mass spectral (GC-EIMS) and ^{1}H-NMR data were consistent with literature data for IAA [21]. Identification of the unknown as IAA was confirmed by comparisons of HPLC, TLC-bioautography, MS, and NMR data from the isolated material with a commercial standard IAA (Sigma I-2886).

Isolation and identification of TSL from strain S11:T:07

Bioautography showed that the crude extract (2.66 g) from 3 l SMB culture of strain S11:T:07 contained an unidentified antifungal compound in addition to the

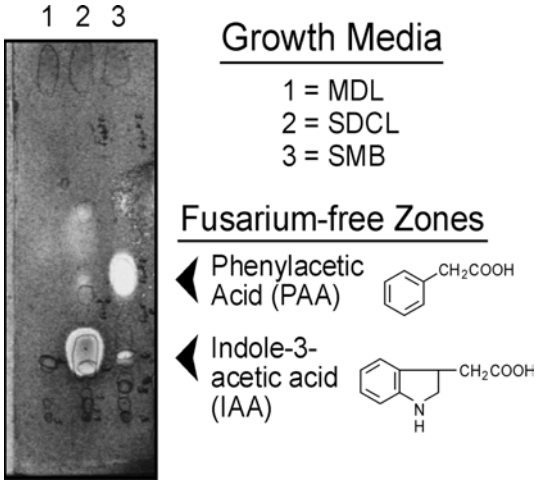

Fig. 1 Thin-layer chromatography (TLC)-bioautography assay of *Enterobacter cloacae* S11:T:07culture extracts against the potato dry rot causative pathogen *Gibberella pulicaris* (*Fusarium sambucinum*), demonstrating medium-dependent antifungal metabolite production. Crude extract (0.9 ml) from a 96-h liquid culture in minimal defined liquid (MDL), semi-defined complete liquid (SDCL), or Sabouraud maltose broth (SMB) medium was spotted in lanes *1*, *2*, and *3*, respectively. The plate was photographed under 254 nm radiation after bioautography. *Light areas* are clearing zones where antifungal compounds exist and prevent the growth of the pathogen. *Dark areas* elsewhere show the uninhibited growth of the pathogen

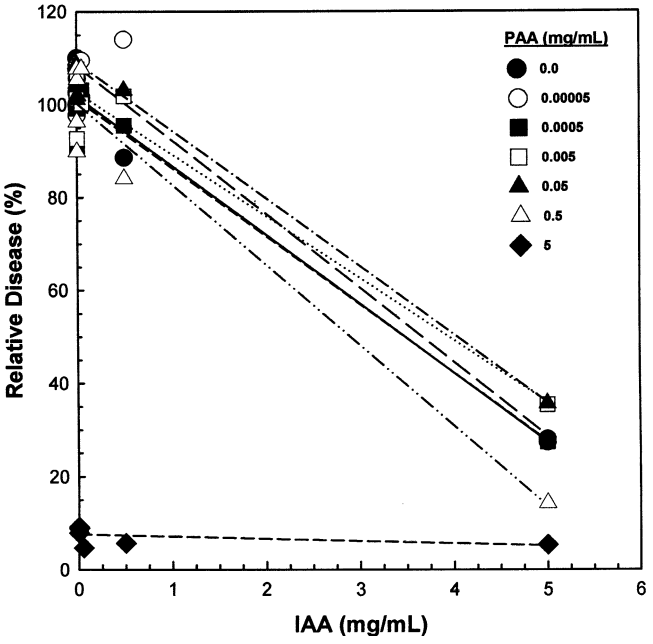

Fig. 2 Response of dry rot disease to the interactive dosage of phenylacetic acid (PAA) and indole-3-acetic acid (IAA). IAA was applied at the same dosages as those listed for PAA. The 0–5 mg/ml concentration series was repeated in eight experiments for PAA and also for IAA. The IAA×PAA combinations were repeated in four experiments. Standard error in the mean relative disease (RD) data was ≤ 4.5 for the PAA concentration series, ≤ 5.2 for the IAA series, and ≤ 13.2 for the IAA×PAA combinations

PAA and IAA identified previously. The unknown compound was partially purified by silica gel flash column chromatography. Further chromatography on acidic TLC plates afforded 1.59 mg purified unknown antibiotic (R_f 0.13 on acidic plates developed in chloroform:methanol 95:5).

The white amorphous solid obtained gave IR, ^{1}H-NMR, ^{13}CNMR, and EIMS spectral data that agreed with data previously reported for TSL [7, 8, 18, 20, 31]. Spectral, chromatographic, and bioautographic comparisons with purchased TSL (4-hydroxyphenethyl alcohol, 98%, Aldrich) confirmed the identification of the isolated antifungal compound as TSL.

Response of dry rot disease to dosage of PAA and IAA

This is the first report of dry rot disease suppression by PAA and IAA dosed in combination (Fig. 2). The diluent matrix (water, pH 4 or pH 7 buffer) used to prepare solutions of PAA and IAA had no impact on dry rot disease development, thus data could be analyzed across all three diluent matrices. Application of 1–5 mg/ml of either compound led to significant reduction ($P < 0.001$) in the relative disease level RD. RD was linearly dependent on IAA concentration, [IAA]: $RD = i + m[IAA]$, where R^2 values ranged from 0.89 to 0.98 for various PAA concentrations [PAA]. The linear decline in RD with [IAA] was not as steep as the linear decline of RD with [PAA]. Application of 5 mg/ml IAA alone reduced RD to 28%, while application of 5 mg/ml PAA reduced it to 8.9%. RD fell below 10% for all IAA concentrations tested when [PAA] was 5 mg/ml. Two-way analysis of variance indicated that the interaction of IAA with PAA was also significant ($P \leq 0.002$). Both the intercept (i) and the slope (m) of RD versus [IAA] declined with [PAA], and both i and m were found to be linearly dependent upon [PAA] with R^2 values of 0.99 and 0.91, respectively: $i = 105.6 - 19.5$ [PAA] and $m = -15.3 + 2.91$ [PAA]. The following predictive model could be constructed based on the data collected: $RD = 105.6 - 19.5$ [PAA] $- 15.3$ [IAA] $+ 2.9$ [PAA][IAA].

TSL enhancement of disease reduction by PAA and IAA

The disease suppressiveness of several combinations of PAA and IAA with and without TSL added at the 0.005 mg/ml level were compared and, in all cases, treatments containing TSL resulted in less disease than those without TSL (Table 1). Furthermore, treatments with TSL exhibited less disease than would be predicted from the above model equation of $RD = f$ ([PAA], [IAA]) for similar treatments containing only PAA and IAA.

Sprout response to IAA dosage

Table 2 shows that, relative to buffer, water, and untreated controls, potato sprouting was inhibited by

Table 1 Enhancement of dry rot disease suppression by addition of 0.005 mg/ml tyrosol (TSL). *PAA* Phenylacetic acid, *IAA* indole-3-acetic acid, *RD* relative disease as a percentage of the disease only control treatment

Treatment composition		TSL present	TSL absent	
PAA (mg/ml)	IAA (mg/ml)	Observed RD (%)	Observed RD (%)	Predicted RD (%)[a]
0.0005	0.0005	74.0	78.2	105.6
0.0005	0.05	36.7[b]	100.6	104.8
0.0005	5.0	2.3	5.8	29.1
0.05	0.0005	92.1	98.3	104.6
0.05	0.05	43.9[b]	118.7	103.9
0.05	5.0	1.7[b]	15.5	28.9
5	0.005	0.9	6.7	8.1
5	0.05	1.7	3.7	8.1

[a]Value of RD% predicted for applied PAA and IAA concentrations based on the model constructed from Fig. 2 data: RD = 105.6−19.5 [PAA]−15.3 [IAA] + 2.9 [PAA][IAA], where [PAA] and [IAA] represent the concentrations of PAA and IAA in mg/ml units

[b]Significantly lower observed RD% when 0.005 mg/ml TSL is present as compared to when it is absent ($P < 0.05$, Student-Newman-Keuls pairwise comparison method)

solutions containing greater than 0.1 mg/ml IAA but not by solutions containing PAA or TSL at ≤ 5 mg/ml. After 2 weeks of storage, application of 0.1 mg/ml IAA reduced sprouting by 37% relative to the untreated control, and by 30% relative to the lumped water-buffer-untreated control group. Treatments of 1 mg/ml IAA reduced sprouting by 52 and 45%, relative to the untreated and lumped control group, respectively. Treatments of 5 mg/ml IAA further reduced sprouting by 84% and 81%, relative to the untreated and lumped control group, respectively; and this level of sprout suppression was statistically similar to that achieved by the CIPC control, which reduced sprouting by 93% relative to the untreated control.

After 4 weeks of bioassay incubation, the 0.1, 1, and 5 mg/ml IAA treatments all demonstrated sprout suppression at levels of 9, 24, and 52%, respectively. The sprout weight percent measurement gave a more quantitative assessment of sprout suppression and allowed for a lower standard deviation and better separation of means upon statistical analysis than did the total sprout length/core length ratio. Based on sprout weight percent, the sprout suppression achieved by the 1 and 5 mg/ml IAA treatments was significant relative to the lumped

Table 2 Solutions of IAA, but not of PAA or TSL, inhibit sprout development of potato eye cores after 2 and 4 weeks storage. Within columns, values with no letters in common are significantly different ($P < 0.05$) based on the Student-Newman-Keuls pairwise comparison method

Treatment	Total sprout length/core length		Sprout weight (%)(4 Weeks)
	2 Weeks	4 Weeks	
IAA (mg/ml)			
0.00001	0.55 CD	1.82 BC	6.36 CD
0.0001	0.58 CD	2.05 C	6.26 CD
0.001	0.72 CD	1.98 BC	7.11 D
0.01	0.65 CD	2.47 C	7.45 D
0.1	0.46 BC	1.94 BC	5.85 CD
1.1	0.35 B	1.90 BC	4.85C [a]
5.0	0.12 A	1.30 B	3.11 B[a]
PAA (mg/ml)			
0.00001	0.83 D	2.53 C	7.61 D
0.0001	0.73 D	2.45 C	7.07 D
0.001	0.71 CD	1.75 B	6.61 CD
0.01	0.60 CD	2.21 C	7.08 D
0.1	0.60 CD	2.20 C	6.04 CD
1.1	0.64 CD	2.11 C	5.75 C
5.0	0.58 CD	1.96 BC	5.56 C
TSL (mg/ml)			
0.00001	0.53 C	1.87 C	6.36 CD
0.0001	0.55 CD	2.32 C	6.33 CD
0.001	0.58 CD	1.87 C	6.44 CD
0.01	0.62 CD	2.10 C	6.69 CD
0.1	0.72 CD	2.16 C	6.34 CD
1.0	0.71 CD	2.01 C	6.80 CD
5.0	0.50 C	2.15 C	6.93 CD
10 ppm CIPC	0.05 A	0.08 A	0.05 A[a]
Buffer	0.57 CD	2.16 C	6.24 CD
Water	0.61 CD	2.39 C	6.58 CD
Untreated	0.73 D	2.04 C	6.41 CD

[a]Values significantly different from the lumped control group, which included untreated, water, and buffer treatments

water-buffer-untreated control group, but was significantly less than the sprout suppression exerted by the CIPC control.

Discussion

Data shown in Figs. 1 and 2 and Table 1 are the first demonstration of the activity of IAA against *G. pulicaris*, the causative agent of Fusarium dry rot of potatoes. This finding adds to the recently evolving literature documenting the antifungal activity range of IAA, a well-known plant growth hormone. Rittich and Pirochtová [36] first reported the antifungal activity of IAA against *Fusarium moniliforme* and *Penicillium expansum*. More recently, Martinez Noel et al. [27] showed that IAA inhibits the in vitro growth of *Phytophthora infestans* and that treatment of potato leaves with IAA solutions reduced the severity of late blight infection following inoculation with *P. infestans*. In potatoes it was first known that IAA plays a role in sprout inhibition [53] and the control of potato dormancy [48]. The more recent literature now suggests that IAA is also involved in disease resistance and the control of disease defense reactions in potatoes [15, 27] and other plant systems, e.g., tomatoes [2]. Thus, our findings make a significant contribution to this developing area of research in plant defense mechanisms.

This is the first report of TSL as a metabolite from the bacterium *E. cloacae*, although other bacterial species, including *Bacillus lactis aerogenes*, *Escherichia coli*, *Proteus vulgaris*, and *Erwinia carotovora* [17, 49, 50, 51] have been previously reported to produce TSL from tyrosine. This is also the first report of the antifungal activity of TSL against *G. pulicaris*, the causative pathogen of Fusarium dry rot. Antibiosis was the property that led to discovery of TSL in extracts from S11:T:07 culture broth. TSL possessed antifungal activity against the potato pathogen *G. pulicaris* in bioautography assays. Furthermore in potato bioassays, TSL significantly enhanced the disease suppressiveness of IAA and PAA combinations (Table 1). Previous reports have described the antifungal activity of TSL against *Penicillium* and *Aspergillus* molds isolated from olives [14], and against *Cladosporium herbarum* in timothy [22, 23] and *Phytophthora citrophthora* in *Citrus* sp. [9], where TSL has been implicated as a possible defense mechanism in diseased plants.

The use of different culture media has revealed that S11:T:07 exhibits nutrient-dependent antifungal metabolite production (Fig. 1). In SMB, which contains tyrosine, the major antifungal metabolites produced were PAA and TSL. In contrast, when S11:T:07 was grown in MDL medium, it produced no detectable antifungal compounds; but in a SDCL rich in amino acids including tryptophan, IAA was the major compound produced. Tryptophan is a key precursor in the microbial biosynthesis of a variety of indole compounds [32]. This and previous research have shown that biocontrol qualities can change with the culture medium used for cell growth, and that quality changes may be related to changes in production of microbial metabolites [42, 44, 45]. To successfully develop strain S11:T:07 as a BCA, it is critical to understand what metabolites it produces, how culture conditions impact metabolism, and how metabolites formulated with the BCA interact to affect biological activities and efficacy.

This is the first report of an *E. cloacae* strain producing the three bioactive compounds IAA, PAA, and TSL, and the first data quantitating their interaction in Fusarium dry rot suppression. Pure compounds of both IAA and PAA have been tested in potato bioassays and shown to have inhibitory activity against dry rot at 0.1–5 mg/ml concentration levels in treatments applied to potato wounds. In parallel studies, IAA at 0.1–5 mg/ml also inhibited sprouting when applied to potato eyes (Table 2). It is notable that the combination of a low level of TSL (0.005 mg/ml) with low levels of PAA (0.0005–0.05 mg/ml) and IAA (0.05 mg/ml) afforded significant disease reduction of 56–63% in our laboratory assays, and that addition of TSL to the PAA/IAA combination significantly improved disease reduction to allow disease control to be possible even at low levels of PAA and IAA. These low levels of IAA, PAA, and TSL are consistent with the accumulation ranges of these compounds as metabolites found in typical S11:T:07 BCA production culture broths (P.J. Slininger, unpublished).

In previous work, we have demonstrated that treatment of postharvest tubers entering storage with *Pseudomonas fluorescens* and *E. cloacae* BCAs can have the dual benefit of inhibiting sprouting and suppressing dry rot of potatoes held in storage over 5 months [47]. While this dual activity is consistent with the bioactivities demonstrated for the metabolites produced by strain S11:T:07, similar efficacies of strain S11:T:07 were observed on both MDL and SDCL medium for disease and sprout suppression in pilot scale whole potato assays [47]. Since MDL medium supports much lower accumulation of PAA, IAA, and TSL compared with SDCL medium, this result suggests that the observed dual biocontrol activities are likely also heavily influenced by other factors, such as BCA colonization of the potato surface environment, and perhaps continued metabolite productions there. The importance of BCA colonization of the potato and the metabolite bioactivity of the broth have indeed also been tested separately, showing that each contributes to the observed biocontrol efficacy [47]. These results suggest that BCA production and formulation environments will together determine the success of maintaining viable cells in storage and of achieving effective colonization and protective metabolite production upon application of the BCA to the potato surface. Future work can now aim at engineering these processes to allow BCAs to make the most of their defense mechanisms upon delivery to the biocontrol arena.

Acknowledgements We are indebted to Linda Ericsson, Dennis Kim, Lori Davis, Andrew Morgan, and Jean Wardrip for excellent technical assistance; to David Weisleder for NMR spectra; and to Ronald Plattner, Janet Snyder, and Gerhard Knothe for mass spectra.

References

1. ASAE Standards (1990) Design and management of storages for bulk, fall-crop Irish potatoes. ASAE, St. Joseph, Mich.
2. Bochow H, Dolej S (1999) Mechanisms of tolerance induction in plants by root colonizing *Bacillus subtilis* isolates. In: Lyr H (ed) Modern fungicides and antifungal compounds II. International Reinhardsbrunn Symposium. Andover, UK, pp 411–416
3. Boyd AEW (1972) Potato storage diseases. Rev Plant Pathol 51:297–321
4. Burkhead KD, Schisler DA, Slininger PJ (1994) Pyrrolnitrin production by biological control agent *Pseudomonas cepacia* B37w in culture and in colonized wounds of potatoes. Appl Environ Microbiol 60:2031–2039
5. Burkhead KD, Schisler DA, Slininger PJ (1995) Bioautography shows antibiotic production by soil bacterial isolates antagonistic to fungal dry rot of potatoes. Soil Biol Biochem 27:1611–1616
6. Burkhead KD, Slininger PJ, Schisler DA (1998) Biological control bacterium *Enterobacter cloacae* S11:T:07 (NRRL B-21050) produces antifungal compound phenylacetic acid in Sabouraud maltose broth culture. Soil Biol Biochem 30:665–667
7. Capasso R, Cristinzio G, Evidente A, Scongnamiglio F (1992) Isolation, spectroscopy and selective phytotoxic effects of polyphenols from vegetable waste waters. Phytochemistry 31:4125–4128
8. Claydon N, Grove JF, Pople M (1985) Elm bark beetle boring and feeding deterrents from *Phomopsis oblongata*. Phytochemistry 24:937–943
9. Del Rio JA, Arcas MC, Botia JM, Baidez AG, Fuster MD, Ortuno AM (2000) Involvement of phenolic compounds in the antifungal defense mechanisms of *Olea europaea L.* and *Citrus* sp. Recent Res Dev Agric Food Chem 4:331–341
10. Desjardins AE, Plattner RD (1989) Trichothecene toxin production by strains of *Gibberella pulicaris* (*Fusarium sambucinum*) in liquid culture and in potato tubers. J Agric Food Chem 37:388–392
11. Desjardins AE, Christ-Harned EA, McCormick SP, Secor GA (1993) Population structure and genetic analysis of field resistance to thiabendazole in *Gibberella pulicaris* from potato tubers. Phytopathology 83:164–170
12. Environmental Protection Agency (1988) Science chapters for chlorpropham. EPA Request Identification Number (RIN) 6169-88
13. Environmental Protection Agency (1996) Registration Eligibility Decision. EPA 738-R-96-023
14. Gourama H, Le Tutour B, Tantaoui-Elaraki A, Benbya M, Bullerman LB (1989) Effects of oleuropein, tyrosol, and caffeic acid on the growth of mold isolated from olives. J Food Protect 52:264–266
15. Hahn K, Strittmatter G (1994) Pathogen-defense gene prp1-1 from potato encodes an auxin-responsive glutathione *S*-transferase. Eur J Biochem 226:619–626
16. Hanson LE, Schwager SJ, Loria R (1996) Sensitivity to thiabendazole in *Fusarium* species associated with dry rot of potato. Phytopathology 86:378–384
17. Hirai K (1918) Formation of tyrosol from L-tyrosine by bacteria. Acta Scholae Medicinalis Universitatis Imperialis in Kioto 2:425–432
18. Ishiguro T, Koga N, Takamura K, Maruyama T (1955) Studies on the components of the flowers of *Osmanthus fragrans*. I. Yakugaku Zasshi 75:781–785
19. Kawchuk LM, Holley JD, Lynch DR, Clear RM (1994) Resistance to thiabendazole and thiophanate-methyl in Canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani*. Am Potato J 71:185–192
20. Kimura Y, Tamura S (1973) Isolation of L-β-phenyllactic acid and tyrosol as plant growth regulators from *Gloeosporium laeticolor*. Agric Biol Chem 37:2925
21. Korhammer SA, Haslinger E (1994) Isolation of a biologically active substance from rhizomes of quackgrass [Elymus repens (L.) Gould]. J Agric Food Chem 42:2048–2050
22. Koshino H, Terada SI, Yoshihara T, Sakamura S, Shimanuki T, Sato T, Tajimi A (1988) Three phenolic acid derivatives from stromata of *Epichloe typhina* on *Phleum pratense*. Phytochemistry 27:1333–1338
23. Koshino H, Yoshihara T, Togiya S, Terada SI, Tsukada S, Okuno M, Noguchi A, Sakamura S, Ichihara A (1989) Antifungal compounds from stromata of *Epichloe typhina* on *Phleum pratense*. Tennen Yuki Kagobutsu Toronkai Koen Yoshishu 31:244–251
24. Leach SS (1978) Quality of stored potatoes improved by chemical treatment. Am Potato J 55:155–159
25. Leach SS, Webb RE (1981) Resistance of selected potato cultivars and clones to *Fusarium* dry rot. Phytopathology 71:623–629
26. Lewis MD, Thornton MK, Kleinkopf GE (1996) Understand why CIPC is a necessity. Potato Grow 25:8–9
27. Martiniez Noel GMA, Madrid EA, Bottini R, Lamattina L (2001) Indole acetic acid attenuates disease severity in potato-*Phytopthora infestans* interaction and inhibits the pathogen growth in vitro. Plant Physiol Biochem 39:815–823
28. Mitchell JW, Livingston GA (1968) Methods of studying plant hormones and growth-regulating substances. Agric Handbook No. 336, USDA, Agric Res Serv (Res Educ Econ), Washington D.C.
29. Mondy NI, Sharada D, Munshi CB, Wurm CM (1992) Effect of storage time, temperature, and cooking on isopropyl N-(3-chlorophenyl) carbamate levels in potatoes. J Agric Food Chem 40:197–199
30. Murdoch AW, Wood RKS (1972) Control of *Fusarium solani* rot of potato tubers with fungicides. Ann Appl Biol 72:53–62
31. Narayanan TK, Rao GR (1976) Production of β-(4-hydroxyphenyl)ethanol and β-(4-hydroxyphenyl)lactic acid by *Candida* species. Can J Microbiol 22:384–389
32. Oberhansli T, Défago G, Haas D (1991) Indole-3-acetic acid (IAA) synthesis in the biocontrol strain CHAO of *Pseudomonas fluorescens*: role of tryptophan sidechain oxidase. J Gen Microbiol 137:2273–2279
33. Pawlak A, Pavek JJ, Corsini DL (1987) Resistance to storage diseases in breeding stocks. In: Jellis GJ, Richardson DE (eds) The production of new potato varieties: technological advances. Cambridge University Press, New York, pp 96–98
34. Powelson ML, Johnson KB, Rowe RC (1993) Management of diseases caused by soilborne pathogens. In: Rowe RC (ed) Potato health management. APS Press, St Paul, Minn., p 156
35. Reiners S, Petzoldts CH (2004) Potatoes. In: Reiners S, Petzoldt CH (eds) Cornell integrated crop and pest management guidelines for vegetable crops, chapter 24, http://www.nys-aes.cornell.edu/recommends
36. Rittich B, Pirochtová M (1990) Chromatographic behavior of aromatic acids in reversed-phase high-performance liquid chromatography. J Chromatogr 523: 227–233
37. Schisler DA, Slininger PJ (1994) Selection and performance of bacterial strains for biologically controlling Fusarium dry rot of potatoes incited by *Gibberella pulicaris*. Plant Dis 78:251–255
38. Schisler DA, Slininger PJ, Bothast RJ (1997) Effects of antagonist cell concentration and two-strain mixtures on biological control of *Fusarium* dry rot of potatoes. Phytopathology 87:177–183
39. Schisler DA, Slininger PJ, Kleinkopf G, Bothast RJ, Ostrowski RC (2000) Biological control of Fusarium dry rot of potato tubers under commercial storage conditions. Am J Potato Res 77:29–40

40. Secor GA, Salas B (2001) Fusarium dry rot and Fusarium wilt. In: Stevenson WR, Loria R, France GD, Weingartner DP (eds) Compendium of potato diseases, 2nd edn. APS Press, St Paul, Minn., pp 23–25
41. Secor GA, Rodriguez D, Rodriguez J, Gudmestad NC (1994) Distribution and incidence of benzimidazole-resistant *Fusarium sambucinum* and *Helminthosporium solani* isolated from potato in North America. BCPC Monogr No 60: Fungic Resist 271–274
42. Slininger PJ, Shea-Wilbur MA (1995) Liquid-culture pH, temperature, and carbon (not nitrogen) source regulate phenazine productivity of the take-all biocontrol agent *Pseudomonas fluorescens* 2–79. Appl Microbiol Biotechnol 43:794–800
43. Slininger PJ, Schisler DA, Bothast RJ (1994) Two-dimensional liquid culture focusing: a method of selecting commercially promising microbial isolates with demonstrated biological control capability. In: Ryder MH, Stephens PM, Bowen GD (eds) Improving plant productivity with rhizosphere bacteria. 3rd International Workshop on Plant Growth-promoting Rhizobacteria, Adelaide, Australia. CSIRO Division of Soils, Glen Osmond, South Australia, pp 29–32
44. Slininger PJ, Van Cauwenberge JE, Bothast RJ, Weller DM, Thomashow LS, Cook RJ (1996) Effect of growth culture physiological state, metabolites, and formulation on the viability, toxicity, and efficacy of the take-all biocontrol agent *Pseudomonas fluorescens* 2–79 stored encapsulated on wheat seeds. Appl Microbiol Biotechnol 45:391–398
45. Slininger PJ, Van Cauwenberge JE, Shea-Wilbur MA, Burkhead KD, Schisler DA, Bothast RJ (1997) Reduction of phenazine-1-carboxylic acid accumulation in growth cultures of the biocontrol agent *Pseudomonas fluorescens* 2–79 eliminates phytotoxic effects of wheat seed inocula without sacrifice to take-all suppressiveness. In: Ogoshi A, Kobayashi K, Homma Y, Kodama F, Kondo N, and Akino S (eds) Plant growth-promoting rhizobacteria present status and future prospects. 4th PGPR Workshop Organizing Committee. Hokkaido University, Sapporo, Japan, pp 464–467
46. Slininger PJ, Burkhead KD, Schisler DA, Bothast RJ (2000) Biological control of sprouting in potatoes. US Patent no. 6 107 247
47. Slininger PJ, Schisler DA, Burkhead KD, Bothast RJ (2003) Postharvest biological control of potato sprouting by Fusarium dry rot suppressive bacteria. Biocontrol Sci Technol 13:477–494
48. Sorce C, Lorenzi R, Ceccarelli N, Ranalli P (2000) Changes in free and conjugated IAA during dormancy and sprouting of potato tubers. Aust J Plant Physiol 27:371–377
49. Spinnler HE, Djian A (1991) Bioconversion of amino acids into flavouring alcohols and esters by *Erwinia carotovora* subsp. *atroseptica*. Appl Microbiol Biotechnol 35:264–269
50. Tanaka T (1958) Decomposition of L-tyrosine by bac. coli-organisms. (I) The decomposition activity and product of L-tyrosine by *B. coli* var. *acidi lactici*. Bull Pharm Res Inst (Osaka Medical College) 17:1–5
51. Tanaka T(1964) The decomposition of L-tyrosine and its derivatives by *Proteus vulgaris*. (1) Production of *p*-hydroxyphenylpropionic acid from L-tyrosine by *Proteus vulgaris*. Bull Pharm Res Inst (Osaka Medical College) 50:1–7
52. Tivoli B, Deltour A, Molet D, Bedin P, Jouan B (1986) Mise en évidence de souches de *Fusarium roseum* var. *sambucinum* resistantes au thiabendazole, isolées àpartir de tubercules de pomme de terre. Agronomie 6:219–224
53. Van Es A, Hartmans KJ (1987) Dormancy, sprouting and sprout inhibition. In: Rastovski A (ed) Storage of potatoes: post-harvest behavior, store design, storage practice, handling, 2nd edn. Pudoc, Wageningen, pp 114–132
54. Wastie RL, Stewart HE, Brown J (1989) Comparative susceptibility of some potato cultivars to dry rot caused by *Fusarium sulphureum* and *F. solani* var. *coeruleum*. Potato Res 32:49–55