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ORIGINAL PAPER

Potato signal molecules that activate pectate lyase synthesis in *Pectobacterium atrosepticum* SCRI1043

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Abstract A new type of plant-derived signal molecules that activate extracellular pectate lyase activity in phytopathogenic bacterium *Pectobacterium atrosepticum* SCRI1043 was revealed. These compounds were characterized and partially purified by means of several approaches including RT-PCR analysis, luminescence bioassay and HPLC fractionation. They were smaller than 1 kDa, thermoresistant, nonproteinaceous, hydrophilic, and slightly negatively charged molecules. Using gene expression analysis and bacterial biosensor assay the mode of activity of revealed compounds was studied. The possibility of their action through quorum sensing- and KdgR-mediated pathways was analyzed.

Keywords *Pectobacterium atrosepticum* · Extracellular enzymes · Pectate lyases · Potato signal molecules

Introduction

The majority of phytopathogenic microorganisms produce a variety of plant cell wall degrading enzymes (PCWDEs) necessary for survival and propagation of bacteria within the host environment. *Pectobacterium atrosepticum* (formerly *Erwinia carotovora* subsp. *atroseptica*) is an economically important "brute force" plant pathogen which causes tissue maceration, resulting in symptoms of soft-rot and blackleg in potato.

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Pectate lyases (Pels) are one of the main virulence factors in soft rot enterobacteria. Expression of the genes encoding Pels is subject to subtle regulation according to environmental conditions such as temperature, pH, and osmolarity, and depending on the growth phase, substrate availability, etc. (Hugouvieux-Cotte-Pattat et al. 1992, 1996; Reverchon et al. 1997; Lautier et al. 2007; Sepulchre et al. 2007; Charkowski et al. 2012). Synthesis of PCWDEs is controlled by quorum-sensing in population density-dependent manner (Whitehead et al. 2002; Crepin et al. 2012; Charkowski et al. 2012). It was well-documented that production of virulence factors is repressed in quorum sensing-deficient mutants (Chatterjee et al. 1995; Liu et al. 2008).

Active production of PCWDEs occurs only when bacteria "sense" their host, namely various host-derived compounds that serve as a signal for bacteria. This characteristic most likely serves to prevent the "useless" expenditure of the endogenous resources for the synthesis of virulence factors while the bacteria reside outside the host plant. The best studied plant-derived inducers of synthesis of bacterial Pels are pectin derivatives (Hugouvieux-Cotte-Pattat and Robert-Baudouy 1989; Roy et al. 1999; Blot et al. 2002). The metabolism of pectic compounds in bacteria includes several main stages. First, by means of bacterial extracellular enzymes, polymers are digested into oligomers, which are transported to the periplasmic space. Then, these oligomers are split into di- or trimers, which are transported through the inner membrane and converted to pyruvate. One of the intermediates of the intercellular metabolism of pectin derivatives—2 keto-3-deoxygluconate (KDG)—displays affinity to KdgR, a transcriptional repressor of many genes, including those encoding PCWDEs and enzymes of intercellular metabolism of pectin derivatives. The interaction of KDG and KdgR results in the upregulation of target genes.

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Pectin derivatives are unlikely to be not the sole host-derived inducers of PCWDEs synthesis because plant metabolites other than pectin were shown to stimulate Pel production (Bourson et al. 1993; Kelemu and Collmer 1993; Brencic and Winans 2005). However, these metabolites were poorly characterized. The induction of expression of PCWDE genes in *P. atrosepticum* in the presence of potato stem or tuber water extracts was demonstrated by Mattinen et al. (2007) by means of microarray analysis. In that study the inducers were shown to be low molecular weight (<5 kDa compounds).

In our study we tried to obtain additional information on the compounds present in the potato extract that enhance extracellular pectate lyase activity in *P. atrosepticum* SCRI1043. These compounds were found to be less than 1 kDa, nonproteinaceous, hydrophilic, slightly negatively charged molecules. The possibility of their action through quorum sensing- and KdgR-mediated pathways was analyzed.

Materials and methods

Bacterial strains, media and growth conditions

Pectobacterium atrosepticum SCRI1043 was grown in a Luria-Bertani medium (LB) with aeration at 28 °C to the late exponential growth phase and then harvested by centrifugation. Cells were washed twice in synthetic M63 medium with glycerol (0.2 %) (Miller 1972) and then diluted to $\sim 10^7$ cell/ml in M63 medium for experimental procedures. The resultant cultures were incubated in M63 medium containing test compounds: potato extract (2–20 µl/ml) or fractions from chromatograph (10–50 µl/ml). When it was needed, polygalacturonate (PGA) (0.25 %) was added to the culture media as a pectate lyase inducer. *Escherichia coli* JLD271 was grown in an LB medium supplemented with tetracycline (50 µg/ml) with aeration at 37 °C.

Pectate lyase, cellulase and protease assay

All assays were performed with supernatants after 24 h of incubation of cells with plant metabolites. Cellulase and protease activities were determined according to Chatterjee et al. (1995). Pectate lyase activity was determined by measuring the degradation of PGA into unsaturated products that absorbed at 234 nm (Moran et al. 1968). The initial linear part of the kinetic curve was used to calculate the rate of enzymatic reaction using Cary Win Kinetics. Specific activity of all enzymes was expressed as units per milligram of bacterial dry weight.

Preparation of potato extract

Crude plant extract was made by grinding 100 g of potato stems with 250 ml MilliQ in a blender. The mixture was incubated for 2 h at 4 °C with gentle agitation, then filtered and centrifuged at 10,000 rpm, 4 °C, for 20 min. The supernatant was further clarified by filtering through a 0.2 µm filter followed by ultrafiltration (Ultra-15 centrifugal filter device, Millipore) to eliminate compounds larger than 3 kDa. Finally, the low-molecular-weight filtrate was lyophilized, re-dissolved in MilliQ (40-fold concentrated sample), filter-sterilized and stored at −20 °C prior to bioassay.

HPLC analysis of potato signal molecules

Low-molecular-weight filtrate was analyzed by HPLC for fractions containing signal activity. 500 µl samples were first injected onto a MilliQ-equilibrated size-exclusion Bio-Select SEC125 column (Bio-Rad). All the obtained fractions were concentrated by lyophilization and, after filter sterilization, were tested for their inducing properties as described above. Inducing fractions of different runs were pooled and concentrated. A further step of purification was performed using a Synergi 4u MAX-RP 80A column (Phenomenex) equilibrated with 5 % acetonitrile in MilliQ and elution was performed with an acetonitrile/MilliQ gradient. The pool of inducing fractions was finally injected onto anion-exchange column UNOQ (Bio-Rad) equilibrated with 20 mM Tris–HCl buffer, pH 8.1 and eluted with linear NH_4 -acetate gradient. The compounds were monitored at A_{260} using BioLogic QuadTec UV/Vis Detector. Flow rate in all cases was 0.5 ml/min.

RNA isolation and reverse transcription

Total RNA was isolated from bacterial cells using the Yellow Solve kit (Sileks) according to the manufacturer's instructions. RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Thermo), and its quality was verified by gel electrophoresis. Total RNA was used for cDNA synthesis. The reaction mixture for reverse transcription contained 1 mM random hexamers, 300 mM dNTPs, 1× Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (RT) buffer, 100 U of M-MLV RT (Sileks), and the required amount of RNAse inhibitor. First, water and random hexamers were mixed with RNA, incubated for 5 min at 70 °C, and cooled immediately on ice. The other components were then added. Incubation was performed using a DNA Engine Thermal Cycler (Bio-Rad). Reverse transcription was performed as follows:

10 min at 25 °C, followed by 1 h at 37 °C, and 10 min at 70 °C. Two microlitres of cDNA were used as the template for real-time PCR.

Quantitative real-time RT-PCR analysis

Real-time RT-PCR was performed using the TaqMan detection method. The primers and probes used (*kduDF*—5′-CAAACAAggTAAAggCggCAA-3′; *kduDR*—5′-CTgCggTCTTCATCggCg-3′; *kduD TaqMan probe*—5′-(FAM-C)TggCTAACgAg(T-BHQ-1)gggCAAAACACggCATCAACg-3′; *pelBF*—5′-CACgCCAgACAATgACACgAC-3′; *pelBR*—5′-gCATgAACCAgACCACCACgC-3′; *pelB TaqMan probe*—5′-(FAM-C)gCAAgCAA(T-BHQ-1)ACggATACAggACgCAATC-3′; *rpoDF*—5′-CTAATgCggAAgAAgACATTgCCCC-3′; *rpoDR*—5′-CTTCTCACgCgCCAgTTCAggATCg-3′; *rpoD TaqMan probe*—5′-(FAM-C)gTCTTCTTC(T-BHQ-1)CgTCTTCgTCTTCTTCCTCgTCg-3′) were designed according to the genome sequence of *P. atrosepticum* SCRI1043 (NCBI GenBank accession number NC_004547) using Vector-NTI Version 9 software (Invitrogen) and synthesized by Sintol. The reaction mixture contained 1x PCR buffer [67 mM Tris–HCl, pH 8.8, 17 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, and 0.1 % Tween 20], 100 mM each dNTP, 0.04 U of Taq DNA Polymerase (Sileks), 0.2 mM each primer, and 0.1 mM TaqMan probe. PCR was performed under the following conditions: 94 °C for 2 min, followed by 45 cycles at 94 °C for 10 s, and at 60 °C for 1 min. The reactions were run and changes in fluorescence emission were detected using an IQ-4 quantitative PCR system (Bio-Rad). The amount of fluorescence was plotted as a function of the PCR cycle using iCycler iQ Optical System Software Version 3.1 (Bio-Rad). The amplification efficiency (E) for all primers was determined using a dilution series of a pool of cDNAs. Additional controls included the omission of RT to measure the extent of residual genomic DNA contamination and omission of template. The *rpoD* gene was chosen as an internal standard (Savli et al. 2003). Three replicates were performed for each reaction. Relative expression levels were determined as the ratio between the quantity of cDNA that corresponded to the target (*kduD*) and reference (*rpoD*) gene transcripts.

Detection of AHL

The level of AHL was analysed using *E. coli* JLD271 that carried the bioluminescence reporter vector pAL103 or the vector pAL104 as a negative control (Lindsay and Ahmer 2005). The luminescence from each probe was detected using a Gel Doc Molecular Imager and measured using Quantity One Analysis Software (Bio-Rad). Commercial *N*-hexanoyl-D,L-homoserine lactone (Sigma) was used as a control.

Results

Effect of potato extract on extracellular pectate lyase, cellulase and protease activities *P. atrosepticum*

No pectate lyase (Pel), cellulase (Cel), and protease (Prt) activities were found in culture supernatants of *P. atrosepticum* grown in LB media (Table 1). This is in accordance with Wei et al.'s assumption that the expression of virulence genes is "silenced" when bacteria are incubated in rich nutritional media (Wei et al. 1992). Minimal M63 medium was used for assays of extracellular enzyme activities. Cel activity in culture supernatants of *P. atrosepticum* grown in M63 medium was below the detectable level (Table 1). The addition of potato extract led to the onset of Cel activity. Prt activity was detected in culture supernatants when *P. atrosepticum* was grown in M63 medium both with and without potato extract; however in the former case the activity was 53 % higher. The most pronounced effect of potato extract was observed for Pel activity, which was about 30-fold higher in M63-potato extract medium (Table 1). Therefore, the enhancement of Pel activity was assessed as an indicator of the presence of potato-derived inducers.

The addition of plant extract led to a dramatic increase of extracellular Pel activity in *P. atrosepticum*, which was regulated in a population density-dependent manner (Fig. 1). The level of *pelB* expression (Fig. 2) was markedly high in the presence of potato extract indicating that overproduction of Pel was regulated at the transcriptional level.

The Pel-inducing effect of plant extract was evident in a concentration-dependent manner (Table 2). Maximum effect was achieved when 20 µl of potato extract was added per 1 ml M63 medium. Pel is known to be upregulated in the presence of pectin derivatives (Bourson et al. 1993; Blot et al. 2002). We compared the effect of PGA and potato extract on the Pel activity of *P. atrosepticum*. Pel activity was 5.5-fold higher when PGA was added to the culture medium as a carbon source (Fig. 3). However, the Pel-inducing effect of potato extract was much more

Table 1 The activities of extracellular enzymes in *Pectobacterium atrosepticum* SCRI1043 cultures, grown in the presence or absence of potato extract

Medium	Cel	Prt	Pel
LB	0	0	0
M63	0	3.16 ± 0.51	3.35 ± 0.49
M63-potato extract	0.12 ± 0.03	4.85 ± 0.55	59.25 ± 6.2

The values represent mean ± SD of three independent measurements. Specific activities are expressed as units per milligram of dry bacterial weight

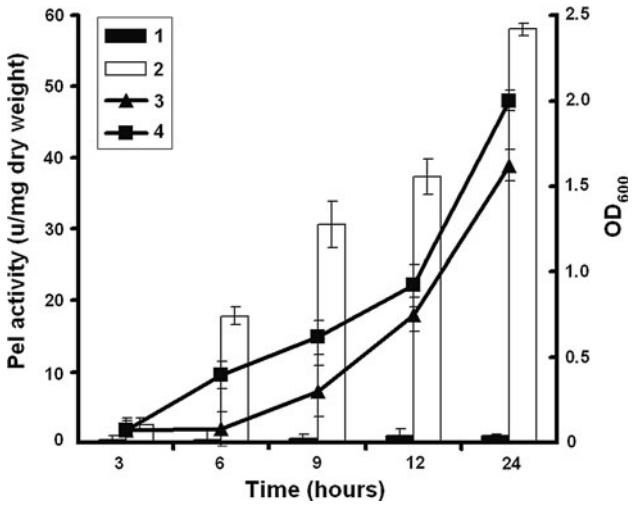

Fig. 1 Effect of potato extract on proliferation (*lines*) and extracellular pectate lyase activity (*column*) of *P. atrosepticum* SCRI1043. Bacterial cells were grown in M63 glycerol medium alone (*1*, *3*) or supplemented with the low-molecular-weight fraction of potato extract (*2*, *4*). The values represent mean ± SD of three independent measurements

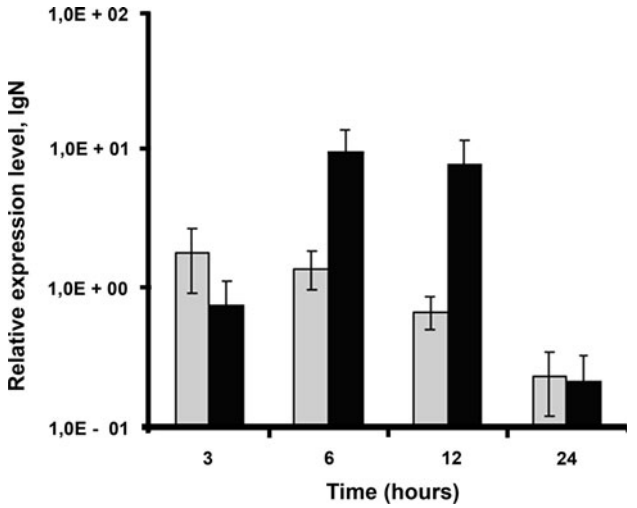

Fig. 2 Expression of *pelB* gene in *P. atrosepticum* SCRI1043 grown in M63 glycerol medium alone (*grey*) or supplemented with the low-molecular-weight fraction of potato extract (*black*). The values represent mean ± SD of three independent measurements

pronounced than that of PGA (25.5-fold higher as compared to the control). A maximal level of Pel activity was observed in the presence of both PGA and potato extract (64.8-fold higher with respect to the control).

PGA-mediated activation of PCWDEs is carried out by an intermediate (KDG), which is formed during intracellular metabolism of pectin degradation products by KduD enzyme (Hugouvieux-Cotte-Pattat and Robert-Baudouy 1989; Barnard and Salmond 2007).

In our experiments the level of *kduD* gene expression increased in the presence of PGA (Fig. 4), irrespective of whether or not potato extract was added to the *P. atrosepticum* cultures. In contrast to PGA, potato extract alone did not induce the expression of *kduD*, suggesting that the amount of pectin derivatives (if any) in the extract was not enough to affect the expression of "pectin-regulated" genes.

Table 2 Extracellular pectate lyase activity in the cultures of *Pectobacterium atrosepticum* SCRI1043 grown at different concentrations of potato extract

Plant extract (µl/ml M63)	Activity (u/mg)
0	3.0 ± 0.17
2	10.2 ± 0.71
5	18.8 ± 1.41
10	29.4 ± 2.12
20	70.6 ± 6.35
30	68.3 ± 5.46

The values represent mean ± SD of three independent measurements

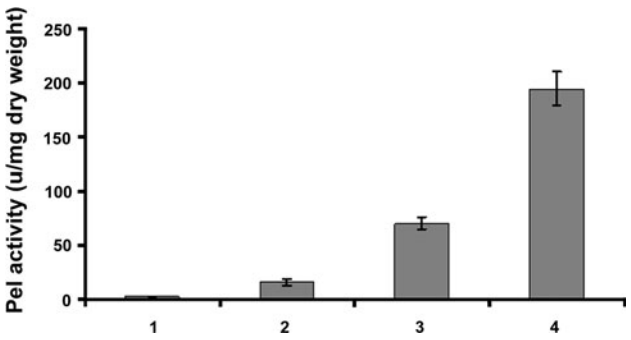

Fig. 3 Effect of polygalacturonic acid (PGA) and potato extract on the extracellular pectate lyase activity in the cultures of *Pectobacterium atrosepticum* SCRI1043. Bacteria were grown in M63 glycerol medium alone (*1*) or supplemented with PGA (*2*), potato extract (*3*) and both PGA and potato extract (*4*). The values represent mean ± SD of three independent measurements

To determine whether the observed increase in Pel activity resulted from the presence of AHL (*N*-acyl homoserin lacton)-mimic compounds in potato extract and/or activation of an AHL-mediated quorum sensing system in *P. atrosepticum,* the AHL-sensor strain of *E. coli* JLD271 carrying a bioluminescence reporter vector (pAL103) was used (Lindsay and Ahmer 2005). Addition of culture supernatants of *P. atrosepticum* to the AHL-sensor strain led to light emission (Fig. 5). The supernatants obtained from *P. atrosepticum* cultures in the presence or absence of potato extract induced luminescence in *E. coli* JLD271+pAL103 to similar levels. The addition of potato extract alone directly to the cultures of *E. coli* JLD271+pAL103 did not result in the luminescence of the AHL-sensor strain. These results indicated that potato extract did not contain the compounds which functionally resemble AHL that are typical for *P. atrosepticum* SCRI1043.

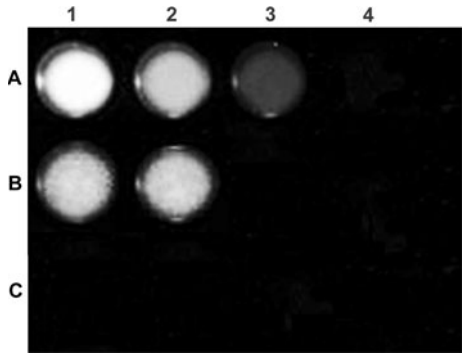

Fig. 4 Semiquantitative assay for C6-AHL and C6-AHL-mimic compounds. The photograph shows wells in a microtitre plate that contained 180 µl of cultures of *E. coli* JLD271, caring vector pAL103 (*line A* and *B*) or pAL104 (*line C*). The wells in *line A* contained a series of dilutions of *N*-(3-oxohexanoyl)-*L*-homoserine lactone (0.1, 0.01, 0.001 and 0 mM, respectively). In the *line B* and *C*, 20 µl of the supernatants of *Pectobacterium atrosepticum* SCRI1043 cultures incubated in the presence (*1*) or absence (*2*) of potato extract were added. Wells *3B* and *3C* contained 20 µl of the potato extract

Properties of potato-derived compounds that enhance Pel activity in *P. atrosepticum*

To characterize the plant-derived *pel*-inducing compounds, we tested their heat stability and solubility in different organic solvents. The *pel*-inducing compounds were thermostable, since after autoclaving and freezing-thawing, the potato extract retained the ability to induce pectate lyase activity in *P. atrosepticum*. Treatment with proteinase K (250 µg/ml) for 3 h at pH 7.0 and 37 °C did not affect *pel*-inducing properties of potato extract. *pel*-inducing properties of the extract also remained intact after extraction with chloroform, isopropanol, or ethyl acetate, indicating that the *pel*-inducing compounds were hydrophilic. After extraction with 80 % methanol for 30 min at 80 °C, or precipitation with 75 % acetone for 60 min at 4°C, *pel*-inducing activity was observed both in precipitate and in supernatant fractions.

To purify plant-derived *pel*-inducing compounds, we fractionated the components of potato extract according to molecular size, hydrophobic and charge properties. Fractionation using centrifugal filter devices showed that the *pel*-inducing compounds had a small molecular size, less than 3 kDa. During fractionation of low-molecular-weight components of plant extract on a Bio-Select SEC125 exclusion column the fractions that enhanced extracellular pel activity in *P. atrosepticum* were eluted as a single peak, with an apparent molecular mass below 1 kDa (Fig. 6a). These fractions were combined, lyophilized and re-dissolved in deionized water with 5 % acetonitrile.

A further step of purification was performed using a Synergi 4u MAX-RP 80A column (Fig. 5b). The inducing compounds did not adhere to the chromatography matrix, because only flow-through fractions induced Pel activity in *P. atrosepticum*. The same was true when a Synergi 4u Polar-RP 80A column was used (data not shown). After lyophilization of active fractions, precipitate was re-dissolved in 20 mM TrisHCl-buffer, pH 8.1 and loaded onto a UNOQ column (Fig. 6c). Three distinct peaks of inducing compounds were obtained. The major portion of activity was linked to the second peak that was eluted at 0.1 M NH_4-acetate and had a λ max value at 228 nm in the absorption spectrum (Fig. 7).

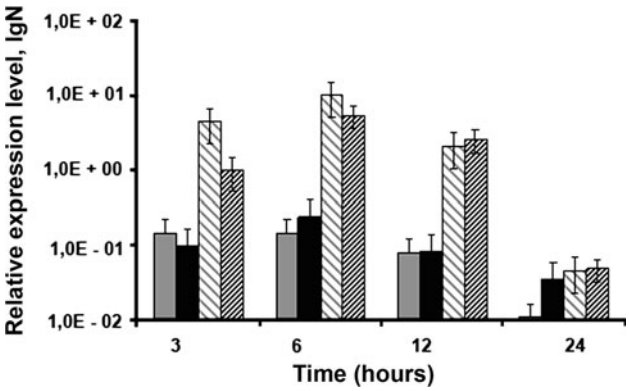

Fig. 5 Expression of *kdu*D gene in *P. atrosepticum* SCRI1043 grown in M63 glycerol medium alone (*grey*), or supplemented with potato extract (*black*), PGA (*hatched grey*), or both potato extract and PGA (*hatched black*). The values represent mean ± SD of three independent measurements

Discussion

In the present study, low molecular weight compounds that induce the activity of extracellular enzymes (Pel, Cel, Prt) in *P. atrosepticum* SCRI1043 were revealed in potato extract. The addition of low molecular weight water-extracted fractions of potato stems to bacterial cultures led to an increase in Prt, Cel and Pel activities, which are known to be crucial for pathogenesis (Pirhonen et al. 1991; Barras et al. 1994; Hugouvieux-Cotte-Pattat et al. 1996; Perombelon 2002). Although the observed effect was evident for all three types of enzymes studied in this work, the degree of induction of activity for the different enzymes varied. The most pronounced effect was observed for Pel. This raises the possibility that plant metabolites may be in charge of both the triggering of different virulence factors and the more specific induction of *pel*-genes. Assay for Pel activity was further used as a test system.

Activation of PCWDEs is well-known to occur in the presence of pectin compounds (Bourson et al. 1993; Chatterjee et al.1995; Blot et al. 2002). Intracellular metabolism of pectin degradation products leads to the formation of an inducer (KDG) of the synthesis of pectin

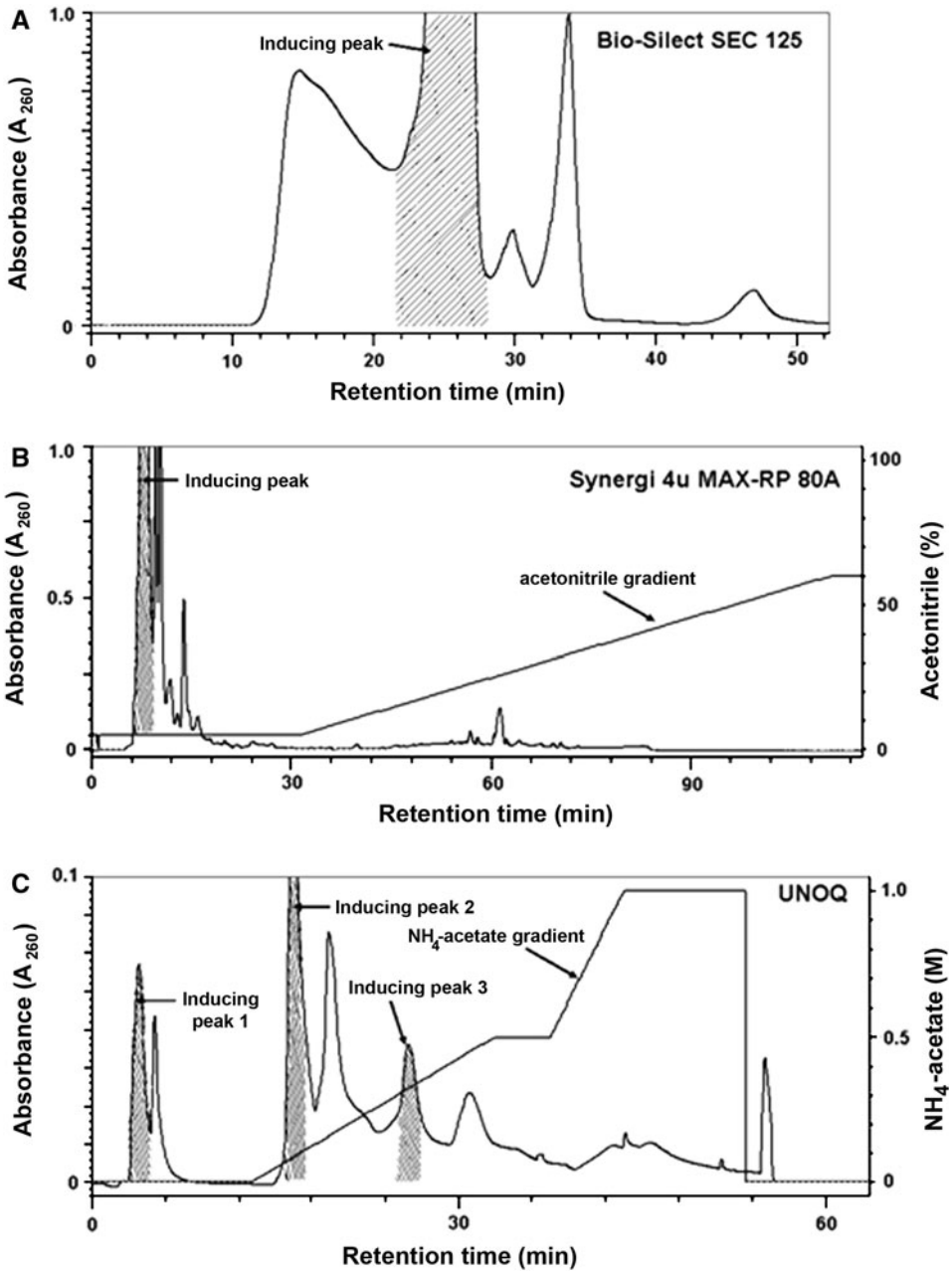

Fig. 6 Sequential separation of potato-derived signal molecules on an exclusion column Bio-Silect SEC 125 (**a**), C_{18} reversed-phase column Synergy 4u MAX-RP 80A (**b**) and anion-exchange column UNOQ (**c**). Fractions, which induced Pel activity in *P. atrosepticum* are indicated as diagonal hatched regions. Results shown are from a single experiment representative of multiple independent experiments

degradation enzymes. KDG nullifies the negative effect of KdgR—the transcriptional repressor of pectin degradation enzymes genes and thus induces their expression. Formation of KDG is carried out by 2-keto-3-deoxygluconate oxidoreductase (KduD). The expression of the *kduD* gene, similar to the genes encoding PCWDEs, is known to be repressed by KdgR and de-repressed in the presence of pectin derivatives, KDG in particular (Nasser et al. 1994; Hugouvieux-Cotte-Pattat et al. 1996). Thus, the expression of both the *pel* and *kduD* genes is upregulated by pectin derivatives in a KdgR-mediated manner.

In spite of upregulation of the *pelB* gene and an increase of pectate lyase activity in *P. atrosepticum* cultures, no increase of *kduD* gene expression was observed in the presence of potato extract. When bacterial growth was supported by PGA, *kduD* gene expression increased irrespectively of the presence or absence of potato extract (Fig. 4). These results indicate that potato extract affect *kduD* gene expression neither positively nor negatively, suggesting that no pectin derivatives in amounts sufficient for upregulation of "KdgR-controlled" genes were present. Thus, the observed effect of induction of pectate lyase activity and *pelB*-gene expression in the presence of potato extract was likely to be unrelated to the presence of pectin compounds (if any) in the obtained plant extract. This observation is supported by the fact that PGA enhanced the *pel*-inducing effect of potato extract.

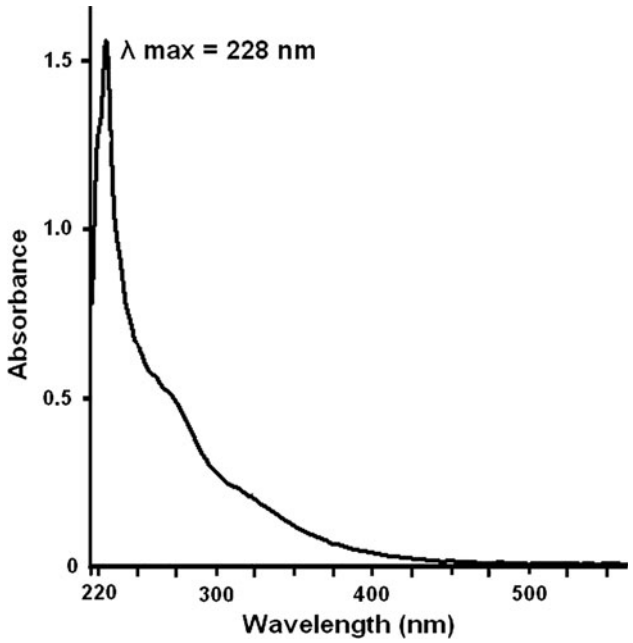

Fig. 7 Absorption spectrum of potato-derived signal molecule eluted from anion-exchange column UNOQ (inducing peak 2)

Potato extract influenced the Pel activity in *P. atrosepticum* in a concentration-dependent manner, reaching the maximum level at a concentration of 20 µl per ml M63 medium. However, if PGA was added to the bacterial culture together with 20 µl/ml of plant extract, the level of the enzymatic activity was 2.8-times higher than in the presence of plant extract alone. Moreover, the PGA alone induced Pel activity to a much lower level than both PGA and potato extract. Thus the inducers that were present in potato extract acted in synergy with pectin-derived inducers. The synergistic effect of PGA and potato extract towards Pel indicates that these two types of inducers likely act through different regulatory networks, supplementing each other and resulting in maximal enzyme activity.

Production of most of the virulence factors in *Pectobacterium* is known to be regulated by quorum sensing (Barnard and Salmond 2007; Crepin et al. 2012; Charkowski et al. 2012). In turn, several plant species were shown to contain the metabolites that interfere with signal molecules—AHLs, leading to the activation of a quorum sensing system (Teplitski et al. 2000; Fray 2002; Mae et al. 2001). In our study we considered the possibility of the presence of AHL-mimic compounds in potato extract. Thus, an AHL-sensor strain *E.coli* JLD271, caring a vector paL103 was chosen. This strain generates luminescence in response to the presence of C6-AHLs which are typical for *P. atrosepticum* SCRI1043. Addition of potato extract to the AHL-sensor strain did not result in light emission. Moreover, AHL production by *P. atrosepticum* in the presence and absence of potato extract was similar (Fig. 5). This indicates that the effect of potato extract on Pel activity was not mediated through the quorum sensing.

To gain additional insights into the physical and chemical properties of plant-mediated *pel*-inducing compounds, fractionation of plant extract was performed. Inducers of Pel activity appeared to be thermostable and resistant to proteinase digestion and had molecular weights below 1 kDa. The presence of *pel*-inducing compounds in the supernatants after methanol or acetone extraction might suggest the possibility of their phenolic nature, especially since ferulic acid was shown to enhance the synthesis of Pel in the presence of PGA in *Dickeya dadantii* which is related to *P. atrosepticum* (Hassan and Hugouvieux-Cotte-Pattat 2011). However, in our study, ferulic acid did not affect pectate lyase activity in *P. atrosepticum* either in the presence or absence of PGA (data not shown). Subsequent fractionation of *pel*-inducing compounds by hydrophobic chromatography using Synergi 4u MAX-RP 80A and Synergi 4u Polar-RP 80A columns provided additional arguments to consider the non-phenolic nature of the target molecules. These were highly hydrophilic and did not adhere to the column matrix, since only the flow-through fractions enhanced Pel activity in the bacterial cultures. After fractionation by means of anion-exchange chromatography, three distinct peaks were found to activate Pel activity, indicating that at least three different compounds in potato extract enhance Pel activity in *P. atrosepticum*. All of these compounds were slightly negatively charged.

Thus, in the study reported herein, additional insights were gained into the diversity of host plant-derived factors affecting the production of virulence factors in *P. atrosepticum* SCRI1043. It was shown that extract of potato stems contains metabolites that increase Prt, Cel and especially Pel activity in *P. atrosepticum*. Plant-derived inducers of Pel were found to be of low molecular weight (<1 kDa), non-proteinaceous, hydrophilic and slightly negatively-charged compounds. These compounds did not resemble any known inducers of Pel (pectin derivatives, AHL-mimic and phenolic compounds) either in their mode of action or chemical properties. The diversity of host plant-derived compounds that influence the production of crucial virulence factors is likely to promote additional ways for *P. atrosepticum* to fine-tune its behavior within the host plant.

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