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THE INDUCTION OF DIFFERENTIALLY EXPRESSED PROTEINS OF *XYLELLA FASTIDIOSA* WITH CITRUS EXTRACT

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ABSTRACT

An *in vitro* system was developed to induce and identify *Xylella fastidiosa* proteins that were differentially expressed in the presence of callus-derived extracts from its host, the citrus cultivar Pêra. To optimize the induction, we first developed a single culture medium for the growth of both, host and bacteria. This medium, CPXPm7, which mimics the citrus xylem sap, showed that *X. fastidiosa* at 72 h post-incubation had 10^8 colony forming units mL^{-1}, while Pêra cells had the highest fresh weight content (0.79 g). After testing various methods of co-cultivation of the bacteria and host callus grown in this single medium, the best induction procedure was to grow *X. fastidiosa* in a solid medium amended with an extract of Pêra callus grown in CPXPm7. Analysis, by two-dimensional electrophoresis, of the *X. fastidiosa* proteins (120 µg of total proteins) grown in the presence of Pêra callus extract revealed 414 differentially expressed protein spots when compared to the protein profile obtained in the absence of the extract. The system developed in this study improves the induction and analysis of differentially expressed proteins of *X. fastidiosa,* which may be involved in pathogenicity.

Key words: 2DE, callus, pathogenicity, protein, *Xylella*-citrus interaction

INTRODUCTION

Xylella fastidiosa (*X. fastidiosa*), a slow-growing, xylem-inhabiting Gram-negative bacterium, is a pathogen that causes Pierce's disease of grapevine (PD), phony peach disease (PPD), leaf scorch of coffee, plum, mulberry, pear, almond, elm, sycamore, oak, maple, and variegated chlorosis (CVC) of citrus (8,33). Brazil is the world's largest producer of citrus with over 70% of that production in the form of orange juice concentrate. Since sweet oranges (*Citrus sinensis* (L.) Osbeck) are the main source for orange juice concentrate and most cultivars are susceptible to CVC, this disease poses a major threat to the Brazilian citrus industry.

X. fastidiosa resides in the xylem vessels of the infected plant and the growth and metabolism of the bacteria result in host-bacterial induced adaptations, which are essential for the disease process and are key points for the investigation and understanding of the host-pathogen interaction (28-30).

In vitro methods are widely utilized to study growth characteristics and nutritional demands of pathogenic bacteria as well as host-pathogen interactions (3). Nutritional media and conditions that provide the appropriate biochemical and biophysical milieu can mimic the host and stimulate the bacteria to express adaptation or pathogenicity factors (10,25). The specific needs and physiological properties of each particular bacteria-host interaction require that these media be tailored specifically to maximize the response.

Due to its fastidious nature, *X. fastidiosa* requires a complex medium for growth under axenic conditions. Different media have been developed to culture these bacteria, and most of

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them have complex nutrients, such as tryptone, yeast extract, soytone, hemin chloride and bovine serum albumin (BSA) (5-7,33). Recently, Chang and Donaldson (4) investigated the nutritional requirement of *X. fastidiosa* from grape and developed a defined growth medium composed mainly of three inorganic acids, two tricarboxylic acids and 17 amino acids. Likewise, Lemos *et al.* (14) developed a defined growth medium for *X. fastidiosa* from citrus. Based upon these studies and others, we hypothesized that it was possible to develop a medium that would induce *X. fastidiosa* from citrus to express, *in vitro*, proteins involved in host pathogenicity. Ideally, this could be done with citrus xylem sap. However, due to the presence of endophytes in the citrus xylem sap (1) and a requirement of relatively large amounts of protein for analysis, the use of sap for *in vivo* studies is limited. To alleviate these problems, citrus and bacteria cells could be cultured axenically, separately or together, to conduct the interaction studies. However, the media used to culture citrus cells *in vitro*, such as those based upon Murashige and Skoog salts (17), do not allow the growth of *X. fastidiosa*. Likewise, the known culture media used to grow *X. fastidiosa* do not support the growth of citrus cells *in vitro*.

The objective of this study was to obtain potential pathogenicity proteins from a CVC isolate of *Xylella fastidiosa* (9a5c) through the induction of differentially expressed bacterial proteins after being co-cultivated with the citrus cv. Pêra. For that, a single culture medium for *in vitro* growth of citrus and *X. fastidiosa* cells was developed and utilized to test various methods of co-cultivation of the pathogen and host cells to find a procedure that optimized the induction of differentially expressed proteins. Additionally, a protein extraction and two-dimensional electrophoresis (2DE) protocols were developed to maximize and improve the analysis of these differentially expressed proteins.

MATERIALS AND METHODS

A single non-defined culture medium, CBXPm7

A single non-defined culture medium, CBXPm7, was developed for the growth of Citrus sinensis (L.) Osbeck cv. Pêra and *Xylella fastidiosa*. This medium was developed to maximize growth and to standardize the analysis of the various culture methods and was formulated from Murashige and Skoog (MS) salts, PW ingredients and the composition of citrus xylem sap (5,6,12,13,16,17,32). This medium was made up of a mixture of two solutions, one autoclaved (A) and the other filtered sterilized (B). The medium composition is listed below and gives the final concentration for each component in the complete medium.

Part A. 4 g L^{-1} Phytone peptone (BBL); 1 g L^{-1} Trypticase peptone (BBL); 0.002 g L^{-1} Hemin Chloride (Sigma); 20 mL L^{-1} glycerol (Sigma); 3 g L^{-1} PVP40 (Calbiochem); 0.125 mM KH_2PO_4 (Merck); 2.1 mM NH_4NO_3 (Merck); 0.3 mM $CaCl_2.2H_2O$ (Merck); 0.02 mM $Na_2FeEDTA$ (Merck); 0.15 mM $MgSO_4.7H_2O$ (Merck); 550 µM myo-inositol (Sigma); 30 µM thiamine (Sigma); 10 µM pyridoxine (Sigma); 15 µM nicotinic acid (Sigma); 0.4 mg L^{-1} 6-benzyl-aminopurine (6-BA) (Sigma); 100 µM H_3BO_3 (Merck); 100 µM $MnSO_4.4H_2O$ (Merck); 30 µM $ZnSO_4.7H_2O$ (Merck); 5 µM KI (Merck); 1 µM $Na_2MoO_4.2H_2O$ (Merck); 0.1 µM $CoCl_2.6H_2O$ (Merck); 0.1 µM $CuSO_4.5H_2O$ (Merck); 2 g L^{-1} phenol red (Merck); and 15 g L^{-1} agar (Merck) (agar was used only when preparing solid medium). The pH was adjusted to 5.9 prior to autoclaving at 121ºC for 20 min.

Part B. 1 g L^{-1} ascorbic acid; 1 g L^{-1} malic acid (ICN); 1 g L^{-1} sodium citrate; 1 g L^{-1} succinic acid (ICN); 2 g l^{-1} L-arginine (Merck); 4 g L^{-1} L-aspartic acid; 2 g L^{-1} L-asparagine (USB); 2 g L^{-1} L-glutamic acid; 2 g L^{-1} L-glutamine; 6 g L^{-1} L-proline; 0.1 g L^{-1} of each (L-cysteine; L-glycine; L-isoleucine; L-leucine; L-lysine; L-methionine; L-phenylalanine; L-serine (Merck); L-threonine; L-tryptophan; L-tyrosine, and L-valine). All reagents were obtained from Sigma unless otherwise mentioned. After adjusting the pH to 5.9, the solution was sterilized through a 0.22 µM filter (Nalgene) and added to the sterile medium (Part A). The two components were mixed and poured into disposable Petri dishes (90 mm x 150 mm), or into sterile flasks. Liquid and plated media were stored for a week at 28ºC in the dark prior to be used to ensure sterility.

Culture of *Citrus sinensis* (L.) Osbeck cv. Pêra in liquid CBXPm7

Citrus callus was derived from nucellar tissues from the sweet orange Pêra, a highly susceptible cultivar to *X. fastidiosa* 9a5c. Cultures of Pêra callus were obtained from the 'Centro Apta Citros 'Sylvio Moreira', Cordeirópolis, SP, Brazil.

During the experimental period, citrus callus was maintained on solid Murashige and Skoog (MS) medium (15 g L^{-1} agar), in Petri dishes (90 x 150 mm), supplemented with 0.4 mg L^{-1} 6-BA at 28ºC without illumination.

To evaluate the percentage of citrus cell growth on CBXPm7 and the period of maximum growth, 0.5 g of 20-day-old cells cultured on solid MS medium were grown in 12 mL of CBXPm7 medium in 50-mL conical-tubes and incubated at 28ºC without illumination at 145 rpm. Ten tubes were incubated to evaluate the fresh weight of citrus cells every 24 h during five consecutive days without adding fresh medium or subcultivation. The experiment was done twice with two replicates each.

Culture of *Xylella fastidiosa* in liquid CBXPm7

The *X. fastidiosa* isolate 9a5c originally obtained from the sweet-orange cultivar Valência and used for the *ONSA* – FAPESP Genome Program (21) was maintained on solid modified PW medium (6). This medium, designated as PWB, differed from the original by containing 4 g L^{-1} L-glutamine (instead of 40 g L^{-1}) and 3 g L^{-1} BSA (instead of 6 g L^{-1}).

The growth curve of *X. fastidiosa* in CBXPm7 was obtained by cultivating this bacterium initially in 25 mL of the same medium (starter culture) for 10 days at 28ºC in the dark at 145 rpm. After this period, 10 mL was added aseptically into an Erlenmeyer flask containing 60 mL of fresh CBXPm7, and the culture was incubated as before. Two mL were taken from this culture at zero time and every 24 h during six consecutive days to evaluate the number of colony forming units (cfu), through serial dilutions (10 to 10^{-6}), on solid CBXPm7. Each dilution had two replicates and the experiment was done twice. Colony forming units were counted 24 h after incubation at the same conditions as stated above utilizing a stereoscope at 40 to 50X magnification (Carl Zeiss/West/Germany).

Induction methods used

Different co-cultivation procedures using Pêra callus in association with *X. fastidiosa* cells were analyzed to determine, which was the most effective at inducing differentially expressed bacterial proteins. Five procedures were tested. 1) Pêra callus was cultured on solid MS medium and the cells were covered with Watman no.2 filter paper. This formed a nurse cell-type culture and the bacteria cells were grown on the surface of the filter paper. 2) Use of MilliCell culture plate inserts (Millipore) to grow bacterial and plant cells simultaneously in a liquid medium. Liquid CBXPm7 medium was used for that because it was the only medium suitable to support the growth of both organisms. 3) Bacterial cells grown surrounded by Pêra callus on solid medium. In this case, the Petri dish was aseptically divided using a stainless steel bar into three sections. The middle section of the plate had solid PWB medium where the *X. fastidiosa* cells were grown, and the two adjacent sections contained solid MS medium which supported the growth of Pêra callus. 4) Bacteria and Pêra cells (separated by dialysis tubing) co-cultivated together in liquid CBXPm7medium. CPXPm7 was used for the reasons stated above. 5) Bacterial cells grown on solid PWB medium amended with an extract derived from Pêra callus (Pc extract – see description below).

Induction studies of *X. fastidiosa* grown on solid PWB amended with Pêra callus extracts

Forty-three g of Pêra callus grown in 70 mL of CBXPm7 (60% w/v) for 3 days was macerated in a mortar and pestle. Afterwards, Pêra cells were disrupted mechanically in the BioNeb® Cell Disruption apparatus (Glas-Col, Terre Haute, IN, USA) under the pressure of 8 kgf cm^{-2} with nitrogen gas at a flow-rate of 17.0 liters min^{-1}. The homogenate was centrifuged at 4,000 x g for 5 min at 20ºC and the supernatant was serially filtered through Whatman paper no. 42 followed by 0.65 and 0.44 µm membrane filters and finally through a 0.22 µm sterile filter. An aliquot of 36 mL of the Pêra callus extract designated “Pc extract” was added aseptically in 300 mL of PWB amended with agar (at approximately 42-45ºC). This medium, designated as “Induced”, was poured into disposable Petri dishes and allowed to solidify. Plates containing PWB medium supplemented with 36 mL of CBXPm7 without citrus extract were designated as “Control”. *Xylella* 9a5c cultured on solid PWB medium was used to streak the Induced as well as the Control media. Plates were incubated at 28ºC/dark, and bacterial cells were harvested from five plates for total protein extraction at various times. These induction studies were repeated at least three times. Five plates containing the PWB induced medium (without bacterial cells) were left for a week at 28ºC/dark to monitor for any possible contamination.

Extraction of whole cell proteins from *X. fastidiosa*

Bacteria cells were collected from the surface of the plates and washed three times with 2 mL of Washing Buffer (10 mM Tris-HCl pH 8.8, 3.0 mM KCl and 50.0 mM NaCl) with a centrifugation for 10 min at 12,000 x g at room temperature (RT) for each wash. Afterwards, cells were resuspended in 1 mL of Extraction Buffer [50.0 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 1.0 mM EDTA pH 8.0, 10.0 mM KCl, 10 mM DTT, 1.0 mM AEBSF (Calbiochem), and 1 g L^{-1} SDS] and disrupted in the BioNeb® Cell Disruption System twice under a pressure of 8 kgf cm^{-2} with nitrogen gas at a flow rate of 17.0 liters min^{-1}. Subsequently, the homogenate was centrifuged for 2 min at 8,000 x g at 4ºC.

The protein concentration was determined with a commercial protein assay kit from BioRad. The solubilized proteins were stored at -80ºC or when necessary, lyophilized (LabConco Freeze Dryer 8, Kansas City, Missouri, USA) and stored at -20ºC prior to 2DE analysis. A total amount of 120 µg of protein from both, the induced and control samples, collected at various times, was analyzed through 2DE.

Electrophoresis

Isoelectric focusing (IEF) was conducted with 18 cm IPG Immobiline DryStrips pH3-10 NL (non-linear) and pH4.5-5.5 (Amersham Biosciences). Strips were rehydrated with 400 µL of IEF solution (9 M Urea; 6% CHAPS; 70 mM DTT; 0.8% IPGbuffer; 0.006% bromophenol blue) containing the soluble *X. fastidiosa* total cell proteins (120 µg) for 8-10 h. The IEF was done with the IPGphor system (Amersham Biosciences) at 50 µA/strip until focusing reached 60-70 kVh (30 V/6h; 150 V/1h; 350 V/1h; 500 V/1h; 1000 V/1h; 3000 V/1h, and 5000 V until the desired focusing time was reached). After electrophoresis, the strips were equilibrated with 50 mM Tris-HCl pH 8.4; 6M urea, 300 mL L^{-1} glycerol; 20 g L^{-1} SDS, and 20 g L^{-1} DTT at RT for 12 min. Subsequently, the strips were equilibrated with the same solution except that DTT was replaced with 30 g L^{-1} iodoacetamide and a trace amount of bromophenol blue at RT for 10 min. Strips were then sealed with 1 g L^{-1} agarose into the top 1.5 mm of a 20 x 20 cm vertical system (BioRad) 8-18% gradient polyacrylamide gel. A molecular mass BenchMark™ Protein Ladder (Gibco-BRL) was used as a standard. PAGE was

performed in the presence of the running buffer (25 mM Tris-HCl, 192 mM Glycine and 1 g L^{-1} SDS) at a constant 30 mA/gel at 10ºC for 5 h or until the bromophenol blue dye reached the bottom of the gels. The gels were rinsed three times with Milli-Q water (Millipore System) for 5 min each and fixed overnight in 400 mL L^{-1} methanol and 100 mL L^{-1} acetic acid. Subsequently, they were silver-stained according to Blum *et al*. (2). 2DE analysis was repeated at least twice with each different protein extraction sample from the induction studies.

Imaging Analysis

After staining, 2DE gels were digitally documented with the Fluor-S MultiImager (BioRad) and analyzed with the Melanie program version 3 (Genebio, Genebra, SW). Spot intensities (as spot volume), i.e., the integrated optical density of the spot within its boundaries, were normalized as a percentage of the total spot volume, and used to quantify the protein spots from gels ran and processed in parallel from the same sample. Proteins were considered differentially expressed, up- or down-regulated, when the mean percentage of the spot volume for a particular protein varied by 1.5-fold or greater when compared to the same protein from the control gel (31).

RESULTS AND DISCUSSION

Studies have shown that interactive growth systems where microorganisms and plant callus tissue are grown together can be used to elicit responses from the host or microorganism (3,18,22,26). In an attempt to create an environment for the induction of potential *Xylella fastidiosa* pathogenicity genes, a single culture medium that closely mimics the components of citrus xylem was developed for the growth of citrus callus and bacteria. CBXPm7 had a mixture of ingredients that favored the growth of both *X. fastidiosa* and Pêra. This m edium had a high content of amino acids, such as arginine, aspartic acid, asparagine, glutamic acid, glutamine and proline, which have been found in sweet orange xylem sap analysis (13,16). Moreover, CBXPm7 contained elevated levels of organic acids such as ascorbic acid, malic acid, citric acid (in the form of sodium citrate) and succinic acid, which were also found in xylem sap (32). The individual growth of *X. fastidiosa* and Pêra cells in CBXPm7 was assessed to determine the period of maximum growth rate of both organisms and these data were used for the induction of *X. fastidiosa* proteins by Pc extracts.

Growth rate of *X. fastidiosa* in CBXPm7

The growth of *X. fastidiosa* in CBXPm7 is given by the number of colony forming units for six consecutive days within the conditions assessed (Fig. 1A). The number of colonies per plate was recorded from the 10^{-5} dilution. Colonies were distinct, smooth, opalescent, and circular. At zero time, the bacterial culture had 10^5 cfu mL^{-1}, placing it at the beginning of the exponential phase. This extended up to 72 h followed by the stationary phase with10^8 cfu mL^{-1}, which remained constant up to 120 h and afterwards dropped to 10^7 cfu mL^{-1} during the death phase (after 120 h). The coefficient for the adjustment of the data (R^2) was 0.9702. Results showed that CBXPm7, which is rich in amino acids and organic acids, favored a faster growth of *X. fastidiosa* when compared to the data collected by Wells *et al.* (33), and Lemos *et al.* (14). Wells *et al.* (33) showed that different isolates of *X. fastidiosa* grown in the PW medium reached 10^8 cfu mL^{-1} at 7 to 14 days after inoculation (stationary-phase). Lemos *et al*. (14) testing different culture media observed that the highest growth rate of *X. fastidiosa* (as µg protein mL^{-1}) occurred at 12 days post-inoculation. While the importance of amino acids and organic acids for *Xylella* growth

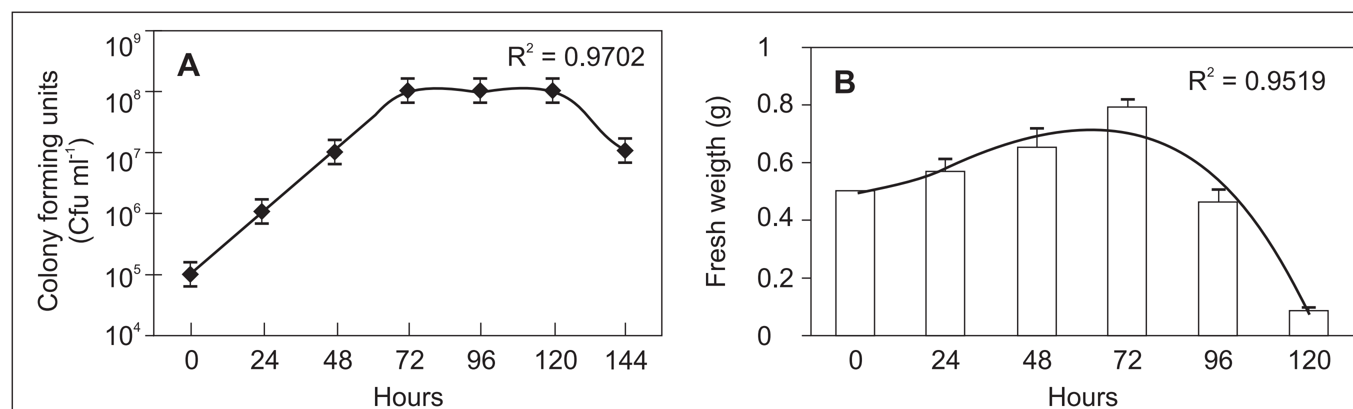

Figure 1. (A) Number of colony forming units of *Xylella fastidiosa* 9a5c grown on solid CBXPm7 for 6 days at 28ºC/dark. (B) Growth of sweet orange cultivar Pêra cell suspension in liquid CBXPm7. Values represent the mean and standard deviation of two replicates from two independent experiments.

has been reported in the literature (5,19), Wells *et al.* (33) reported that the growth of *X. fastidiosa* was stimulated in the presence of amino acids in an isolate depending manner. Furthermore, CBXPm7 contains hemin chloride, which is also known to enhance the growth of *X. fastidiosa* (7). Therefore, based on the accelerated growth, the medium CBXPm7 apparently met the nutritional requirements of the *X. fastidiosa* isolate 9a5c.

Growth rate of Pêra callus in CBXPm7

Pêra cells were grown in liquid CBXPm7 medium for 120 h without adding fresh medium or subcultivation. Fig. 1B illustrates the fresh weight (g) of Pêra cells, which during the first 72 h varied from 0.5 to 0.79 g. Afterwards, the fresh weight of the culture decreased to 0.465 at 96 h and to 0.065 at 120 h post-incubation. The coefficient for the adjustment of the data (R^2) was 0.9519. To ensure that only metabolically active citrus cells were utilized for the extraction of exudates, cells were collected at 72 h post-inoculation to obtain Pc extracts because during the growth, plant cells synthesize and release metabolites, which can modify the general physical and physiological properties of the medium. Growing cell suspension is very complex since the medium ingredients can be converted into growth factors that promote plant cell division and growth (9). The rapid early growth rate of Pêra cells found in this study was expected due to the presence of a high content of nitrogenous compounds (amino acids, phytone peptone, trypticase peptone and organic acids), which are known to promote and increase cell growth (9,20,27). Furthermore, the relatively short culture period assessed (120 h) showed the occurrence of Pêra cell death, which was probably followed by cell lysis as indicated at 96 and 120 h. This observation may be the consequence of the accumulation of toxic extracellular products such as proteases, cell fragments or secondary metabolites, and nutrient limitations in the culture medium since subcultivation or addition of fresh medium was not conducted. These findings were also observed by Eriksson (9), who evaluated the growth rate of *Haplopappus gracilis* in 25-mL-cell suspension and by Steward *et al.* (24), who assessed the cell growth rate of *Medicago sativa* cultivated in a bioreactor. The overall pattern of the growth rate of Pêra cell grown in liquid CBXPm7 corroborates with Steward *et al.* (24), who found the highest growth rate of *M. sativa* in a bioreactor occurred on the third day, which was followed by the occurrence of cell death and lysis.

Effect of Pc extract on the expression of *Xylella fastidiosa* proteins

Preliminary results showed that procedures 1 through 4 (see Materials and Methods, *Induction methods used*) either did not produce enough protein to conduct the 2DE analysis; caused poor growth of bacterial cells; yielded total bacterial proteins that were contaminated with plant proteins, or produced bacterial proteins lacking any response to the presence of Pc extract. Only strategy 5, in which *Xylella* 9a5c was cultivated in solid PWB supplemented with Pc extract produced from Pêra callus cultivated in liquid CBXPm7, resulted in the most effective and reproducible yields of induced bacterial proteins.

Based upon our studies, we found that a 60% (w/v) extract (Pc extract) from Pêra cells added to solid PWB was able to stimulate the induction of differentially expressed proteins of *X. fastidiosa*. PWB medium amended with the Pc extract produced faster growth and a higher yield of proteins than the CBXPm7 solid medium amended with Pc extract (data not shown). Conversely, extract derived from citrus cells grown in MS and amended to either PWB or CBXPm7 media inhibited the growth of *X. fastidiosa* (data not shown), which could be due to the high salt content of MS, and thus, confirmed the need of a growth medium that supported the growth of both organisms. The effect of Pc extracts over time on the expression of *X. fastidiosa* proteins grown on solid PWB and analyzed via 2DE (8-18% PAGE) (120 mg of total proteins resolved by IEF pH 3-10NL) is illustrated in Fig. 2A, which shows the highest number of total proteins was on the sixth day in culture. Fig. 2B illustrates representative gels with protein spots between pH 3.5 to 9.5 and molecular weight from 8 to 100 kDa (1-, 6-, and 10-day-old cultures). Fig. 3 shows gels with the same protein samples resolved at an IEF pH 4.5-5.5 to illustrate some of the novel, up- and down-regulated proteins. Over the total analyzed period (1 to 14 days), 414 differentially expressed proteins were found, of which 180 were new, 154 were up-, and 80 were down-regulated. Within the tested conditions, only 227 protein spots were well defined and could be easily cut from the gel for future identification. Furthermore, the 2DE analysis of the Pc extract revealed no protein spots in common with those from the bacteria, confirming that no citrus proteins were carried-over during the extraction procedure (data not shown).

Over the development of this study, the extraction and 2DE protocols were optimized to take advantage of changes that have been shown to improve the quality and quantity of the *X. fastidiosa* proteins. The protocols listed in the Materials and Methods represent the most current improvements. Moreover, the 2DE protein gradient separation system used to analyze proteins obtained from bacterial cultures over time revealed that the Pc extract affected the expression of 414 *X. fastidiosa* proteins. Plant cells in suspension are known to be a source of biologically active factors (23). Cells in culture have advantages over whole plants due to their consistent nature, which results in a uniform well-synchronized response with the production of, for example, secondary metabolites. These metabolites may play a role in the interaction of plants with biotic and abiotic environmental factors (23). Other studies that supplemented bacterial culture media with plant leaf extract or plant flavonoids have shown that compounds derived from plant can stimulate differential expression of bacterial proteins (11,15).

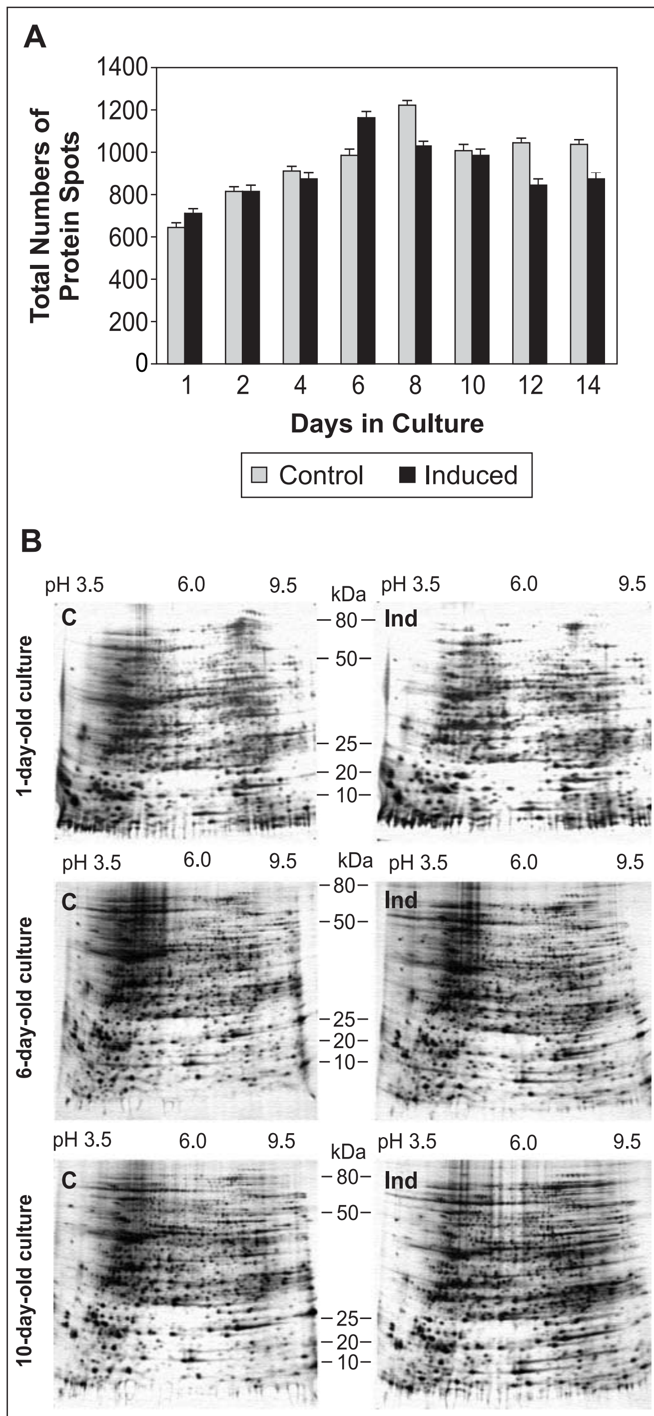

Figure 2. (A) The total number of protein spots obtained, over time, from *X. fastidiosa* 9a5c grown on solid PWB amended with Pc extract (**Ind**uced) and on solid PWB without Pc extract (**C**ontrol). Total bacterial protein (120 µg) was analyzed via 2DE (IEF pH3-10NL and 8-18% PAGE). Values are the mean of two replicates from two independent experiments. (B) Representative gels with bacterial protein between pH 3.5 to 9.5 and 8 to100 kDa.

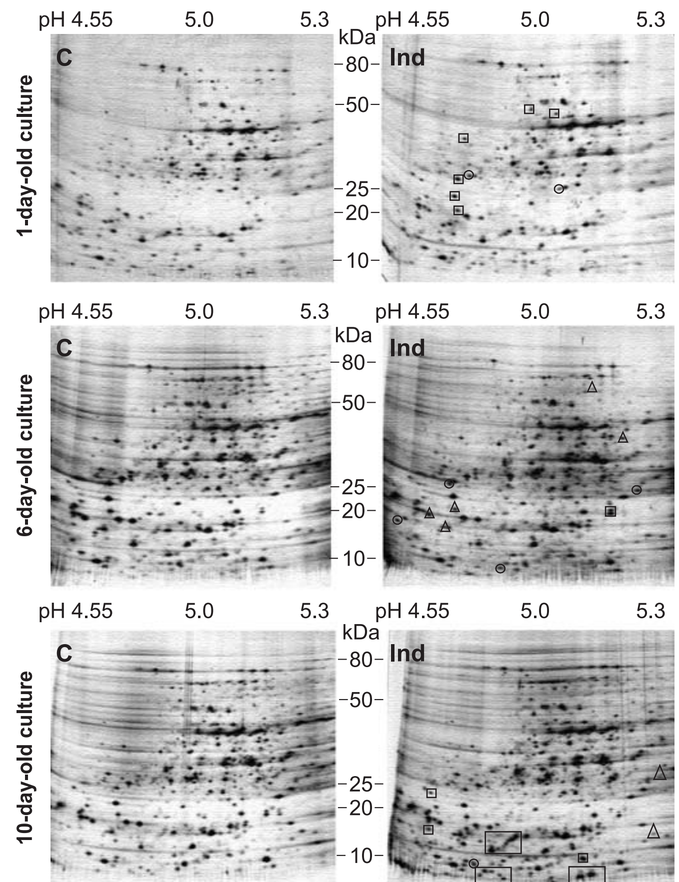

Figure 3. 2DE (IEF pH4.5-5.5 and 8-18% PAGE) analysis of *X. fastidiosa* 9a5c proteins (120 µg). (**Ind**uced) is in the presence and (**C**ontrol) is in the absence of Pc extract. Circles are novel; squares are up-, and triangles are down-regulated proteins when compared to the control gels.

The protocols used in this study for the protein extraction and 2DE analyses as well as the culture medium developed for the induction of *X. fastidiosa* proteins showed promising results and the 227 protein spots will be processed for Maldi-TOF analysis. This *in vitro* study could shed additional light on the disease process of citrus variegated chlorosis (CVC) by analyzing the expression, function, and mechanism of induction/ repression as well as the synthesis of *de novo* proteins of *Xylella* genes when in the presence of metabolites derived from the host cells.

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RESUMO

Indução de proteínas de *Xylella fastidiosa* expressas diferencialmente com extrato de citros

Estudos *in vitro* foram desenvolvidos para obter proteínas de *Xylella fastidiosa* expressas diferencialmente na presença de calos do hospedeiro, citros cultivar Pêra. Para otimizar a indução, desenvolveu-se um meio de cultura comum, o qual foi baseado na seiva do xilema de citros, para cultivar a bacteria e os calos de Pêra. Dados mostraram, após 72 h de cultivo neste meio, 10^8 unidades formadoras de colônias de *X. fastidiosa* por mL, e 0,79 g de peso seco de células de Pêra. Após testar diferentes métodos de co-cultivo da bactéria com calos de Pêra neste meio, observou-se que a melhor taxa de indução ocorreu quando *X. fastidiosa* foi cultivada em meio sólido enriquecido com um extrato derivado dos calos de Pêra. Análise em gel bidimensional (2DE) de *X. fastidiosa* (120 µg) cultivadas na presença do extrato revelou 414 proteínas expressas diferencialmente quando comparado com o perfil proteico obtido na ausência do extrato.

Palavras-chave: calos, interação *Xylella*-hospedeiro, 2DE, patogenicidade, proteína

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