BRAZILIAN JOURNAL OF MICROBIOLOGY

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Genetics and Molecular Microbiology

A protein expression system for tandem affinity purification in *Xanthomonas citri* subsp. citri

Giordanni C. Dantas[a,1], *Paula M.M. Martins*[a,1], *Daniela A.B. Martins*[b], *Eleni Gomes*[c], *Henrique Ferreira*[a,*]

[a] *Depto. de Bioquimica e Microbiologia, Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, SP, Brazil*
[b] *Depto. de Bioquímica e Tecnologia Química, Instituto de Química, Universidade Estadual Paulista, Araraquara, SP, Brazil*
[c] *Depto. de Biologia, Universidade Estadual Paulista, São Jose do Rio Preto, SP, Brazil*

ARTICLE INFO

Article history:
Received 7 May 2015
Accepted 23 October 2015
Available online 2 March 2016
Associate Editor: Solange Ines Mussatto

Keywords:
Citrus canker
Expression vectors
TAP-tag

ABSTRACT

Citrus canker, caused by the Gram-negative bacterium *Xanthomonas citri* subsp. citri (Xac), is one of the most devastating diseases to affect citrus crops. There is no treatment for citrus canker; effective control against the spread of Xac is usually achieved by the elimination of affected plants along with that of asymptomatic neighbors. An in depth understanding of the pathogen is the keystone for understanding of the disease; to this effect we are committed to the development of strategies to ease the study of Xac. Genome sequencing and annotation of Xac revealed that ~37% of the genome is composed of hypothetical ORFs. To start a systematic characterization of novel factors encoded by Xac, we constructed integrative-vectors for protein expression specific to this bacterium. The vectors allow for the production of TAP-tagged proteins in Xac under the regulation of the xylose promoter. In this study, we show that a TAP-expression vector, integrated into the *amy* locus of Xac, does not compromise its virulence. Furthermore, our results also demonstrate that the polypeptide TAP can be overproduced in Xac and purified from the soluble phase of cell extracts. Our results substantiate the use of our vectors for protein expression in Xac thus contributing a novel tool for the characterization of proteins and protein complexes generated by this bacterium *in vivo*.

* *Corresponding author.*
E-mail: henrique.ferreira@linacre.oxon.org (H. Ferreira).
[1] These authors contributed equally to this work.
http://dx.doi.org/10.1016/j.bjm.2016.01.026

Introduction

Citrus canker, one of the most important diseases affecting citrus crops worldwide, is caused by a Gram-negative bacterium, *Xanthomonas citri* subsp. citri (Xac). The complete genome sequence of Xac, which was reported more than a decade ago,[1] has significantly contributed to improving our understanding of the molecular processes of this plant pathogen.[2–5] Of the 4313 Xac ORFs that have been annotated, nearly 63% exhibit homology to genes of known function whereas ~37% correspond to hypothetical ORFs that could potentially code for novel polypeptides.[1] Such a distribution pattern of ORFs is a common feature in microbial genomes and it is hypothesized that a significant amount of information regarding adaptation and pathogenicity may be encoded within such hypothetical proteins. The biggest challenge in characterizing these hypothetical ORFs is that the phenotypes generated by them are usually unknown.

Among techniques that have the potential to be used for the characterization of hypothetical proteins we cite: two dimensional (2-D) protein gels allied to mass spectrometry, DNA microarrays, and next generation sequencing of nucleic acids (NGS).[6–15] All of these methods allow for high-throughput processing of candidate genes/proteins and the data obtained by analyzing patterns of co-expression and co-repression (exhibited by a particular gene/polypeptide during different growth or development conditions) in turn helps in the attribution of function. In addition to the aforementioned techniques, *in vivo* protein localization *via* fluorescence microscopy has also proven to be a powerful tool for annotating function to unknown protein factors.[16,17] However, the most promising techniques in this field are those that are capable of demonstrating a direct contact between hypothetical proteins and (an) other cellular factor(s). One such method is the yeast-two-hybrid (YTH) technique and its variant the bacterial-two-hybrid (BTH) system.[18,19] One of the first major successes of the YTH system was the construction of a complex protein–protein interaction map of the human pathogen *Helicobacter pylori*.[20] In Xac this method has been applied for the identification and characterization of several previously unknown protein components of the type III and IV secretion systems.[2,3] In spite of the obvious value and utility of the YTH technique, the methodology suffers from certain serious disadvantages such as the need to construct a genomic/cDNA expression library of the organism under study. Another technique, an interesting alternative to the YTH/BTH methods, is the Tandem Affinity Purification strategy (TAP-tagging).[21,22] TAP-tagging was developed to aid in the purification of active macromolecular complexes from yeast; it differs from the two-hybrid systems in the aspect that it does not require the construction of protein expression libraries to be screened with baits. The basis of TAP-tagging is the expression of a fusion-protein comprising of the protein of interest fused to a TAP-tag either at the C- or the N-terminus. Expression of this fusion protein inside a cellular environment wherein the expression of the protein under study is indigenous causes the TAP-tagged protein to interact with other cellular factors in the same fashion as an untagged protein would. Following cell propagation, protein complexes comprising of TAP-protein fusion and their associated cellular factors are to be purified and identified by mass spectrometry. The Tap-tagging technique has been successfully adapted to a variety of organisms including the rice pathogen *Xanthomonas oryzae* pv. oryzae.[23–35]

Herein, we describe a protein expression system for tandem affinity purification in Xac (TAP expression vectors). The vectors involved in this system have the capability to stably integrate into a specific genomic region (the *amy* locus) of Xac without altering pathogenicity or virulence. Finally, we expressed and purified the TAP-tag from Xac, corroborating the use of our expression vectors for protein studies in this bacterium.

Materials and methods

Bacterial strains and growth conditions

The *X. citri* subsp. citri used in the study[36,37] was the sequenced strain 306.[1] *Escherichia coli* strain DH10B (*F*$^-$ *mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80lacZΔM15 ΔlacX74 recA1 endA1 araΔ139 Δ(ara, leu)7697 galU galK λ-rpsL* (Str^R) *nupG*) was used for cloning. DH10B was cultivated at 37 °C in LB[38] and Xac at 30 °C in NYG (Peptone 5 g/L, yeast extract 3 g/L and glycerol 20 g/L). For the amylase tests, soluble starch was added to NYG-agar at a final concentration of 0.2%. During the cloning procedure, ampicillin (20 μg/mL) and kanamycin (10 μg/mL) were added to the media to allow for selection of plasmid-carrying colonies.

General methods

Basic molecular biology experiments were conducted as per prescribed protocols.[38] Electrotransformation of Xac was performed as described previously.[39] DNA polymerases and restriction and modifying enzymes were obtained from Fermentas (Thermo Scientific). The construction of pHF5Ca (GenBank FJ562210) has been previously described in the literature[40]; pHF5Na (GenBank FJ573043) is a variant of pHF5Ca in which the *tap1479* has been replaced by the *tap1761*[21] (Fig. 1A). The *tap1761* cassette was isolated by PCR using pBS1761 as template[21] and the primer pair PBS1761F 5′-TGA GGA TCC ATG ATA ACT TCG TAT AGC ATA C and PBS1761R 5′-TCA TTC TAG ACT ATA GGG CGA ATT GGG TAC C. Following PCR, the purified fragment was digested with *Bam*HI/*Xba*I and ligated to the backbone of pre-digested pHF5Ca. For detecting the presence of pHF5Ca integrated into the Xac genome diagnostic PCR was performed. The primer pair used for this purpose was: pxyl 5′-GTA CTT ACT ATA TGA AAT AAA ATG and 1479R 5′-ATC AAG CTT CAG GTT GAC TTC CCC G.

Protein expression

Strains of *E. coli*/pHF5Ca and Xac *amy*::pHF5Ca were activated by the inoculation of 2–3 isolated colonies in 3 mL of LB or NYG, respectively, along with the required antibiotics. The inoculums were cultured for 8 h at 30 °C and 180 rpm. At the end of the designated growth period, the cultures were diluted 1:50 (*E. coli*) or 1:10 (Xac) in Erlenmeyer flasks containing 50 mL LB or NYG broth plus antibiotics. Cultures were grown for 14 h at

30 °C and 180 rpm. At the end of the time period, strains were again diluted 1:50 (*E. coli*) or 1:10 (Xac) in Erlenmeyer flasks containing 50 mL LB or NYG broth (for Western blotting) or 500 mL LB or NYG (for protein purification). Cultures were incubated at 30 °C and 180 rpm till an OD_{600} value of 0.5 was reached. For induction of protein expression, xylose was added to the growth medium to a final concentration of 0.5%; time period for induction was 1 h (*E. coli*) and 4 h (Xac) using conditions identical to those employed for culture growth. Subsequent to induction, cells were harvested by centrifugation at 4000 × *g* for 10 min followed by washing with cell wash buffer (30 mM Tris–HCl, 200 mM NaCl); cell pellets were stored at −80 °C till use.

Western blotting

Total protein extracts were prepared by resuspending 20 mg of each cell pellet in 100 μL of SDS-sample buffer. Sample processing, SDS-PAGE, and transfer to PVDF membrane was conducted using standard methodology.[38] For detection of the immobilized TAP-tag, the PVDF membrane was washed with PBS-Tween-20 (0.2%) containing 5% skimmed milk and incubated with a secondary antibody coupled to HRP (rabbit Anti-Horse IgG; SIGMA A6917) in a dilution of 1:3000. The principle underlying this methodology is that Protein A, which is part of the TAP-tag, is known to bind IgG isotypes directly. Following incubation with the antibody, the

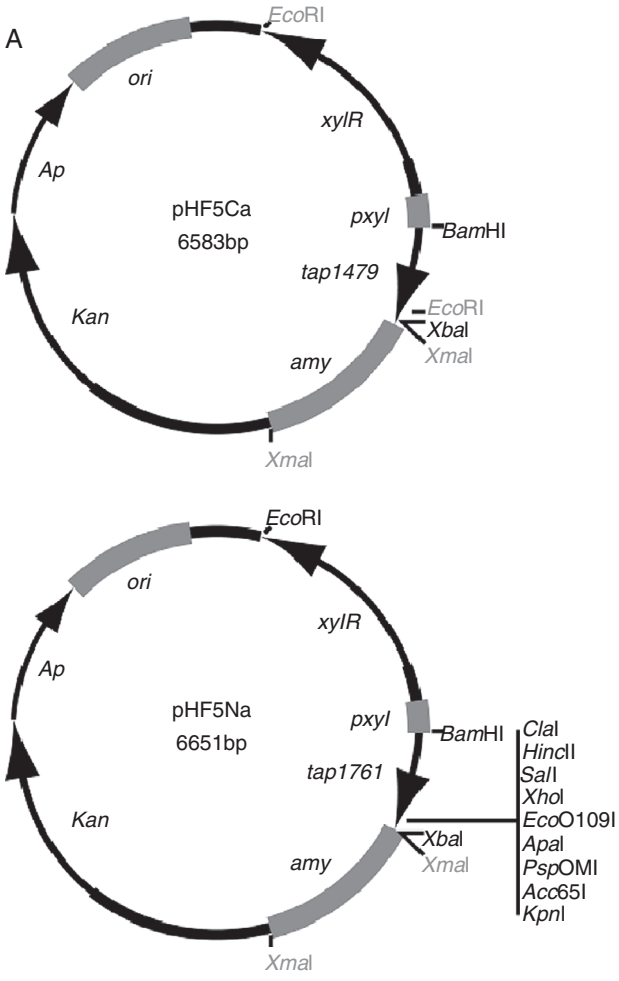

Fig. 1 – Expression vectors for Tandem Affinity purification in Xac. (A) Schematic representation of pHF5Ca and pHF5Na: the protein expression vectors for TAP-tagging in Xac. Each vector carries a different TAP-coding DNA, *tap1479* and *tap1761* respectively, which produce TAP fusions to either the C- or N-terminal ends of proteins under study. Both vectors are based on the pCR2.1-TOPO backbone which carries a pUC replication origin and DNA cassettes for ampicillin (Ap) and kanamycin (kan) resistance. (B) DNA sequence of the xylose promoter region common to pHF5Ca and pHF5Na showing the location of the ribosome binding-site (RBS) and the start codon (ATG). (C) The DNA sequences of the 3′-ends of *tap1479* and *tap1761*. Underneath each sequence is the schematic view of the protein fusion that can be produced using the tag. Note that the organization of the modules that compose the TAP-tags (CBP, TEV, and ProtA) differ with respect to TAP1479 and TAP1761 (see text); for the C-terminal fusions, the TAP1479 has ProtA located at the extreme C-terminus of the polypeptide, while ProtA is at the beginning of the protein in the N-terminal fusions. Unique restriction sites for DNA cloning are shown in black; non-unique sites in gray. CBP, calmodulin binding-protein; TEV, Tobacco Etch Virus Protease recognition sequence; ProtA, Protein A from *Staphylococcus aureus*; and EK, enterokinase recognitions sequence (DDDDK, present only in TAP1761); *pxyl*, xylose promoter; *xylR*, the xylose repressor gene; *amy*, an 800 bp fragment of the alpha-amylase gene from Xac.

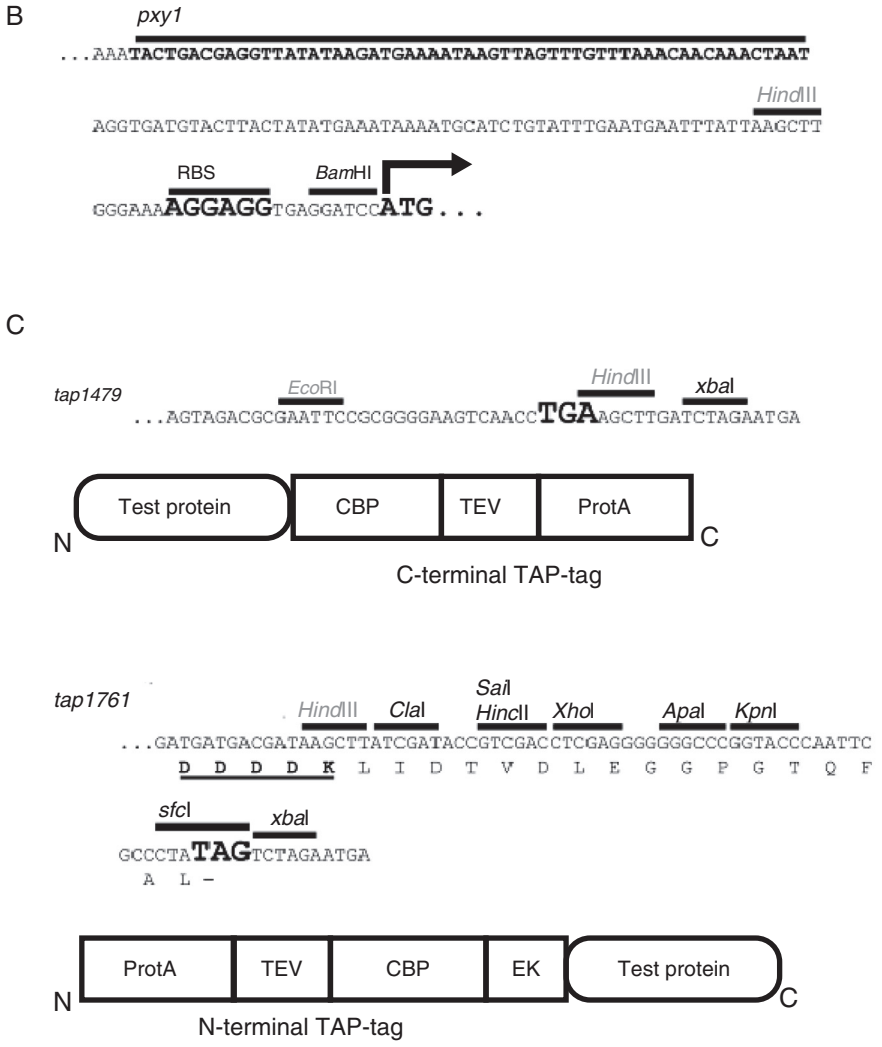

Fig. 1 – (*Continuación*)

western blot was developed by immersing the membrane in ECL detection reagents (Amersham ECL Western Blotting System kit-GE). The chemiluminescence produced was captured on X-ray film (Hyperfilm, GE) for further analysis.

Protein purification

Cell pellets obtained from 500 mL cultures were dissolved in pre-chilled 10 mL TST (50 mM Tris–HCl pH 8.0, 150 mM NaCl, and 0.05% Tween 20) containing a one-fourth of a tablet of EDTA-free protease inhibitors (Roche 1.873.580). Cells were lysed by sonication using a Vibra-Cell VCX 130 (Sonics) (10 pulses of 10 s each with intervals of 1 min) and the sonicated samples were then centrifuged at 48,000 × *g* for 30 min at 4 °C. Batch binding of proteins to the affinity resin was done by incubating the clarified suspensions with 500 μL of IgG-Sepharose (previously equilibrated in TST; GE 17-0969-01) on an ice bath with gentle agitation through a rotary shaker (120 rpm). After a 2 h incubation, resin was transferred to a 10 mL disposable column (Poly-prep BioRad 731-1550) and washed 3–4 times with 20 column-volumes of TST using gravity flow. Samples of 100 μL were collected throughout the process for SDS-PAGE analysis. The TAP-tag was released from the resin by acid elution using 0.5 M acetic acid (pH 3.4; adjusted with 10 M ammonium acetate). Eluate fractions of 1 mL each were precipitated using TCA (3% final) and centrifuged for 10 min using a microcentrifuge at maximum speed; protein pellets were washed once with absolute ethanol and then dissolved in 100 μL of SDS sample buffer for SDS-PAGE.

Pathogenicity tests

The plant host used for this study was the sweet orange cultivar 'Bahia' (*Citrus sinensis* L. Osbeck). Orange trees were cultivated under green-house conditions. Cells to be tested were cultivated in the appropriate medium until OD_{600} reached ~0.6 (~10^8 CFU/mL). Subsequent to growth, cell suspensions or dilutions were used to inoculate leaves on the abaxial surface with the help of hypodermic syringes (1 mL) fitted with needles. Symptoms were observed in the course of three weeks from the day of inoculation.

Results

The TAP expression vectors for Xac

In this study, the two TAP expression vectors used for Xac, pHF5Ca and pHF5Na, carry the xylose promoter (*pxyl*), the xylose repressor (*xylR*), and the TAP encoding sequences *tap1479* or *tap1761* (Fig. 1A). These components are separated by a short stretch of DNA containing a Ribosome Binding Site (RBS) whose sequence is a consensus for both *Bacillus subtilis* as well as *E. coli*[41] (Fig. 1B). The xylose promoter used has been previously evaluated by our group in Xac and found to function satisfactorily.[17,40] The two TAP sequences, *tap1479* and *tap1761*, encode for tags used in Tandem Affinity Purification of protein complexes and are composed of three components (Fig. 1C): (a) Two domains of Protein A (ProtA; *Staphylococcus aureus*) that is known to have a strong affinity for IgG; (b) A peptide that binds calmodulin (CBP); (c) A short peptide stretch containing the recognition sequence for the protease TEV (Tobacco Etch Virus) separating the above mentioned components.[21,22] The expression vectors pHF5Ca and pHF5Na differ only in the TAP-tags that they encode; pHF5Ca carries the *tap1479* and is used for the expression of protein fusions containing the peptide TAP1479 at the C-termini whereas the pHF5Na carries the *tap1761* and is used to produce fusions of TAP1761 to the N-termini of proteins of interest. Additionally, both the vectors used are integrative as they carry the *amy106-914* fragment of Xac which drives their integration into the *amy* locus of the bacterium *via* a single crossover event. Upon integration, the *amy* gene of Xac is disrupted and the bacterium becomes incapable of degrading starch.

Xac mutants harboring the TAP expression vectors colonize citrus

In a previous study, we demonstrated that Xac mutant strains carrying the GFP expression vectors pPM2a or pPM7g integrated into the *amy* locus were capable of inducing disease symptoms in citrus plants.[17] Herein, in continuation of the previous study, we wanted to evaluate if the presence of the TAP coding DNA altered or affected the virulence/pathogenicity associated with Xac. To test this hypothesis, pHF5Ca was electroporated into Xac; and kan^R transformants (candidates potentially carrying the vector pHF5Ca) were selected for further characterization. To certify that the TAP expression vector had integrated into *amy* locus, a set of putative mutants were tested for their ability to degrade starch on a test plate.[17] The results clearly demonstrated that the candidate mutants were deficient in starch degradation hence proving that pHF5Ca was stably integrated into the *amy* locus of Xac such that these mutants were unable to produce alpha-amylase (data not shown). Alongside the starch degradation test, we also carried out diagnostic PCRs on a selection of six kan^R mutants so as to certify the presence of pHF5Ca (Fig. 2). The diagnostic PCRs detected a DNA fragment of ~640 bp corresponding to the *tap1479* in all the mutants analyzed (compare lanes 2–7 with the positive control in lane 8 wherein pHF5Ca was used as DNA template). No bands were detected in the negative control (lane 1, wild type Xac).

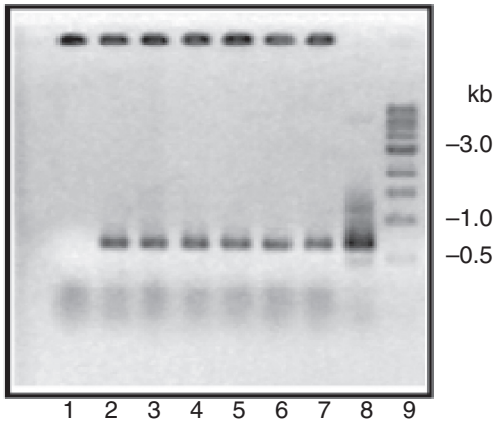

Fig. 2 – Colony PCR to detect the presence of pHF5Ca integrated into the chromosomal DNA of Xac. Subsequent to electrotransformation of Xac with the expression vector pHF5Ca, six kan^R mutants were selected and subjected to colony PCR using the primers PBS1479F/R specific for the amplification of the *tap1479* (approximately 640 bp). The PCR amplicons were visualized after migration through a 0.7% agarose gel. Lane: 1, WT Xac (negative control); 2–7, the six selected mutants; 8, PCR using pHF5Ca as the DNA template (positive control); and 9, DNA molecular weight marker (1 kb DNA ladder, NEB).

In order to verify if Xac *amy*::pHF5Ca was capable of causing disease symptoms in a susceptible host, two independently selected mutants were inoculated into leaves of sweet orange (Fig. 3A–C). Seven days post-inoculation, the borders of the area inoculated with Xac clearly exhibited the typical water-soaking symptom associated with the disease (Fig. 3D–F). These areas developed brownish canker-like lesions during the course of the next three weeks (data not shown). A point to note is that the mutant strains displayed the same distinct lesion pattern as the wild type. In contrast, the area inoculated with NYG-medium (negative control) showed only a mild chlorosis. In summary, the results obtained in this study clearly demonstrated that the ability to cause disease remained unaffected following integration of the Xac *amy*::pHF5Ca mutants.

TAP-tag expression

The production of a TAP-tag can be detected in Western blotting assays by exploiting the ability of ProtA to bind to the IgG antibody.[42] A strain of *E. coli* carrying pHF5Ca (multicopy) vector and two Xac *amy*::pHF5Ca mutant strains (harboring a single copy of the expression cassette integrated into the *amy* locus of Xac) were cultivated and induced with xylose. Total protein extracts were separated by SDS-PAGE (Fig. 4A) and transferred onto a PVDF membrane so as to facilitate detection of TAP1479 using antibody reactions. The bands of the size expected for the TAP1479 (~21 kDa) were detected for both the mutant strains of Xac as well as for *E. coli* transformed with pHF5Ca (Fig. 4B, lanes 1–2, and 5–8). The results also showed

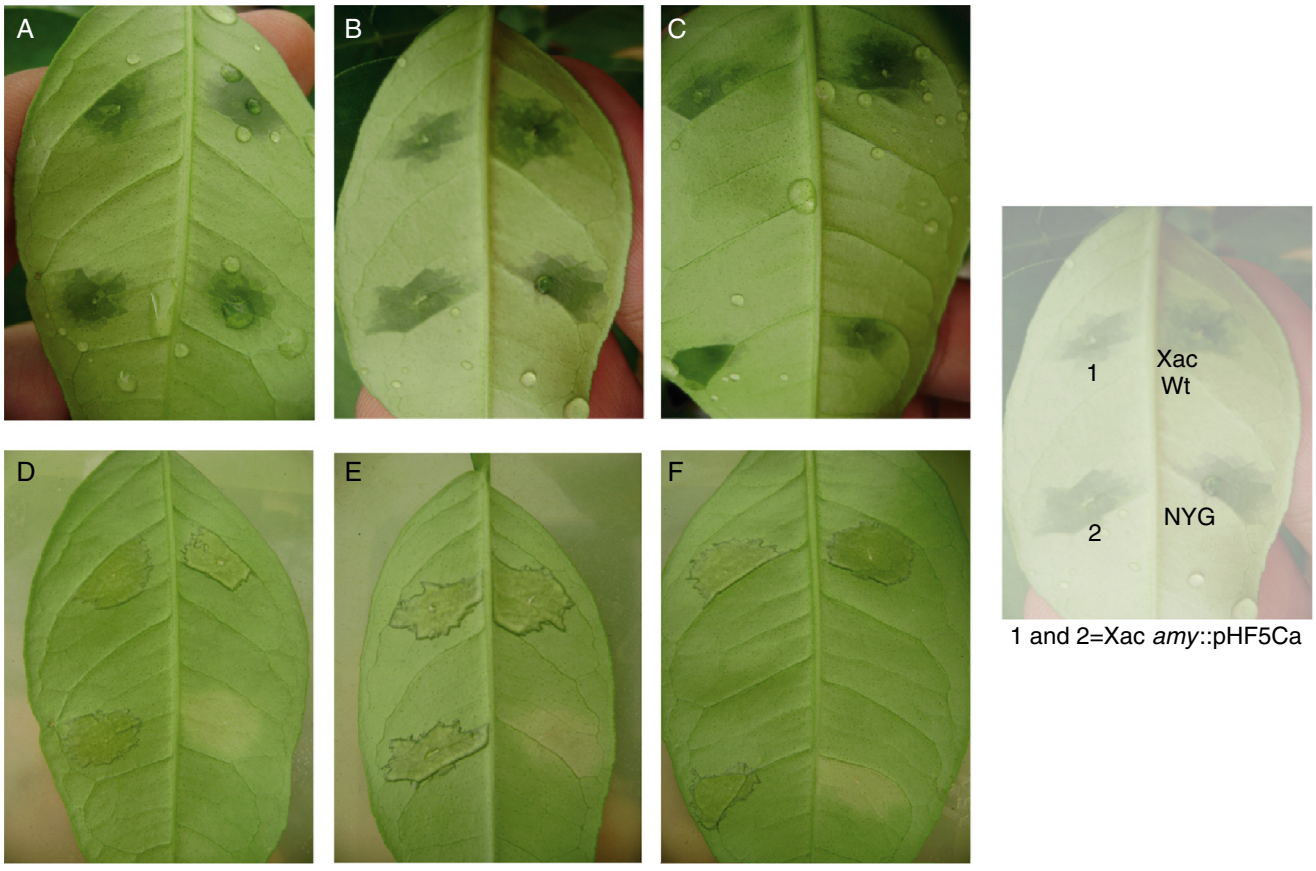

Fig. 3 – The integration of the TAP expression system into the chromosome of Xac does not alter its pathogenicity. Two independently selected mutants of Xac *amy*::pHF5Ca (1 and 2) were inoculated into leaves of sweet orange Bahia alongside the wild type strain (Xac wt) and a sample of NYG-medium (negative control). A map of the inoculation is shown on the right-hand side of the figure. Pictures were taken immediately after inoculation (A–C) and also 7 days post-inoculation (D–F). The test was conducted in triplicate (A–C).

that the production of TAP1479 was responsive to induction by xylose (lanes 1 and 2, 5 and 6, 7 and 8). For the negative control, wild type Xac, no signal corresponding to the band size of TAP1479 was detected (lanes 3 and 4). It was observed that *E. coli* produced more of the tag than the Xac mutants, a result that is consistent with the multicopy condition of pHF5Ca in *E. coli*. The results obtained clearly show that the expression system is functional in both bacteria and also that an intact and active ProtA moiety was produced in Xac.

Purification of the TAP-tag from Xac

The TAP-tag detected in the previous section appeared to be a resilient polypeptide capable of retaining the property of IgG-binding even after treatment with SDS and boiling as is mandatory for sample preparation in SDS-PAGE electrophoresis. However, results obtained by western blotting do not guarantee function, as the positive outcome can easily be explained as a byproduct of residual IgG binding activity of ProtA. Additionally, for the protein complexes produced in Xac to be useful, it is essential to check if the TAP1479 could be produced in larger amounts and in a soluble form. In order to evaluate this, an attempt was made to purify TAP1479 by affinity separation. An *E. coli* strain carrying pHF5Ca and a Xac *amy*::pHF5Ca mutant were cultivated and induced with xylose. Following expression of the TAP-tag, cells were lysed by sonication and clarified cell extracts were subjected to affinity chromatography using IgG-immobilized resin. TAP1479 was successfully purified from the soluble phase of both extracts (Fig. 5; see arrows). As observed in the Western blotting experiments, *E. coli* produced a relatively higher amount of the tag (a tag concentration of ~1.4 mg/mL was estimated for the sample in lane 8) as compared to the yield from Xac (~0.2 mg/mL, lane 7). However, the amount of tag produced by Xac, though less, should suffice for mass spectrometry identification. The contaminant bands above the TAP1479 represent denatured human IgG which was stripped out from the matrix by the acid elution together with other bacterial proteins that might be interacting with the resin. These results corroborate the use of the expression vectors pHF5Ca and pHF5Na as integration/protein expression systems for TAP-tagging in Xac.

Discussion

In the present work we characterized a novel protein expression system intended for high-throughput analysis of protein complexes in Xac. These vectors are integrative and allow

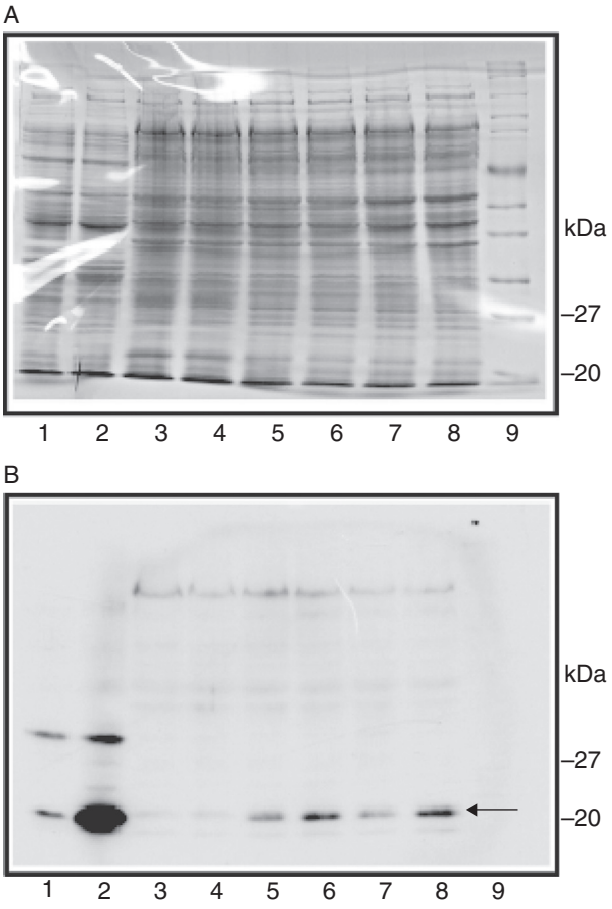

Fig. 4 – Western blotting analysis to detect expression of TAP1479 by Xac *amy*::pHF5Ca mutants. The functionality of the TAP expression system was verified by the ability of an *E. coli* DH10B strain, with pHF5Ca and Xac kan^R mutants, carrying the vector integrated into the chromosome, to produce the TAP1479 (approximately 21 kDa) (details of the induction procedure have been described in "Materials and methods" section). (A) Coomassie blue-stained 10% SDS-PAGE showing the separation of proteins from cell extracts of *E. coli* and Xac; lanes: 1, *E. coli*/pHF5Ca; 2, *E. coli*/pHF5Ca + 0.5% xylose; 3, Xac (WT); 4, Xac WT + 0.5% xylose; 5, Xac *amy*::pHF5Ca mutant 1; 6, Xac *amy*::pHF5Ca mutant 1 + 0.5% xylose; 7, Xac *amy*::pHF5Ca mutant 2, 8; Xac *amy*::pHF5Ca mutant 2 + 0.5% xylose; and 9, protein molecular weight marker. (B) X-ray film displaying bands detected by the Western blotting analysis. Proteins in a replica of the gel shown in A were transferred to a PVDF membrane and exposed to a secondary antibody coupled to HRP. Lanes are the same as in (A). The position of the TAP1479 is marked with a black arrow on the right hand side of the film.

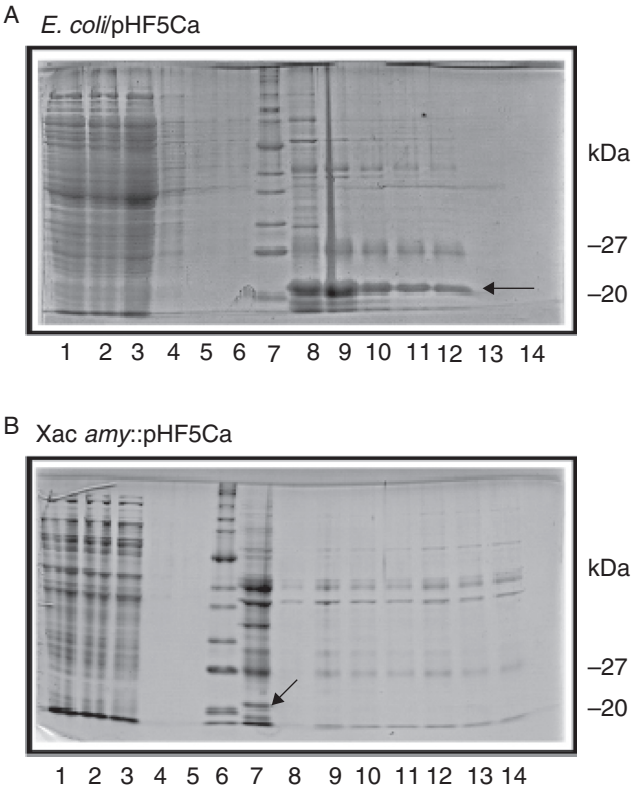

Fig. 5 – Purification of TAP1479 from *E. coli*/pHF5Ca and Xac *amy*::pHF5Ca. *E. coli* DH10B carrying pHF5Ca and a kan^R mutant of Xac harboring pHF5Ca integrated into its chromosome were induced for the production of the TAP1479. Following expression, cells were lysed and protein extracts subjected to affinity chromatography through IgG-sepharose. (A) *E. coli*/pHF5Ca; and (B) Xac *amy*::pHF5Ca, Coomassie blue-stained 10% SDS-PAGE showing the separation of proteins from the fractions collect throughout the process. (A) lane: 1, clarified lysate; 2, flow through; 3–6, washes (20 column-volumes each); 7, protein molecular weight marker; 8–14, elution fractions. (B) Lane: 1, clarified lysate; 2, flow through; 3–5, washes; 6, protein molecular weight marker; 7–14, elution fractions. The position of the TAP1479 is marked with black arrows in both gels.

for the production of fusion proteins tagged with the TAP-tag either at the C- or at the N-terminal ends.[21,22] Integration of the expression vectors into the chromosome of the bacterium may occur site-specifically into the *amy* locus (as shown here) or into the ORF as demonstrated previously in a preliminary characterization of pHF5Ca.[40] Integration mediated by a crossover between ORFs (ligated in the vector and its chromosomal copy) puts the expression of the TAP-tagged peptide under the control of the native promoter of the chromosomal ORF. Hence, use of pHF5Ca is recommended so as to have a C-terminal TAP-tagged protein. The integration into the *amy* locus is the preferable site of integration as it eliminates the possibility of perturbations of chromosomal regions containing the ORFs under investigation. Genome integration also guarantees that the expression cassette will be propagated as a single copy per cell, allowing for a better control of protein expression under the xylose promoter.[43,44] Finally, in this study, we have shown that the introduction of a TAP coding DNA into Xac had no detectable effect on virulence, which emphasizes and underlines the utility of the TAP-expression vectors for protein interaction studies in this plant pathogen.

Result obtained from this study proved that the TAP-tag could be stably expressed and purified from the soluble phase of Xac cell extracts. Currently, we are in the process of testing a number of TAP-protein fusions (hypothetical, previously characterized and control factors) in our model organism in an attempt to conduct functional assignments using the TAP-tagging strategy. Apart from its use in TAP-tagging experiments, the expression vectors pHF5Ca and pHF5Na can also be applied for protein expression and purification from *Xanthomonas* spp. thus eliminating the need for heterologous expression in *E. coli*. An example of this application is the strategy for expression and purification of TAP1479 outlined above. Another feasible application would be in genetic complementation tests *in vivo*. In such experiments, the TAP coding DNA may be removed during the cloning steps such that the polypeptide produced in Xac will not carry any tag.

Although extensively explored in eukaryotes, the utility of tandem affinity purification for protein–protein interaction analysis in prokaryotes is limited.[24,26,45–49] TAP-tagging was originally used in *E. coli* as a tool for exploring the biological roles of components of protein complexes involving ACP and other cellular factors, as well as for the characterization of YbdB (EntH), a thioesterase produced in *E. coli* under iron starvation.[26,45,47,48] A broader high-throughput protein interaction study using TAP-tag has been reported in *E. coli* in which 857 proteins were tagged[24]; more than six hundred proteins were successfully purified; of which, 530 were found to be a part of protein–protein complexes. Protein interactants were subsequently identified by mass spectrometry and the resultant data were compiled to build a network of protein–protein associations covering a vast variety of biological activities. Additionally, the network raised the possibility of annotating uncharacterized factors with their designated function. More recently, TAP-tagging has been used to label factors in *X. oryzae* pv. oryzae[34,35] allowing for the quantitative analyses of protein expression and secretion. TAP-labeling was also used by our group, in a previous study, to detect the expression of the chromosome segregation protein ParB in Xac.[40] In conclusion, the results outlined above have demonstrated the stability of the TAP moiety and the expression vectors described herein have the potential to develop into valuable tools for exploring protein networks in Xac especially where factors of unknown function may be participants.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

GCD and PMMM received scholarships from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2013/19806-5 and 2006/59494-9) and CAPES/Brazil. This work was funded by FAPESP grant numbers 2004/09173-6, and 2013/50367-8.

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