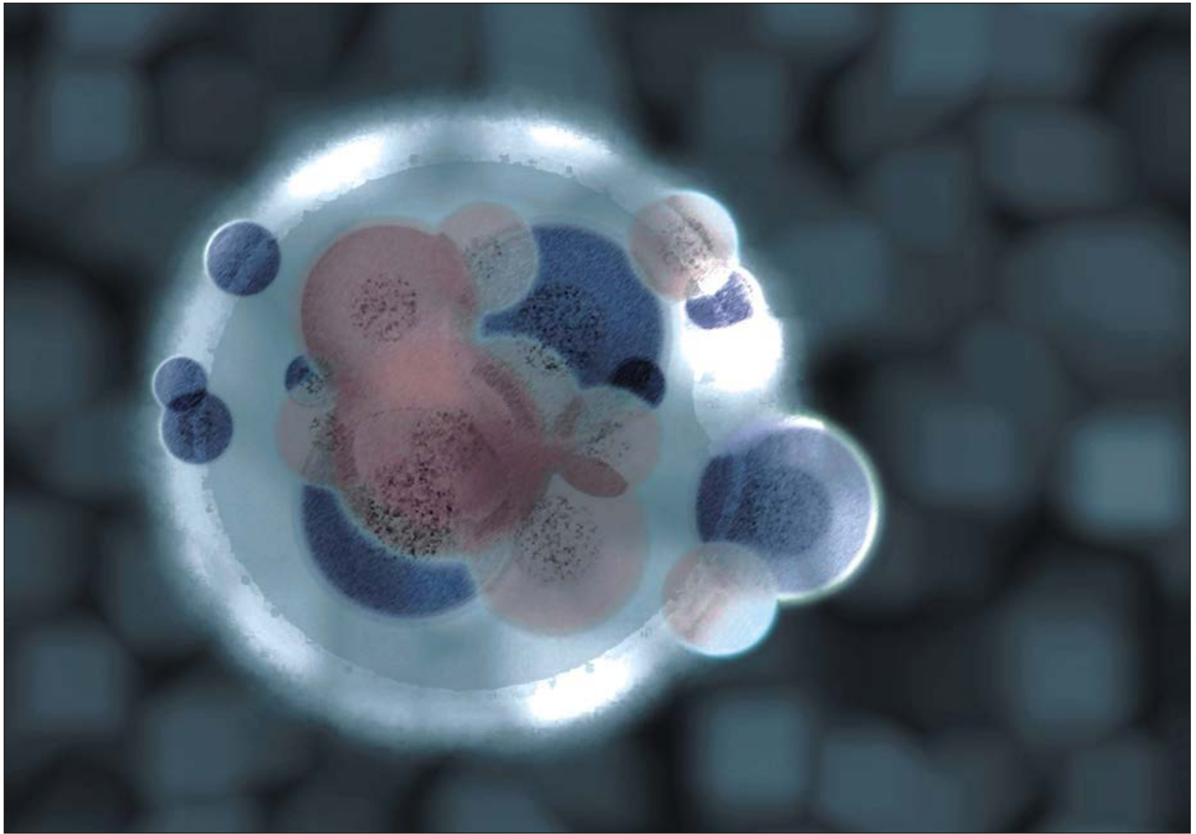

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Disruption of *drrA* Gene Affects the *Xanthomonas axonopodis* pv. *citri* Response to Oxidative Stress

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Abstract: The deduced amino acid sequence of *drrA* gene (XAC3759) from *Xanthomonas axonopodis* pv. *citri* (*Xac*) is similar to a variety of earlier described functionally uncharacterized ABC (ATP-Binding Cassette) transporters. In order to investigate the biological process in which this gene is involved, we constructed a *drrA* deletion mutant strain and tested its susceptibility to drugs with different mechanisms of action and to oxidative stress and also its capability to infect *Citrus sinensis*. The results showed that *Xac* responses to seven drugs (tetracycline, penicillin, ampicillin, chloramphenicol, acriflavine, ethidium bromide and doxorubicin chloridrate) and to host infection are independent upon *drrA* expression. However, Northern Blot analyses revealed that *drrA* gene is induced as short response to H_2O_2 exposure, only at the early phase of bacterial growth. Furthermore, the *drrA* disruption rendered the mutant more sensitive to oxidative stress than the control strain, suggesting that this transporter plays an important role in modulating oxidative stress susceptibility in *Xac*. Also, the *drrA* gene is co-transcripted with two hypothetical ORFs (Open Read Frame) as an operon cluster (named here *drrABC*) when *Xac* wild type strain was submitted to oxidative stress. The sequence analysis and topology prediction of the deduced proteins from these two neighboring hypothetical ORFs (XAC3758 and XAC3757) show that they have 6 transmembrane segments which are characteristic of permeases, supporting the structure of ABC transporter systems with an ABC subunit and two permeases.

Key words: Microbiology, genomics, molecular biology, gene expression, ABC transporter, antibiotics

INTRODUCTION

Xanthomonas axonopodis pv. *citri* (*Xac*) is a soil Gram-negative bacterium that causes citrus bacterial canker, a serious disease of most of citrus species and cultivars in many citrus-producing areas worldwide. This disease leads to the defoliation and premature abscission of affected fruit causing considerable losses for growers and for the economy of citrus-producing countries (Verniere *et al*., 2003).

Xac genome was sequenced and 4313 ORFs (Open Read Frame) were identified in 5,175,554 bp (Da Silva *et al*., 2002). The comparative genomic showed that 62.8% of these ORFs have an assigned function inferred by computational analyses, which provides only a general prediction of biochemical function through the identification of the family-specific sequence pattern (family signature). ATP-binding cassette systems (ABC transporters), for instance, are easily identified in the annotation process of sequenced genomes through their three conserved motifs known as the Walker A and B sites and the linker peptide with the signature motif LSSGQ, facilitating the protein clustering even without biological function knowledge (Dassa and Bouige, 2001; Ren and Paulsen, 2005; Biemans-Oldehinkel *et al*., 2006). However, in order to establish the biological role genes, direct experimentations are necessary.

The gene *drrA* of *Xac* (drug resistance gene) (XAC3759, GenBank accession No. AAM38602) was previously identified by the *Xac* complete genome sequencing (Da Silva *et al*., 2002) and was characterized as an ATP-binding protein from an ABC transporter, supposedly involved with drug resistance by drug efflux pump. However, an updated sequence similarity analysis using BLASTx program (Basic Local Alignment Search Tool) reveals a higher similarity of XAC3759 nucleotide sequence with functionally uncharacterized proteins from phytopathogenic and environmental Gram-negative

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bacteria than with drug efflux system (97% identity with *X. campestris* pv. *vesicatoria* GenBank accession No. CAJ25609; 92% identity with *X. oryzae* pv. *oryzae* GenBank accession No. BAE67328; 86% identity with *X. campestris* pv. *campestris* GenBank accession No. AAM42975 and 65% identity with *Sphingomonas* sp. GenBank accession No. EAT09413).

As it has been shown that ABC transporters play an important role in bacterial lifestyle, virulence and survival besides their involvement on drug resistance mechanism (Burse *et al*., 2004; Brencic and Winans, 2005; Piddock, 2006), the aim of this study was to investigate the biological process in which *drrA* gene is involved in *Xac*. For so, we constructed a *drrA* deletion mutant strain (Δ*drrAXac*) to determine whether the inactivation of *drrA* gene would affect *Xac* susceptibility to drugs and to oxidative stress as a simulation of the induced stress conditions at the pathogen-plant interaction when reactive oxygen species (ROS: hydroxyl radical, superoxide anion and hydrogen peroxide) are produced by the host and readily reacted with various pathogen cellular components inhibiting microbial survival (Lamb and Dixon, 1997). Furthermore, we evaluated *Xac* capability to infect and develop disease in citrus.

MATERIALS AND METHODS

Bacterial strains, plasmids and growth conditions: The *Xac*306 strain, isolated from citrus canker lesions of sweet orange leaves (*Citrus sinensis*), was obtained from the culture collection of Instituto Agronômico do Paraná (IAPAR) (Londrina, PR, Brazil) and its genome has been completely sequenced (Da Silva *et al*., 2002). *Xac*306 and derived strains were routinely grown in nutrient broth (NYGB) medium (Daniels *et al*., 1984) or NYGB agar plates for 24-48 h at 28°C. *Escherichia coli*, DH5α, used during the construction of the disruption cartridge, was grown on Luria-Bertani (LB) agar medium or in LB liquid medium for 12-24 h at 37°C. *E. coli* DH5α and *Xac* competent cells were prepared for the heat shock (Sambrook *et al*., 1989) and electroporation (White and Gonzales, 1991) transformation methods, respectively. Kanamycin and ampicillin, both 50 μg mL^{-1}, were used to maintain selection for resistance markers. The vectors were extracted using the QIAGEN Plasmid Mid Kit (QIAGEN).

Construction of a Δ*drrAXac* mutant: Standard techniques for molecular cloning were used as previously described by Sambrook *et al.* (1989). Genomic DNA from *Xac* was isolated by protein K lysis method (Ausubel *et al*., 1987). A Δ*drrAXac* mutant was constructed by the disruption of the *drrA* gene from *Xac* with a kanamycin resistance cartridge (Kan^r). The p*drrA* vector was constructed into pGEM®-T Easy vector by cloning a 1275 kb PCR amplification product of *Xac* genomic DNA using the primers Up1: 5'-GGAGACAGGGAAAGCATTGG-3' and Dn1: 5'-GGGCGATAGATGAAATGG-3', which annealed at 295 nt upstream and 255 nt downstream of *drrA* start and stop codon, respectively. The p*drrA* was digested with *Bal*I and *Stu*I to remove 537 bp from the cloned *drrA* in which two conserved motifs of the ABC transporter were localized (Walker A and Signature ABC). A 1283 bp *Eco*RI fragment encoding a kanamycin resistance gene from the pUC4K vector (GE Healthcare) replaced the removed fragment to generate the plasmid pGEM-Δ*drrA*. The construction was confirmed by DNA automatic sequencing (ABI Prism®377 DNA Sequencer System). The disruption cartridge was recovered from pGEM-Δ*drrA* on a 2021 bp fragment by *Not*I enzymatic restriction. Subsequently, it was inserted into a single *Eco*RV site present within the polylinker of pKX33 (Baldini *et al*., 1999) generating the plasmid pKX33-Δ*drrA*, which was used to electrotransform the *Xac*306 strain as previously described (Ferreira *et al*., 1995). The *Xac*306 strain was also electrotransformed with plasmid pKX33 and the resultant strain (*Xac-KX33*) used as a control. Klenow *E. coli* DNA polymerase I (USB Corporation) was used to fill protruding ends, when necessary.

The confirmation of *drrA* deletion by homologous recombination was done by the identification of a 2622 bp PCR amplification product of Δ*drrAXac* genomic DNA by using primer Up2: 5'-AACACCTCCAGCGACAGC-3' and primer Dn2: 5'-TAACCCAGGAAGACCAGC-3', which annealed 603 nt upstream and 449 nt downstream of *drrA* start and stop codon, respectively. This region corresponds to the *Xac* chromosomal sequence flanking the targeted sequence cloned in the pGEM-T easy vector that was absent in the vector and in the disruption cartridge. The deletion in the Δ*drrAXac* mutant was also confirmed by probing a DNA gel blot with both *Bal*I-*Stu*I deleted fragment and disruption cartridge gene Kan^r by Southern Blotting method (Sambrook *et al*., 1989).

Antibiotic and H_2O_2 susceptibility assay: The *Xac* strains were spread separately over NYGB agar broth. Sterile 3 MM filter paper disks (0.65 cm^2) were impregnated with 10 μL of drug solution and placed on the surface of the inoculated NYGB agar plates. The concentrations of drug solution were: tetracycline, 10 μg μL^{-1} penicillin, 50 μg μL^{-1}; ampicillin, 50 μg μL^{-1}; chloramphenicol, 100 μg μL^{-1}; acriflavine, 20 μg μL^{-1}; ethidium bromide, 10 μg μL^{-1} and doxorubicin chloridrate, 4 μg μL^{-1} (antibiotic from the daunorubicin anthracycline family)

(Adriblastina RD®-Pharmacia and Upjohn). Following 48 h incubation at 28°C, the diameter of the bacterial growth inhibition zones surrounding the filter disc was measured. The relative susceptibility of different strains to antibiotics was evaluated based on zone of inhibition around filter discs.

The bacterial growth was also monitored spectrophotometrically after adding H_2O_2 at two concentrations (1 and 2 mM H_2O_2) and at two bacterial growth phases: early-phase ($OD_{640\,nm}$ = 0.3-0.5 before log phase of bacterial growth) and exponential-phase ($OD_{640\,nm}$ >0.5 at exponential log phase culture). This assay was performed with 2 mL of each strain overnight cultures inoculated separately into 30 mL of NYGB broth at 28°C. *Xac* cultures were grown with shaking for 2-3 h until they reached the desired growth phase before H_2O_2 addition. Samples (2 mL) were then removed at 0, 2, 6, 8, 10 h intervals after H_2O_2 addition and optic density was measured.

Determination of *drrA* gene expression conditions: Northern Blot analyses (Sambrook *et al.*, 1989) were performed with *Xac*306 cultures after drug and H_2O_2 treatments at early- and exponential-phase cells. In both cases, the cultures were maintained under shaking and aliquots were removed after 5, 10 and 20 min of exposure to drug treatment and 5, 10, 20, 30 and 60 min of H_2O_2 treatment. The drug concentrations used were: tetracycline, 5 μg mL^{-1}; penicillin G, 100, 200, 350 μg mL^{-1}; ampicillin, 100 μg mL^{-1}; acriflavine, 0.5 μg mL^{-1}; doxorubicin chloridrate, 0.5, 2.5, 5, 10 μg mL^{-1}. The H_2O_2 concentrations were 1.5, 2, 3 and 6 mM. The total RNA was isolated with a RNA isolation system (Promega). The *Bal*I-*Stu*I fragment obtained during the cartridge construction was used as probe.

***In vivo* infection assay:** Analyses of host symptoms and bacterial population dynamics in plant tissue were carried out as described previously (Mehta and Leite, 2001) and conducted at IAPAR (Londrina, Paraná State) in 2005. Bacterial suspensions at 10^5 cfu mL^{-1} were used to infiltrate young leaves of *C. sinensis*, a susceptible host. The plants had been pruned to induce new leaf growth and were maintained under greenhouse conditions. Each bacterial strain was inoculated in leaves of different plants by hypodermic syringe carrying out the infiltration. In addition, other plants were also assayed with the three bacterial strains inoculated in different leaves. Approximately 10 leaves per plant were inoculated with bacterial suspensions of each *Xac* strains. The bacterial growth was determined by sampling 3 foliar disks of 0.63 cm^2, which were macerated in 1.5 mL of distilled water. Samplings were made at 0, 1, 2, 4, 8 and 16 days after the inoculation and each suspension obtained was diluted to appropriate concentrations and 100 μL were plated onto the culture medium. The plates were then incubated for 2-3 days.

Sequence analysis of the *drrAXac* locus and neighborhood: Nucleotide and protein sequence analyses were performed using the InterPro database (Mulder *et al.*, 2003) and Conserved Domain Architecture Retrieval Tool (CDART; of NCBI website) to identify domains and functional sites in the deduced proteins. HMMTOP, a biotool from the Transporter Classification Data Base (TCDB version 2.0.1) (Ren *et al.*, 2004) was used for the transmembrane segment prediction of proteins.

RESULTS AND DISCUSSION

The *drrA* mutant strain was constructed to perform mutant and wild comparison trials (Fig. 1A). The obtained PCR amplification patterns with primers Dn2 and Up2 showed that the genomic DNA of mutant strain was 740 pb longer than wild type (Fig. 1B) assuring the *drrA* disruption by deletion replacement with cartridge gene Kan^r integration after homologous recombination. The Southern blotting analysis of total DNA of *Xac* transformed cells confirmed the mutant strain isolation. *Bal*I-*Stu*I deleted fragment and cartridge gene Kan^r were used as probe (data not shown).

The effect of *drrA* gene inactivation on the capacity of *Xac* to response to different stress conditions and to infect citrus developing disease was evaluated through the comparison among the three bacterial strains (Δ*drrAXac, Xac*306 wild type and *Xac-KX33*).

The relative susceptibilities of Δ*drrAXac*, *Xac*306 and *Xac-KX33* strains were determined by the agar diffusion technique to seven growth inhibitors (tetracycline, penicillin G, ampicillin, chloramphenicol, acriflavine, ethidium bromide and doxorubicin chloridrate). No significant difference in drug susceptibility was observed among the three *Xac* strains (Table 1). In addition, the Northern blotting analyses showed that *drrA*

Table 1: Diameter measurement (cm^2) of bacterial growth inhibition zones

	Growth inhibitors[a]						
Strains	Tet	Pen	Amp	Cm	Acr	Etbr	Dox
*Xac*306	2.4	2.3	2.0	0.9	1.6	1.6	1.0
Δ*drraXac*	2.3	3.2	2.0	no halo	1.8	1.5	1.1
Xac-KX33	2.5	3.0	2.2	no halo	1.6	1.6	1.1

[a]The stock solution concentrations impregnated on sterile filter disks were: tetracycline (Tet), 10 μg μL^{-1}; penicillin (Pen), 50 μg μL^{-1}; ampicillin (Amp), 50 μg μL^{-1}; chloramphenicol (Cm), 100 μg μL^{-1}; acriflavine (Acr), 20 μg μL^{-1}; ethidium bromide (Etbr), 10 μg μL^{-1} and doxorubicin chloridrate (Dox), 4 μg μL^{-1}. The reported values are representative of two experiments

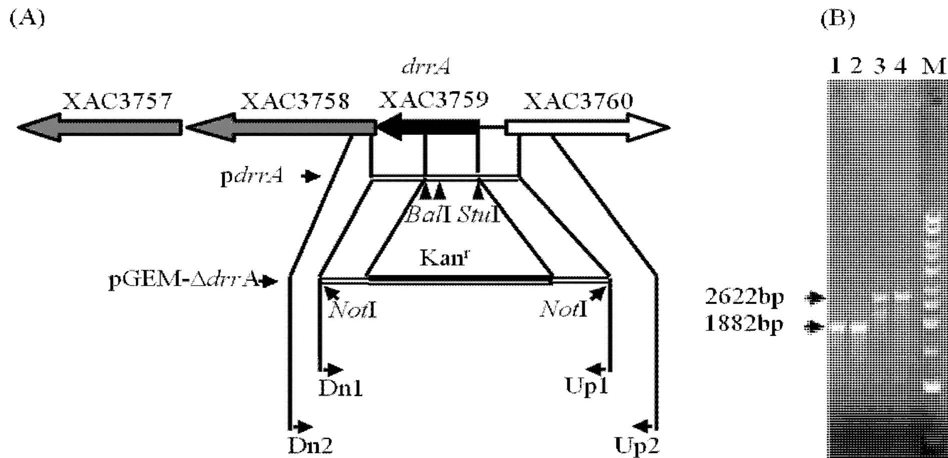

Fig. 1: Disruption of *drrAXac* by allelic exchange with a kanamycin resistant cassette. (A) The diagram illustrates the steps of the disruption cartridge assembling for a Δ*drrA* deletion mutant construction. The region of *drrA* gene cluster was amplified by primers Dn1 and Up1 and cloned into pGEM®-T Easy vector to give rise to plasmid p*drrA*. The p*drrA* was digested with *Bal*I and *Stu*I and the fragment was substituted by a kanamycin resistance cartridge (Kan^r) resulting in pGEM-Δ*drrA* carrying the disruption cartridge. Arrowed boxes represent the direction of the putative genes in the *Xac* genome. (B) PCR amplification of genomic DNA of *Xac* strain transformed with pKX33-Δ*drrAXac* vector. Primers used were Up2 and Dn2. (lanes 1, 2) *Xac*306 strain (control); (lanes 3, 4) Δ*drrAXac* strain and (M) Kilobase DNA marker (GE Healthcare)

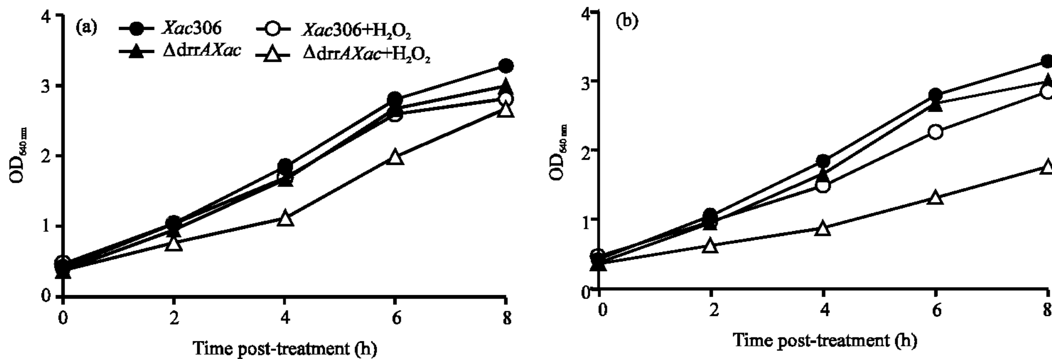

Fig. 2: Effect of H_2O_2 treatment on *Xac*306 and Δ*drrAXac* cultures. H_2O_2 was added to bacterial culture at early-phase growth ($OD_{640nm} \leq 0.5$) to record the absorbance at different times after exposition to oxidative stress. (A) 1 mM H_2O_2 and (B) 2 mM H_2O_2. The experiment was assayed in triplicate and a single representative experiment is illustrated here

gene was not required for *Xac* resistance to these seven drugs since *drrA* gene expression was not detected after exposure to each drug (data not shown). Hence, the disruption of the *drrA* gene does not affect the *in vitro* susceptibility to these drugs under the conditions employed.

As *drrA* has not seemed to be involved with *Xac* drug resistance, we assessed the role of *drrA* in *Xac* response to H_2O_2 exposure as a simulation of the induced stress conditions at the pathogen-host interaction. For so, we assayed Δ*drrAXac* mutant and control strains under different experimental conditions of H_2O_2 concentration and bacterial growth phases. The results showed that when H_2O_2 was added at exponential-phase culture ($OD_{640\ nm}>0.5$) mutant and wild strains were similarly tolerant to both H_2O_2 concentrations assayed (1 mM and 2 mM H_2O_2) data not shown. However, when H_2O_2 was added at early-phase growth ($OD_{640\ nm} \leq 0.5$) Δ*drrAXac* strain was more sensitive to oxidative stress than the wild type showing that *drrA* gene disruption altered the response to oxidative stress in the mutant strain, an effect dependent on H_2O_2 concentration (Fig. 2). Northern blotting analyses showed that *drrA Xac*306 gene is up-regulated after 20 min of exposition to different H_2O_2

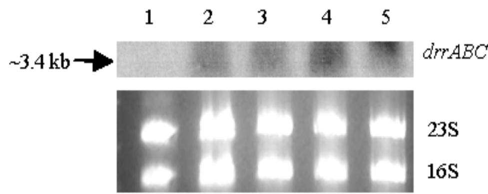

Fig. 3: Northern analysis of the *drrABC* operon using total RNA from *Xac*306 strain after 20-minute exposure to 1.5 mM (lane 2), 2 mM (lane 3), 3 mM (lane 4) and 6 mM of H_2O_2 ($OD_{640\,nm} \leq 0.5$). Lane 1 corresponds to the untreated culture. A co-transcript of about 3.4 kb, including XAC3759 (*drrA* gene), XAC3758 (*drrB* gene) and XAC3757 (*drrC* gene), was revealed using the *Bal*I-*Stu*I fragment of *drrA* gene (see Fig. 1A) as the probe. Ethidium bromide-stained rRNA bands are shown for comparison of the quantities of loaded RNA

concentrations, as a short and quick response, only at early phase of bacterial growth. ($OD_{640\,nm} \leq 0.5$) (Fig. 3). At exponential and stationary phases of bacterial growth and in the absence of H_2O_2 treatment, the *drrA* gene expression was not detected.

Due to the oxidative tolerance decrease as a consequence of *drrA* disruption, we tested if the *drrA* mutation had any effect on growth and survival of *Xac* in plants. However, unexpectedly, no difference in host symptom development or vesicle size was observed between Δ*drrAXac* and control strains when bacterial suspensions were inoculated on leaves of *C. sinensis*. Typical lesions of citric canker began to be visible in all inocula simultaneously after 5 days. The collapse of the infiltrated area was followed by a premature abscission of the inoculated leaves (Fig. 4A). Also, the monitoring of the *Xac* population in leaves of *C. sinensis* during the first sixteen days post-inoculation revealed no variation in growth rate *in vivo* or *in vitro* (Fig. 4B). Thus, these results suggest that *drrA* was not essential for the process of *Xac* pathogenicity in the plant model tested here.

It has been reported that to several bacteria including *Xanthomonas* species (Farr and Kogoma, 1991; Mongkolsuk *et al.*, 2005), at exponential and stationary phases of bacterial culture, an enzymatic detoxification mechanism is induced to promote the cellular protection against harmful metabolic products of essential cellular process. Multiple isozymes are induced at later bacterial cultures. Isozymes as catalases (KAT) are induced by carbon excess and by the lack of nutrients during the late phases of bacterial culture and superoxide dismutase (SOD) is responsible for the natural elimination of

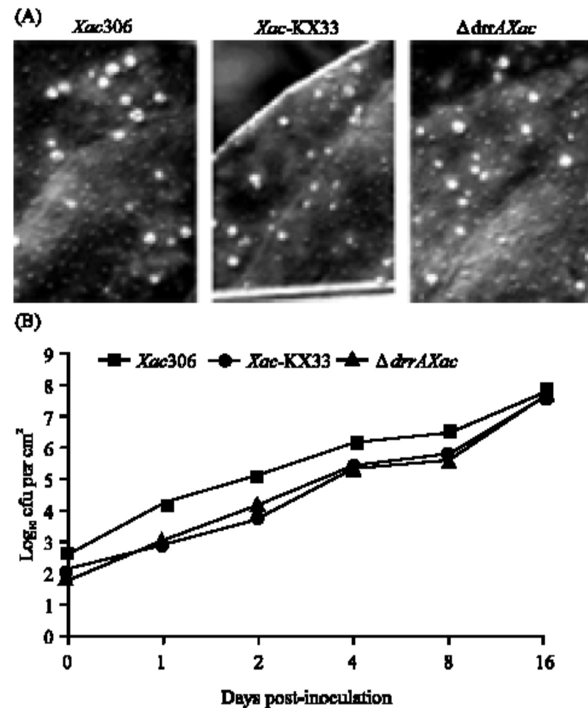

Fig. 4: *In vivo* infection assays. (A) Comparison of canker symptoms (light spots on surface leaves) evoked by the Δ*drrAXac*, *Xac*306 and *Xac-KX33* strains 10 days post-inoculation in *C. sinensis*. (B) Total population of bacteria per cm^2 of *C. sinensis* tissue leaves at different times post-inoculation. The result illustrated here is a single representative experiment from replayed experiments

peroxides and superoxides produced by essential aerobic cell metabolism (Farr and Kogoma, 1991; Fridovich, 1997). Then, indirectly, the action of these enzymes enhances the tolerance to several stress form including subsequent H_2O_2 stimuli as a cross-protection response. Thus, we presume that the DrrA transporter plays an important role in modulating susceptibility to oxidative stress before the enzymatic detoxification is activated, at early phase of bacterial growth, followed by the inhibition of *Xac drrA* gene expression when the enzymatic detoxification mechanism is induced.

Furthermore, in plant-pathogen interaction process, the Hypersensitive Response (HR) has been described as the first pathogen-induced defense host response (Bonas and Van den Ackerveken, 1999). HR is triggered in resistant plants by specific plant-pathogen recognition followed by a wide range of physiological changes including ROS production (Lamb and Dixon, 1997). Considering the negative results obtained by *in vivo* assays to evaluate the essentiality of *drrA* gene to growth

and the survival of *Xac* in plant host, we supposed that neither plant-pathogen recognition nor ROS took place due to the lack of HR activation in consequence of *C. sinensis* susceptibility to *Xac* infection. Consequently, without oxidative stress induction, *drrA* expression was not required and the mutation effect could not be assessed. However, we can not rule out the possibility of the *drrA* gene playing a role in bacterium-environmental interaction as part of a defense system against environmental oxidative stress conditions and not being required for bacterium-host interaction.

Interestingly, the gene expression analysis revealed a long RNA transcript with a length of almost 3400 pb, what we deduced to be the co-transcription of three ORFs (XAC3759, *drrA* gene, 723 pb; XAC3758, hypothetical protein, 1437 pb and XAC3757, hypothetical protein, 1269 pb) as an operon cluster (operon *drrABC*) (Fig. 1, 3). The sequence analysis and topology prediction of the deduced proteins from these two neighboring hypothetical ORFs (XAC3758 and XAC3757) show that they have 6 transmembrane segments which are characteristic of permeases, supporting the structure of ABC transporter systems with an ABC subunit and two permeases.

CONCLUSION

The growth phenotype observed for Δ*drrAXac* mutant suggests that the *drrAXac* gene is not essential for the survival of *Xac* in the absence of oxidative stress. Moreover, we presume that DrrA transporter plays an important role in modulating susceptibility to oxidative stress in *Xac* and that the two co-transcripted hypothetical ORFs should participate of this process.

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REFERENCES

Ausubel, F., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smithand and K. Struhl, 1987. Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley-Interscience and John Wiley, Sons, New York, USA.

Baldini, R.L., S.T. Tahara and Y.B. Rosato, 1999. A rolling-circle miniplasmid of *Xanthomonas campestris* pv. *glycines*: The nucleotide sequence and its use as a cloning vector. Plasmid, 42 (2): 26-133.

Biemans-Oldehinkel, E., M.K. Doeven and B. Poolman, 2006. ABC transporter architecture and regulatory roles of accessory domains. FEBS. Lett., 580 (4): 1023-1035.

Bonas, U. and G. Van den Ackerveken, 1999. Gene-for-gene interactions: Bacterial avirulence proteins specify plant disease resistance. Curr. Opin. Microbiol., 2 (1): 94-98.

Brencic, A. and S.C. Winans, 2005. Detection of and response to signals involved in host-microbe interactions by plant-associated bacteria. Microbiol. Mol. Biol. Rev., 69 (1): 155-194.

Burse, A., H. Weingart and M.S. Ullrich, 2004. The phytoalexin-inducible multidrug efflux pump AcrAB contributes to virulence in the fire blight pathogen, *Erwinia amylovora*. Mol. Plant Microb. Interact., 17 (1): 43-54.

Da Silva, A.C., J.A. Ferro, F.C. Reinach, C.S. Farah, L.R. Furlan and R.B. Quaggio *et al*., 2002. Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. Nature, 417 (6887): 459-463.

Daniels, M.J., C.E. Barber, P.C. Turner, M.K. Sawczyc, R.J. Byrde and AH. Fielding, 1984. Cloning of genes involved in pathogenicity of *Xanthomonas campestris* pv. *campestris* using the broad host range cosmid pLAFR1. EMBO. J., 3 (13): 3323-3328.

Dassa, E. and P. Bouige, 2001. The ABC of ABCS: A phylogenetic and functional classification of ABC systems in living organisms. Res. Microbiol., 152 (3-4): 211-229.

Farr, S.B. and T. Kogoma, 1991. Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. Microbiol. Rev., 55 (4): 561-585.

Ferreira, H., F.J.A. Barrientos, R.L. Baldini and Y.B. Rosato, 1995. Electrotransformation of three pathovars of *Xanthomonas campestris*. Applied Microbiol. Biotechnol., 43 (4): 651-655.

Fridovich, I., 1997. Superoxide anion radical (O_{2}.), superoxide dismutases and related matters. J. Biol. Chem., 272 (30): 18515-18517.

Lamb, L. and R.A. Dixon, 1997. The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol. Plant Mol. Biol., 48 (1): 251B275.

Mehta, A. and R.P. Leite, 2001. New studies on non-pathogenicity of *Xanthomonas axonopodis* pv. *aurantifolii* strain C to sweet orange. Summa Phytopathol., 27 (1): 53-56.

Mongkolsuk, S., J.M. Dubbs and P. Vattanaviboon, 2005. Chemical modulation of physiological adaptation and cross-protective responses against oxidative stress in soil bacterium and phytopathogen, *Xanthomonas*. J. Ind. Microbiol. Biotechnol., 32 (11-12): 687-690.

Mulder, N.J., R. Apweiler, T.K. Attwood, A. Bairoch, D. Barrell, A. Bateman and D. Binns *et al*., 2003. The InterPro Database, brings increased coverage and new features. Nucleic Acids Res., 31 (1): 315-318.

Piddock, L.J., 2006. Multidrug-resistance efflux pumps-not just for resistance. Nat. Rev. Microbiol., 4 (8): 629-636.

Ren, Q., K.H. Kang and I.T. Paulsen, 2004. TransportDB: A relational database of cellular membrane transport systems. Nucleic Acids Res., 35 (Database issue): D284-288.

Ren, Q. and I.T. Paulsen, 2005. Comparative analyses of fundamental differences in membrane transport capabilities in prokaryotes and eukaryotes. PLOS. Comput. Biol., 1 (3): e27.

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Verniere, C.J., T.R. Gottwald and O. Pruvost, 2003. Disease development and symptom expression of *Xanthomonas axonopodis* pc. *citri* in various citrus plant tissues. Phytopathology, 93 (7): 832-843.

White, T.J. and C.F. Gonzales, 1991. Application of electroporation for efficient transformation of *Xanthomonas campestris* pv. *oryzae*. Phytopathology, 81 (5): 521-524.