Assessment of *Xanthomonas axonopodis pv. manihotis* Genes Expressed During Plant Infection

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INTRODUCTION

Cassava bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam)* is a major disease endemic in Latin America and Africa. Changes in the pathogen's population structure may lead to the overcoming of the deployed resistance.

We wished to determine the molecular basis of pathogenesis of *Xanthomonas axonopodis pv. manihotis* using molecular approaches. DNA microarray analysis is a valuable tool that allows evaluation of gene expression and provides information about DNA sequences that are simultaneously expressed in specific environments. For that, anonymous sequences, if the studied organism is not sequenced, can be screened using mRNA or cDNA as a probe.

The present study aims to elucidate *Xanthomonas axonopodis* pv. *manihotis* (Xam) genes that are expressed during cassava infection. A genomic library was constructed comprising 3450 clones. Different techniques such as RNA extraction from bacteria recovered from infected tissues challenged by *Xam* and total bacterial RNA labeling as the construction of a *Xam* micorarray were standardized at CIAT. Preliminary results are presented below.

RESULTS and DISCUSSION

Genomic library and probes

Genomic DNA from CIO46 strain was extracted and partially digested with *Mse*I. Adapters corresponding to the same enzyme were ligated to digested fragments and then amplified using an adapter as primer. Amplification product was run in agarose gel and purified lately to be cloned into pGMT-Easy vector (Promega).

3450 clones harboring inserts between 500 and 1500bp were obtained and were amplified directly from bacterial culture to construct the DNA chip.

Total RNA was extracted from cassava plants infected with *Xam* strain CIO46 24, 48, and 72hours; 5 and 7 days after inoculation. Pull of RNA obtained at different times post-inoculation was used as treatment probe and total RNA from bacteria grown in culture media

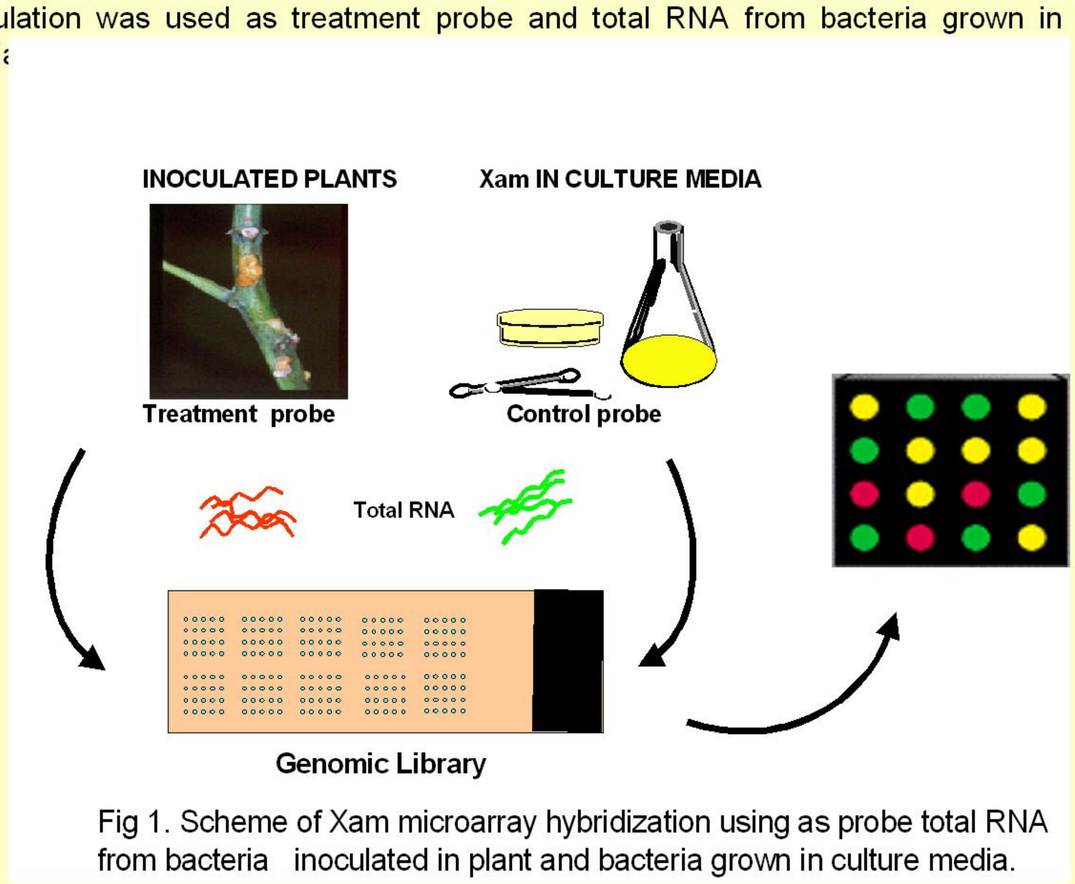

Fig 1. Scheme of Xam microarray hybridization using as probe total RNA from bacteria inoculated in plant and bacteria grown in culture media.

Microarray analysis

Slides containing four replications of each clone were hybridized with 5µg of RNA labeled directly with Cye3 and Cye5 dyes (Amersham). Spot intensities from scanned slides were quantified using ArrayPro 4.0 software (Fig.2).

Background correction was made only over signals that were higher than two standard deviation of local background besides, intensity-dependent normalization (LOESS) was performed and differentially expressed genes were detected using SAM (Significance Analysis of Microarrays).

We found that of 3450 elements, 77 were expressed in bacteria present in the infected plant, and 16 were expressed in bacteria grown over culture media.

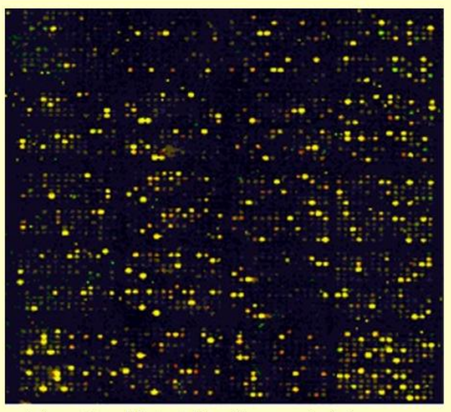

Fig 2. Hybridization of *Xam* CIO 46 genomic library with 5µg of RNA labeled directly with cye3 and cye5 dyes (Amersham). Spot intensities from scanned slides were quantified using ArrayPro 4.0 software

Putative Function	Expect
conserved hypothetical protein NMB1543 [imported] - Neisseria meningitidis (strain MC58 serogroup B)	3.00E-18
hypothetical protein NMA1792 [imported] - Neisseria meningitidis (strain Z2491 serogroup A)	3.00E-18
probable transposase, 39K - Escherichia coli insertion sequence IS5	1.00E-92
probable transposase, 39K - Escherichia coli insertion sequence IS5	3.00E-92
hypothetical protein CP0987 [imported] - Chlamydophila pneumoniae (strain AR39)	3.00E-48
hypothetical protein TC0129 [imported] - Chlamydia muridarum (strain Nigg)	3.00E-46
O-antigen biosynthesis protein homolog rfbC - Myxococcus xanthus	6.00E-21
teichuronic acid biosynthesis tuaG [imported] - Bacillus halodurans (strain C-125)	3.00E-09
hypothetical protein TC0114 [imported] - Chlamydia muridarum (strain Nigg)	2.00E-11
hypothetical protein TC0130 [imported] - Chlamydia muridarum (strain Nigg)	2.00E-11
type I restriction enyzme ECOR124/3 I M homolog - Methanococcus jannaschii plasmid pURB800	1.00E-10
EcoE type I restriction modification enzyme M subunit - Escherichia coli	4.00E-09
hypothetical protein PH0217 - Pyrococcus horikoshii	4.00E-05
artifact-warning sequence (translated ALU class C) - human	2.00E-21
artifact-warning sequence (translated ALU class A) - human	2.00E-19
conserved hypothetical protein XF1862 [imported] - Xylella fastidiosa (strain 9a5c)	2.00E-18
hypothetical protein XF1863 [imported] - Xylella fastidiosa (strain 9a5c)	5.00E-10
probable aminotransferase protein (EC 2.6.1.-) [imported] - Sinorhizobium meliloti (strain 1021) megaplasmid	1.00E-23
adenosylmethionine--8-amino-7-oxononanoate transaminase (EC 2.6.1.62) bioA - Bacillus subtilis	2.00E-20
fimbrial assembly protein XF0373 [imported] - Xylella fastidiosa (strain 9a5c)	3.00E-60
hypothetical protein HI0435 (transformation locus) - Haemophilus influenzae (strain Rd KW20)	1.00E-24
chr partitioning protein, ParA family - Deinococcus radiodurans (strain R1)	6.00E-12
hypothetical protein - Synechocystis sp. (strain PCC 6803)	1.00E-11
probable permease of ABC-2 transporter PA2678 [imported] - Pseudomonas aeruginosa (strain PAO1)	3.00E-13
probable ABC transporter system integral membrane protein - Serratia marcescens	1.00E-12
conjugal transfer protein XFa0007 [imported] - Xylella fastidiosa (strain 9a5c)	2.00E-05
hypothetical protein TC0128 [imported] - Chlamydia muridarum (strain Nigg)	1.00E-05
type 4 fimbrial biogenesis protein PilY1 PA4554 [imported] - Pseudomonas aeruginosa (strain PAO1)	2.00E-08
pilY1 protein - Pseudomonas aeruginosa	2.00E-08
hypothetical protein 1 - Pseudomonas cepacia insertion sequence IS407	5.00E-11
conserved hypothetical protein PA0987 [imported] - Pseudomonas aeruginosa (strain PAO1)	4.00E-10
type II site-specific deoxyribonuclease (EC 3.1.21.4) BsuBI - Bacillus subtilis	2.00E-08
type II site-specific deoxyribonuclease (EC 3.1.21.4) PstI - Providencia stuartii	9.00E-06
probable ClpA/B-type proteinase PA2371 [imported] - Pseudomonas aeruginosa (strain PAO1)	5.00E-14
probable ClpA/B-type chaperone PA0090 [imported] - Pseudomonas aeruginosa (strain PAO1)	7.00E-14
ABC transporter ATP-binding protein XF0944 [imported] - Xylella fastidiosa (strain 9a5c)	3.00E-18
ABC transporter, ATP-binding protein CC2148 [imported] - Caulobacter crescentus	2.00E-10

Analysis of differential clone sequences

Clones that showed altered expression were sequenced and compared with PIR and GenBank databases using Blast program.

Cell wall modification

A differentially expressed clone showed homology to a gene involved in teichuronic acid biosynthesis pathway (tuaG). TuaG synthesis plays an important role in integrity of the bacterial cell. Bacterial surface polysaccharides appear to be involved in virulence and immunity process.

Effector mechanisms

Two clones shared homology with typeI restriction enzyme ECOR124/3 I M and EcoE, and other with PstI (*Providencia stuartii*) restriction enzyme. Besides two DNA clones shown homology to a type II site-specific deoxyribonuclease. These enzymes could be involved in plant DNA damage as effector mechanism in *Xam* attack. In addition, we found a clone that encodes a putative ClpA/B-type proteinase that could represent other bacterial effector.

Metabolic changes

A clone shows homology to a DAPA aminotransferase (EC 2.6.1.62), that corresponds to a bacterial enzyme (gene bioA) and catalyzes an intermediate step in the biosynthesis of biotin. Biotin has been associated to metabolic and energy pathways (Jitrapakdee S, Wallace JC., 2003).

Transport

Two clones encoding a probable permease of ABC-2 transporter and ABC transporter system were found.

We also found genes of unknown function or showing no homology with sequences in the databases (41 clones). They may represent new genes putatively involved in the *Xam* infection process.

PERSPECTIVES

We identified several clones from *Xanthomonas axonopodis* pv. *manihotis* Cio 46 that are specifically expressed during infection phase. They represent potential pathogenesis factors which are expressed or triggered only as response to plant components and are associated to the establishment of the *Xam*-cassava compatible interaction.

Real-Time PCR analyses are in course to validate a selected number of clones. This will allow us to establish the time post-inoculation at which each sequence is expressed during the infection process.