Pathology workshop
Nanning - China
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Identification of genes involved in epiphytic survival of *Xanthomonas albilineans*, the causal agent of leaf scald of sugarcane

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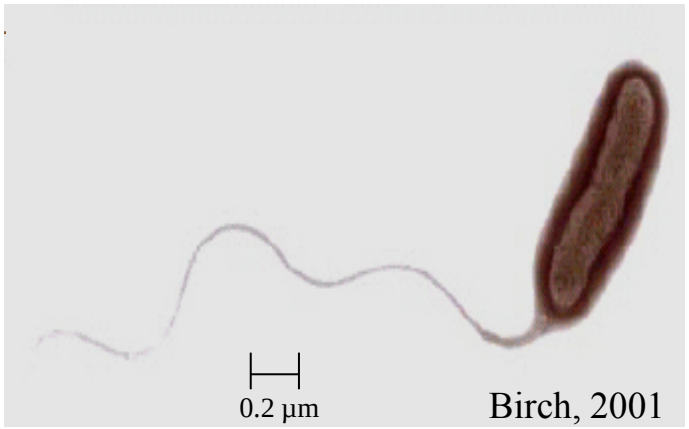
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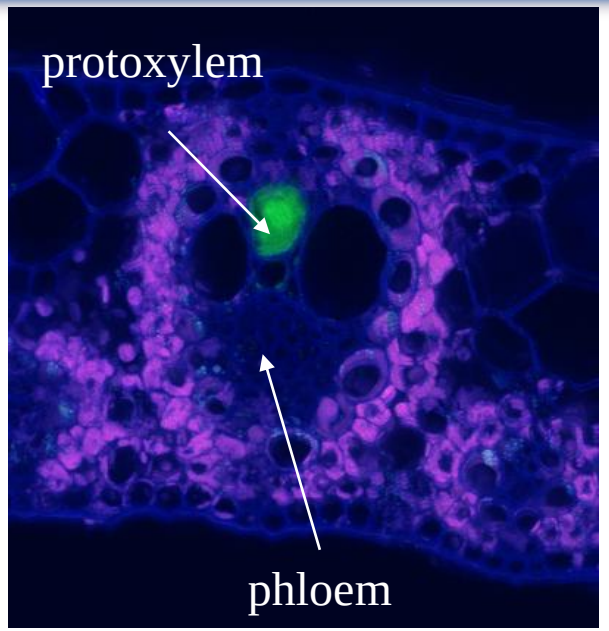


Research team :
Genomics and Molecular Analysis of Pathogenicity of Plant Pathogenic Bacteria

Introduction

Xanthomonas albilineans



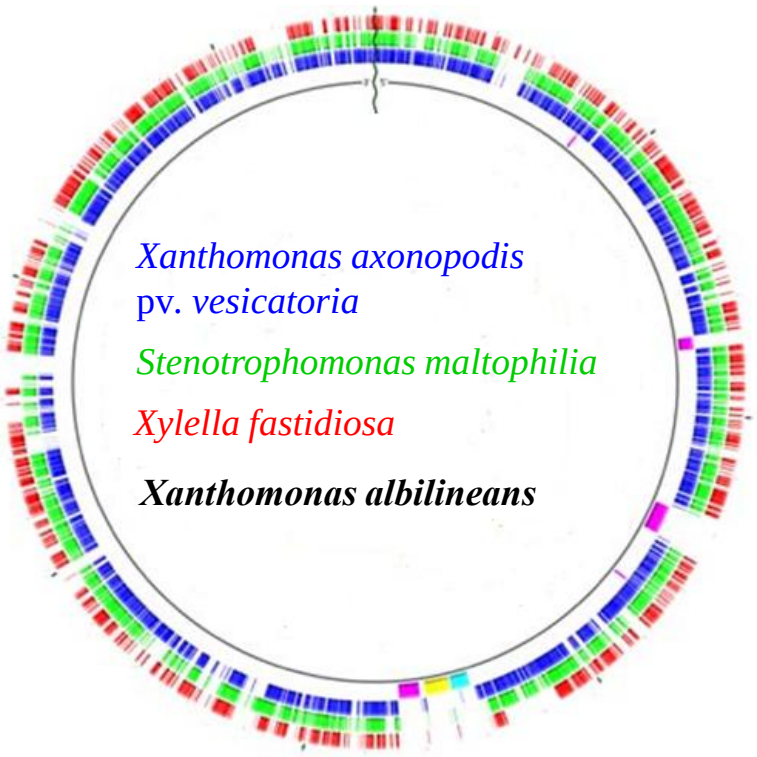








Xanthomonas albilineans : An original and particular species of *Xanthomonas*



The chromosome of *Xanthomonas albilineans* strain GPE PC73 (from Guadeloupe)

Genome size = 3.7 Mb (*Xanthomonas* = 5 Mb)

X. albilineans does not possess:

- *gum* genes (no production of xanthan gum)
- A Hrp type III secretion system

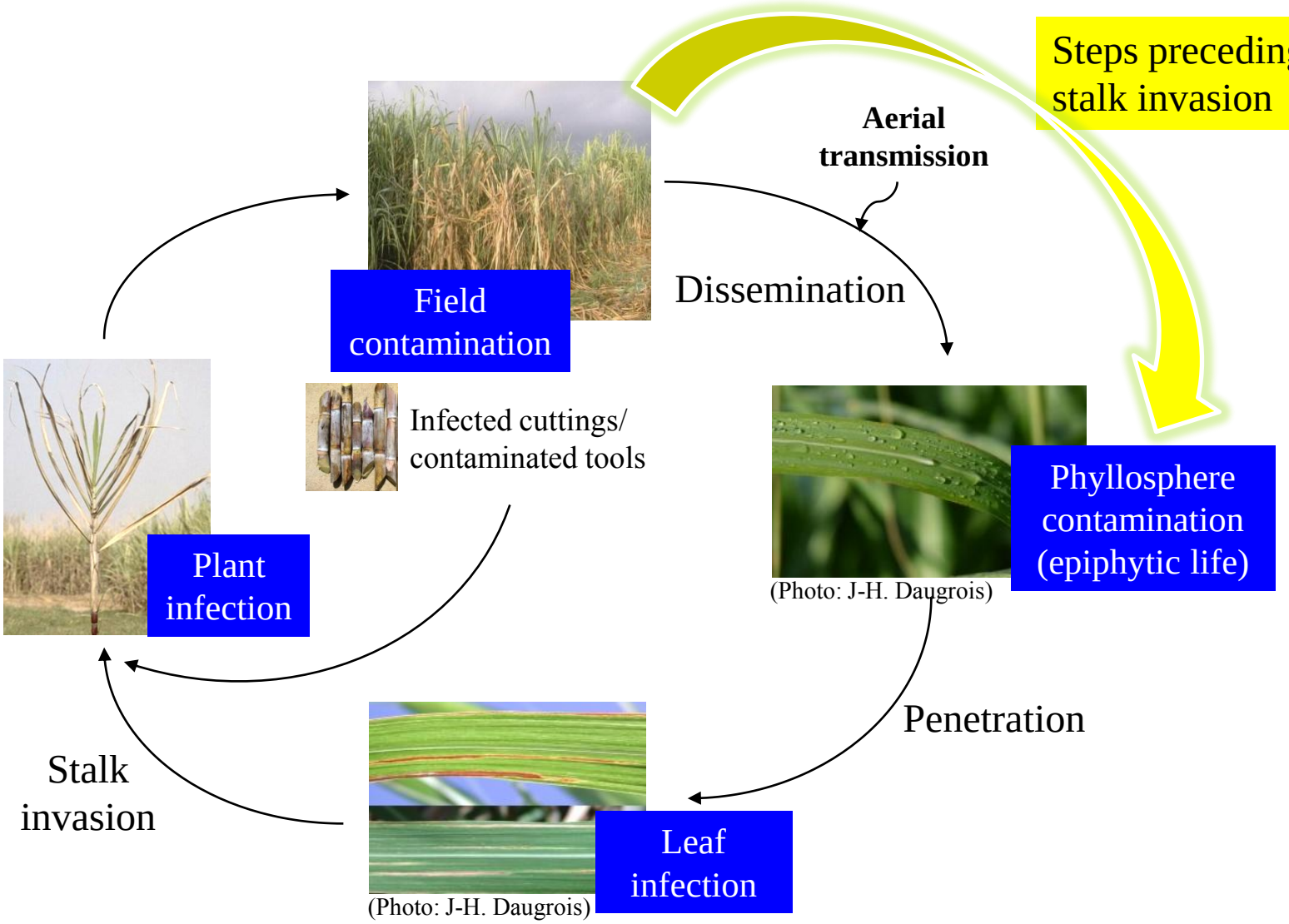
X. albilineans does possess:

- An animal pathogen-associated type III secretion system of the SPI-1 family («*Salmonella* Pathogenicity Island-1»)
- NRPS («Non Ribosomal Peptide Synthetase») genes involved in biosynthesis of small molecules
- A gene cluster called XALB1 involved in albicidin biosynthesis

Pieretti *et al*. 2009 *BMC Genomics*; Royer *et al*. 2004 *MPMI*.

What are the other molecular determinants involved in pathogenicity of *Xanthomonas albilineans* ?

Scientific context: Infection cycle



Objective: To identify molecular factors involved in the epiphytic life of *X. albilineans*

Strategy 1: Multiple candidate gene approach

Mutants of pathogenicity

Mutant	Extent of stalk colonization
Production of surface polysaccharides (*xanB, gmd, rmd, galU,…*)	none
Outer membrane protein A (*ompA*)	none
Production of albicidin + other small NRPS molecules (*albXXI*)	= WT except in the upper part of the stalk
Type III secretion system SPI-1 (*xsaO*- injectisome assembly protein)	= WT
Quorum sensing (*rpf* - cell to cell communication)	= WT or > WT
Twitching motility (*pilY* - type IV pilus biogenesis protein pilY)	= WT

Strategy 2: WT strain approach

Wild-type strains with different characteristics

Strain	Aerial transmission	Pathogenicity
Xa23R1	-	+
Xa GPE PC73	+	+
XaFL07-1	+	+
XaS3 (close to *X. albilineans*)	+	-

Materials and methods



Tissue cultured plantlets

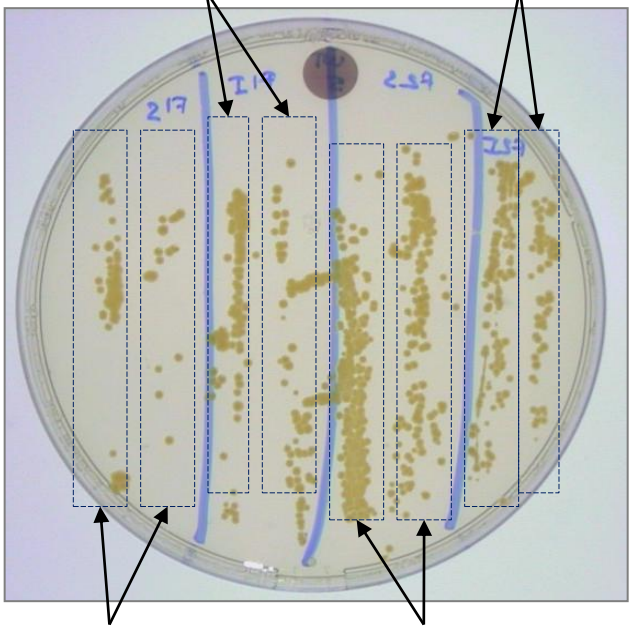
Immersion in a bacterial suspension

5 seconds



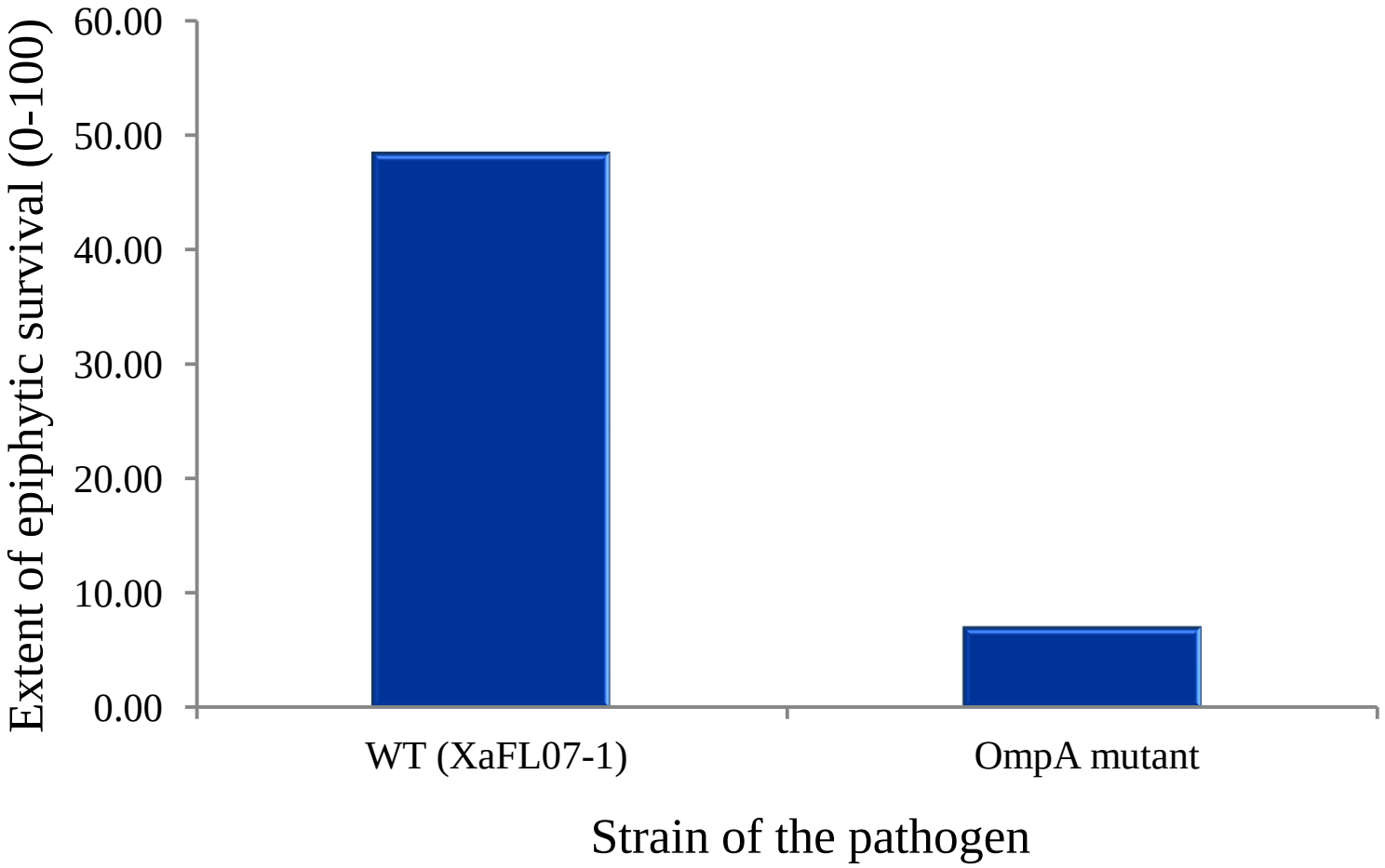
Incubation for two weeks

12h of light
28°C



Imprints of sugarcane leaves on selective medium

Results



Extent of epiphytic survival of wild type strain XaFL07-1 and mutant XaOmpA

Results

1. Mutants of pathogenicity

Mutant	Extent of stalk colonization	Phyllosphere colonization
Production of surface polysaccharides (*xanB, gmd, rmd, galU,…*)	none	none
Outer membrane protein A (*ompA*)	none	none
Production of albicidin + other small NRPS molecules (*albXXI*)	= WT except in the upper part of the stalk	= WT
Type III secretion system SPI-1 (*xsaO*- injectisome assembly protein)	= WT	Slightly < WT ?
Quorum sensing (*rpf* - cell to cell communication)	= WT or > WT	= WT or < WT
Twitching motility (*pilY* - type IV pilus biogenesis protein pilY)	= WT	= WT

Results

2. Wild-type strains from different geographic origins

Strain	Aerial transmission	Pathogenicity	Phyllosphere colonization
Xa23R1	-	+	+ ?
Xa GPE PC73	+	+	++
XaFL07-1	+	+	++
XaS3 (close to *X. albilineans*)	+	-	++

Conclusion

➢ Features involved in epiphytic survival of *X. albilineans*

- Surface polysaccharides and outer membrane protein A: critical cell surface molecules
- Rpf quorum sensing (precise role to be determined: Biofilm?)
- T3SS SPI-1 (to be confirmed)

➢ Features apparently not involved in epiphytic survival of *X. albilineans*

- NRPS molecules (albicidin and other small molecules)
- Twitching motility

➢ Strains of *X. albilineans* involved in aerial transmission: more adapted to epiphytic survival than strains not aerially transmitted ?

Perspectives

- Replication of experiments and complementation studies to confirm phenotypes
- Study of additional WT strains (aerially vs. non aerially transmitted strains)
- Genetic comparison of aerially vs. non aerially transmitted strains
- Identification of additional genes involved in epiphytic life by transposon or site-directed mutagenesis

ACKNOWLEDGMENTS

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THANK YOU FOR YOU ATTENTION