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Genome sequencing of pathogens for a better understanding and management of sugarcane diseases

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Genome sequencing

What's a genome (NCBI : National Center for Biotechnology Information)?

«Life is specified by **genomes**. Every organism, including humans, has a genome that contains all of the biological information needed to build and maintain a living example of that organism**.** The biological information contained in a genome is encoded in its **deoxyribonucleic acid (DNA)** and is divided into discrete units called **genes**. Genes code for proteins that attach to the genome at the appropriate positions and switch on a series of reactions called gene expression **»**

NCBI data base:

In 2012, sequence data from the whole genomes of over 1,000 organisms available, including genomes of several viral and bacterial sugarcane pathogens

Sequenced sugarcane pathogens (whole genome)

Viral pathogen	Disease	Genome size
Fiji disease virus (FDV)	Fiji leaf gall	27-30 Kb
Peanut clump virus (PCV)	Red leaf mottle	10.4 Kb
Sugarcane bacilliform virus (SCBV)	Leaf fleck	7.3-7.4 Kb
Sugarcane mosaic virus (SCMV), *Sorghum mosaic virus* (SrMV)	Mosaic	9.6 Kb
Sugarcane streak virus (SSV), *Sugarcane streak Egypt virus* (SSEV), *Sugarcane streak Mauritius virus* (SSMV) = *Sugarcane streak Reunion virus* (SSRV)	Streak	2.7 Kb
Sugarcane streak mosaic virus (SCSMV)	Streak mosaic	9.8 Kb
Sugarcane striate mosaic associated virus (ScSMaV)	Striate mosaic	8.1 Kb
Sugarcane yellow leaf virus (SCYLV)	Yellow leaf	5.8-5.9 Kb

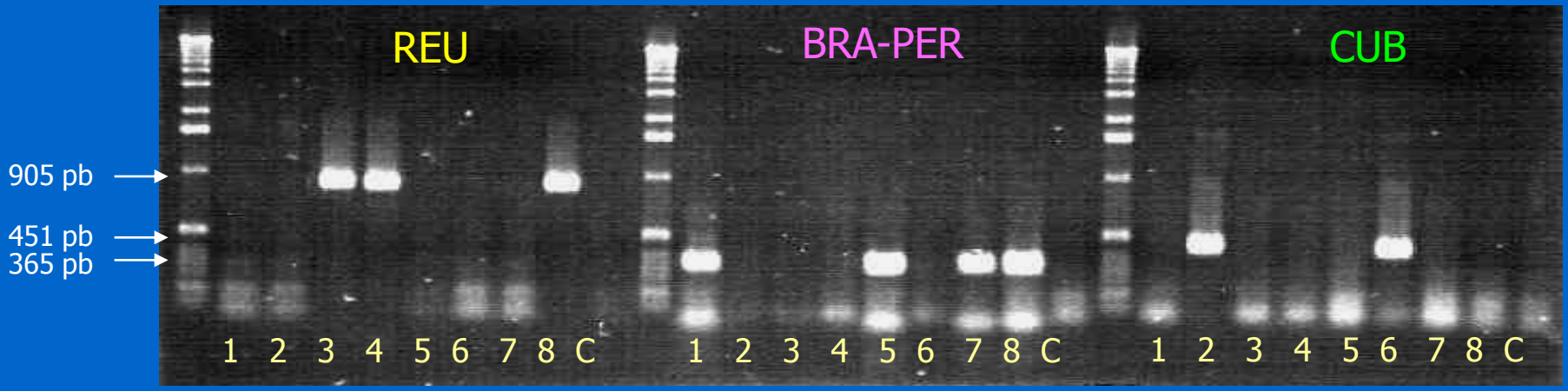
Sequenced sugarcane pathogens (whole genome)

Bacterial pathogen	Disease	Genome size
Leifsonia xyli subsp. *xyli*	Ratoon stunt	2.6 Mb
Xanthomonas albilineans	Leaf scald	3.7 Mb
Xanthomonas sacchari	?	4.9 Mb

Fungal pathogen	Disease	Genome size
Sporisorium scitamineum	Smut	18.3 Mb
?		

Genome sequencing of pathogens: objectives

Development of molecular diagnostic methods (PCR, RT-PCR, etc.) for the identification and detection of sugarcane pathogens, including genomic variants (no need for whole genome sequence):



Detection of SCYLV genotypes by RT-PCR

Efficient control of pathogens during movement of sugarcane germplasm (quarantine)

Genome sequencing of pathogens: objectives

Furthermore

Whole genome sequencing and whole genome comparisons very useful to understand
- molecular evolution
- molecular ecology
- mechanisms of pathogenicity of sugarcane pathogens:

2 examples

Xanthomonas albilineans and *Sugarcane yellow leaf virus*

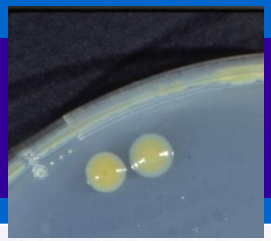
Xanthomonas albilineans

The causal agent of leaf scald,
one of the major diseases of sugarcane





The pathosystem





(R. Birch)

Xanthomonas albilineans

Sugarcane leaf scald disease



End of 19th century (Fiji, Java)

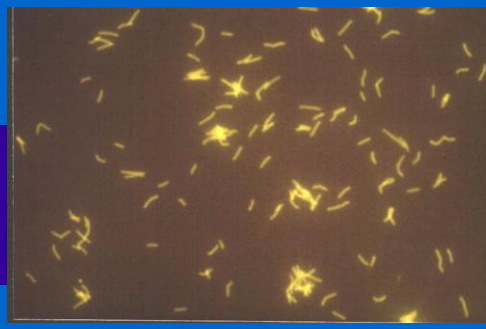
- 1911: First description of the disease in Australia

- control by use of resistant cultivars (*Saccharum officinarum* x *S. spontaneum*)

- 1980s: outbreaks in several locations (Florida, Guadeloupe,…)

Beginning of 21st century (more than 60 countries)

Xanthomonas albilineans



A xylem-invading pathogen



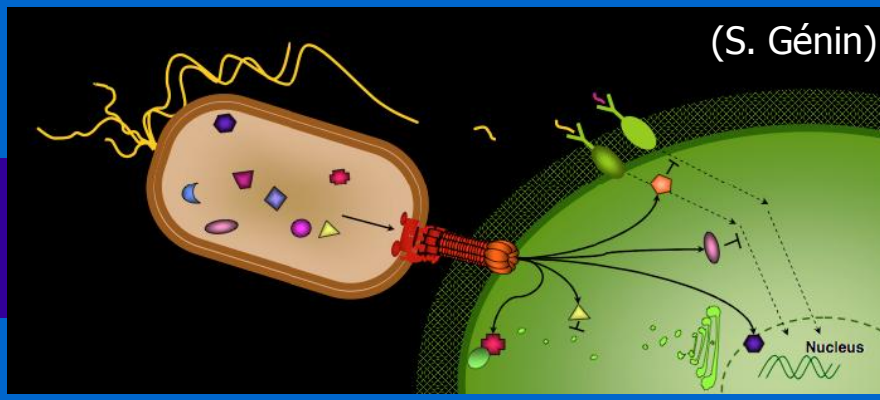




X 262 (Martin *et al.*, 1932)



X. albilineans



An unusual species of *Xanthomonas*....

- *X. albilineans* does not possess an Hrp type III secretion system (only pathogenic xanthomonad without Hrp TTSS)
- *X. albilineans* does not produce xanthan gum (non mucoid on sucrose rich media)
- *X. albilineans* colonies appear in culture after 4-5 days of growth
- Sugarcane can be infected by *X. albilineans* for weeks or months without showing symptoms (latency phase)
- *X. albilineans* produces a unique toxin: albicidin



Pathogenicity of *X. albilineans*: albicidin and "?"

- Pathogenicity of *X. albilineans* is not based on albicidin production only:

Tox- mutants do not produce symptoms, but are still able to colonize the sugarcane stalk



X 262 (Martin *et al.*, 1932)

- What are the other pathogenicity factors of *X. albilineans* ?

- New objective: to sequence the entire genome of *X. albilineans*.... and we did, in 2006

Genome sequence of a pathogenic strain of *X. albilineans* from Guadeloupe

- Genome size: 3,768,695 bp
- 3 plasmids (32, 27 et 25 kb)
- % GC = 63%
- 3115 putative ORFs including 2014 with putative functions
- 83% ORFs conserved in other sequenced xanthomonads
- 522 ORFs that are absent in other sequenced xanthomonads

X. campestris (5.1 Mb)

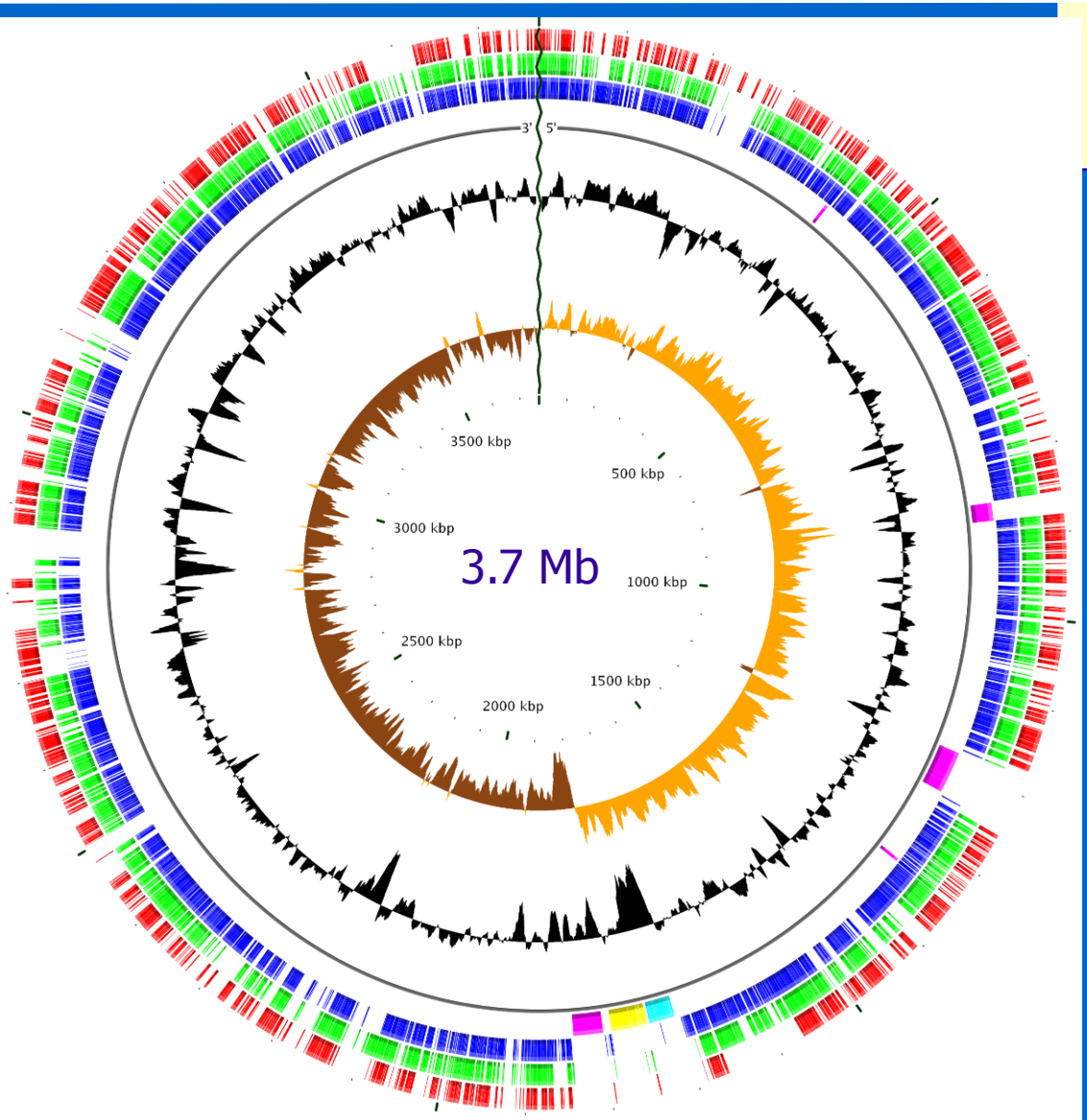
X. citri (5.2 Mb)

X. vesicatoria (5.1 Mb)

X. oryzae (4.9 Mb)

Pieretti et al. 2009. BMC Genomics 10:816

Genome of *Xanthomonas albilineans*



Xylella fastidiosa
Stenotrophomonas maltophilia
Xanthomonas axonopodis* pv. *vesicatoria

TTSS of the SPI-1 family (Salmonella pathogenicity Island-1) mainly found in animal pathogens

Albicidin biosynthesis gene cluster XALB1

NRPS genes involved in biosynthesis of small molecules

Pieretti et al. 2009. BMC Genomics 10:816

The chromosome of *X. albilineans* strain PC73 from Guadeloupe

Identification of new pathogenicity factors of *Xanthomonas albilineans*

What can you do with the genome sequence (functional genomics): Starting 2007, application of two methods to identify new pathogenicity factors:

- Site-directed deletions of candidate genes identified in the genome of *X. albilineans*
- Random Tn*5* (transposon) mutagenesis

Both methods followed by inoculation of sugarcane plants with newly introduced mutations

Identification of new pathogenicity factors of *Xanthomonas albilineans* by transposon mutagenesis

- Random Tn*5* mutagenesis:
  1,216 Tn*5* mutants screened in sugarcane

- 61 mutants affected in pathogenicity (no or less symptoms or no or less colonization of the sugarcane stalk compared to wild type strain)

- Cloning and sequencing of the Tn*5* insertion sites, and identification of the Tn*5* insertion site using the entire genome sequence of *X. albilineans* strain PC73 from Guadeloupe

Identification of new pathogenicity factors of *Xanthomonas albilineans* (Random Tn*5* mutagenesis)

61 mutants affected in pathogenicity:

- 17 mutants in the ribosome machinery
- 44 mutants in 33 loci

Pathogenicity-related loci predicted to encode proteins involved in a variety of functions:

- EPS and LPS biosynthesis (6 loci)
- Fatty acid biosynthesis (2 loci)
- Amino acid biosynthesis (1 locus)
- Purine biosynthesis (1 locus)
- Carbohydrate metabolism (1 locus)
- Nitrogen metabolism (1 locus)
- Regulatory and cell signaling (2 loci)
- Secretion systems (1 locus)
- Transport (5 loci)
- Catalytic activity (1 locus)
- DNA binding (2 loci)
- Drug resistance (1 locus)
- Unknown function (9 loci)

Identification of new pathogenicity factors of *Xanthomonas albilineans* (Random Tn*5* mutagenesis)

Pathogenicity-related loci predicted to encode proteins with unknown functions:

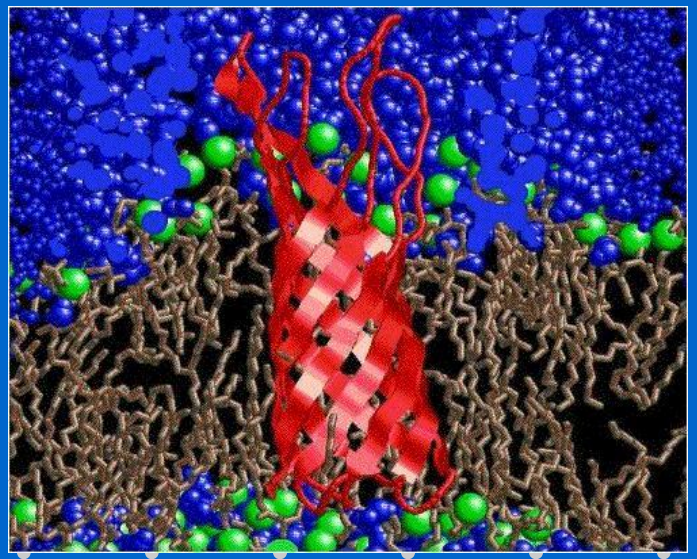
- 6 loci found in other xanthomonads or in other bacterial species
- 2 loci found only in *X. albilineans*
- 1 locus found only in *X. albilineans* and *X. axonopodis* pv. *vasculorum*, another sugarcane pathogen

➢ 5 mutants affected in pathogenicity carried Tn*5* insertions in locus XAL_c0557 of *X. albilineans* predicted to encode an outer membrane protein OmpA (named XaOmpA1)

Role of outer membrane proteins

- Outer membrane structural integrity (Beta barrel with peptidoglycan attachment)
- Nutrient uptake
- Sensing changes in environment
- Porins

OmpA from *E. coli* in a lipid bilayer environment (biop.ox.ac.uk/www/lj2000/sansom/sansom_7.jpg)

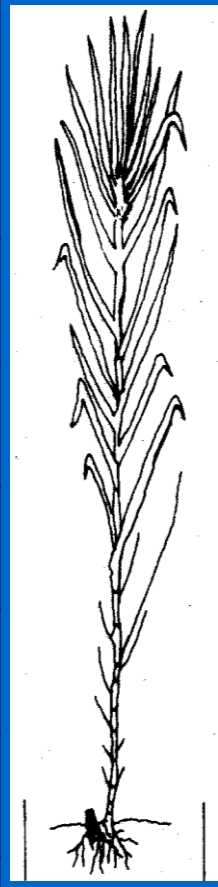






OmpA1 phenotypes in *X. albilineans*

	OmpA1 mutants of Xa	Xa wild type
Symptoms	-	+
Leaf colonization	+	+
Stalk colonization	-	+
Albicidin	+	+
Biofilm	-	+
Motility	-	+



OmpA1 phenotypes in *X. albilineans*



(D. Gargani)

Wild type
X. albilineans



(D. Gargani)

OmpA1 mutant of
X. albilineans

Pathogenicity factors of *Xanthomonas albilineans* as potential targets for genetic-based control methods

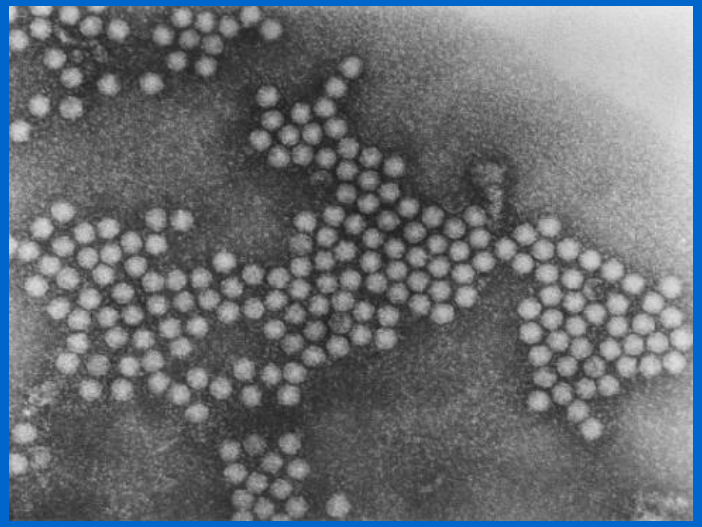
- **Toxin albicidin** (no symptoms but stalk colonization): anti-pathogenesis approach (Birch, 2001), but not a major factor in all strains or under all environmental conditions

- **XaOmpA1** (no symptoms and no stalk colonization): a new and apparently essential fitness factor in *X. albilineans*

- **Surface polysaccharides** (reduced symptoms and reduced stalk colonization)

Sugarcane yellow leaf virus

The causal agent of yellow leaf of sugarcane



SCYLV particules (B. Lockhart)



Yellow leaf of sugarcane

- First described in Hawaii in 1989 (Yellow leaf syndrome)
- Yellow wilt in East Africa in the 1970s?
- Symptoms non specific and unreliable for diagnosis
- Severe yield losses in Brazil in the 1990s (cv. SP71-6163)
- Found in most sugarcane producing countries in 2012
- Transmitted through setts and aphids



Yellow leaf of sugarcane





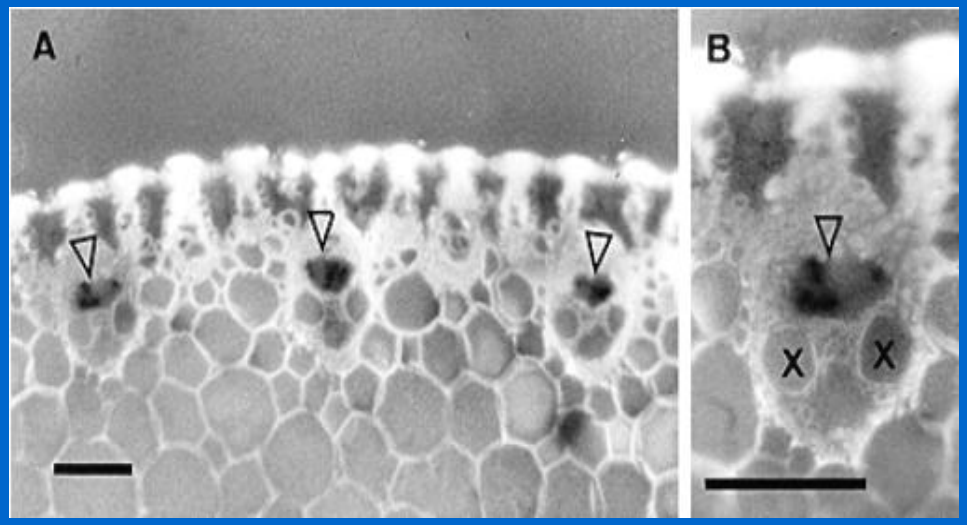
(J. Daugrois)

Melanaphis sacchari

Yellow wilt, Zimbabwe, 1969
(Zimbabwe Sugar Association)

The causal agent of yellow leaf

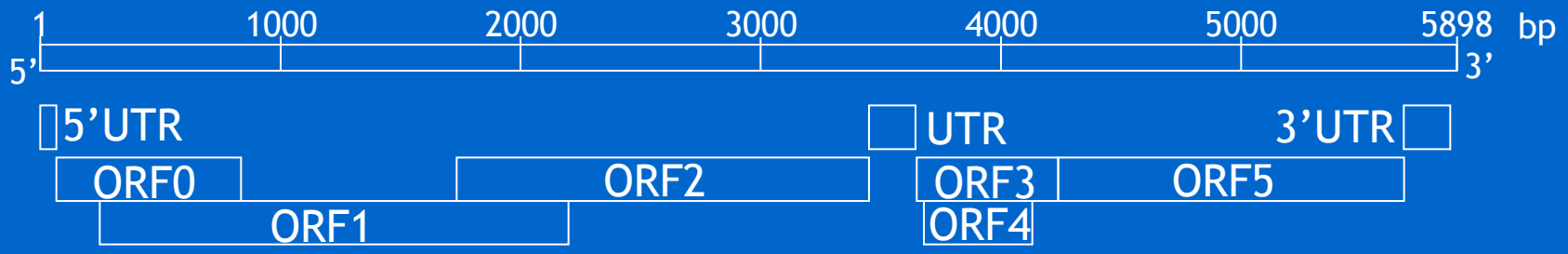
- *Sugarcane yellow leaf virus* (SCYLV)
- A polerovirus of the *Luteoviridae* family
- Occurrence of genetic diversity within the virus (Borg & coll., 2001; Moonan & Mirkov, 2002; Abu Ahmad & coll., 2006)



Location of virus particules in phloem vessels (from Vega & coll., 1997)

Genome organization of SCYLV

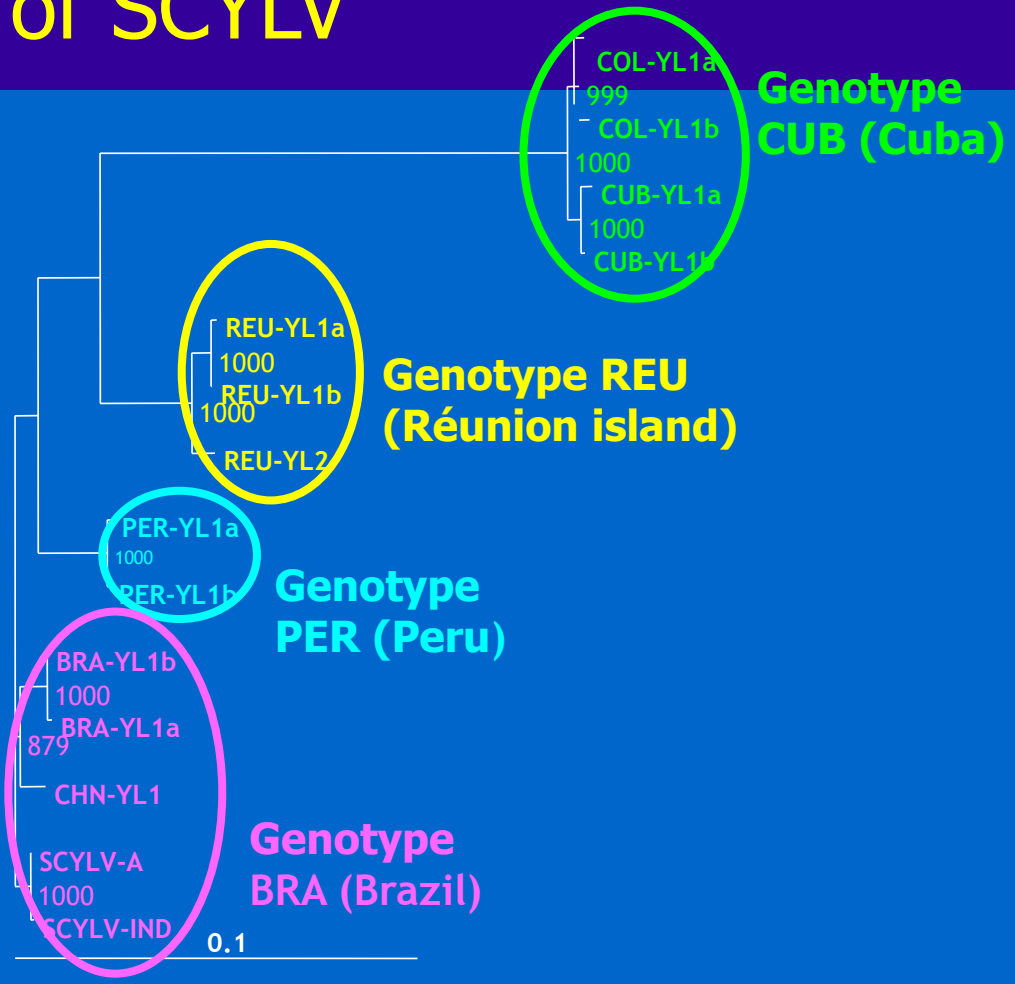
Monopartite, positive-sense single stranded RNA of 5,898 nucleotides (6 ORFs)



ORF0 = suppressor of post-transcriptional gene silencing (symptom expression)
ORF1 = multifunction protein (serine proteinase and putative genome-linked viral protein)
ORF2 = RNA-dependant RNA-polymerase
ORF3 = capsid protein
ORF4 = movement protein
ORF5 = read-through protein associated with virus transmission by aphids

Genetic diversity of SCYLV

Comparison of whole genomes of SCYLV isolates: Identification of four genetic groups
(Abu Ahmad et al., 2006)



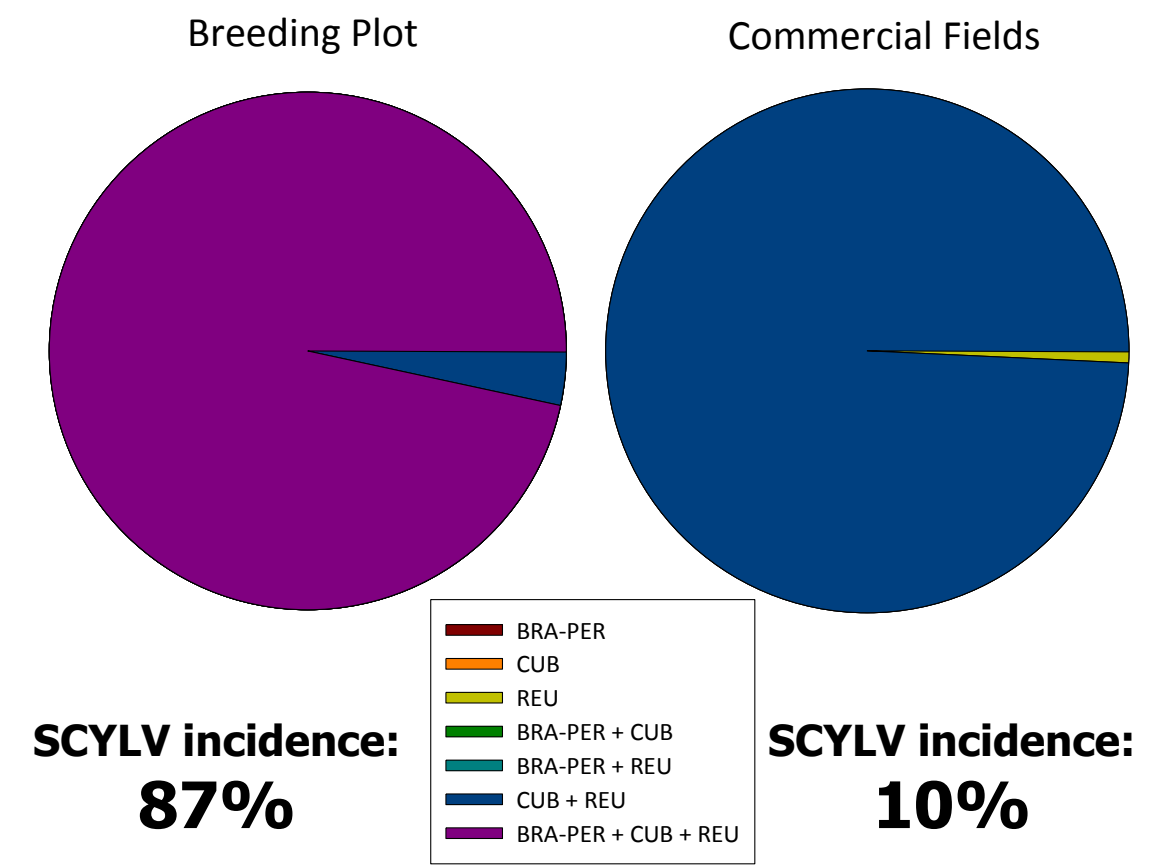
Two other genotypes (IND and CHN) reported in India (Viswanathan et al., 2008) and in China (Wang and Zhou, 2010), respectively

Variability in pathogenicity of SCYLV

- Differences in the capacity of producing yellowing symptoms:
  - Genotypes BRA-PER produced more yellow leaf symptoms in Guadeloupe and Réunion than REU
- Differences in the capacity to infect plants:
  - Genotype CUB better transmitted than other genotypes (Guadeloupe)
  - Intra-genotype variations suspected: Genotype REU transmitted to sugarcane cultivar R570 in Guadeloupe but not in Réunion

Impact of genetic diversity of pathogens on management of sugarcane diseases :

Knowing the genetic diversity of SCYLV (or other viruses/pathogens) in a given location is essential for efficient screening of resistant plant material



SCYLV incidence and genotype distribution in Guadeloupe (FWI)

Genome sequencing and the discovery of unknown or undescribed pathogens (especially viruses)

Several virus pathogens of sugarcane are already known and extensively studied

HOWEVER:

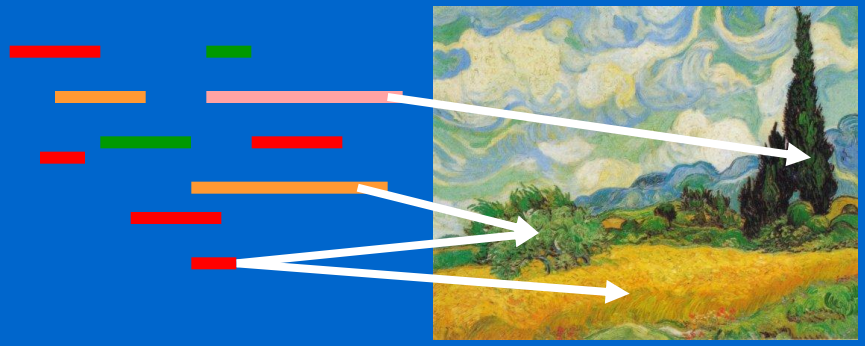
It is assumed that unknown pathogens are still to be discovered, especially if these pathogens do not cause symptoms that can be easily observed (such as SCYLV)

Metagenomics



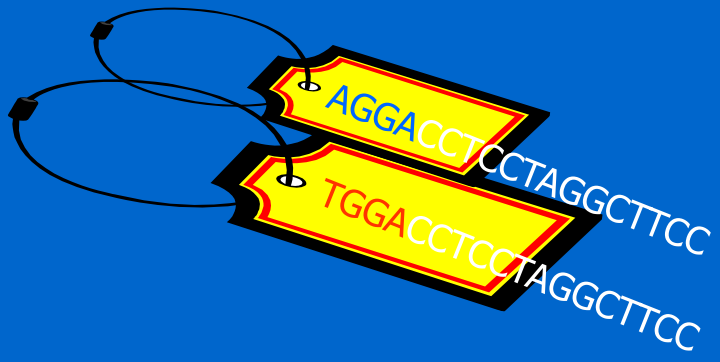
- Metagenomics = the study of genetic material recovered directly from environmental samples and a study of collective genomes of microorganisms (as opposed to clonal culture)

From metagenomics to ecogenomics



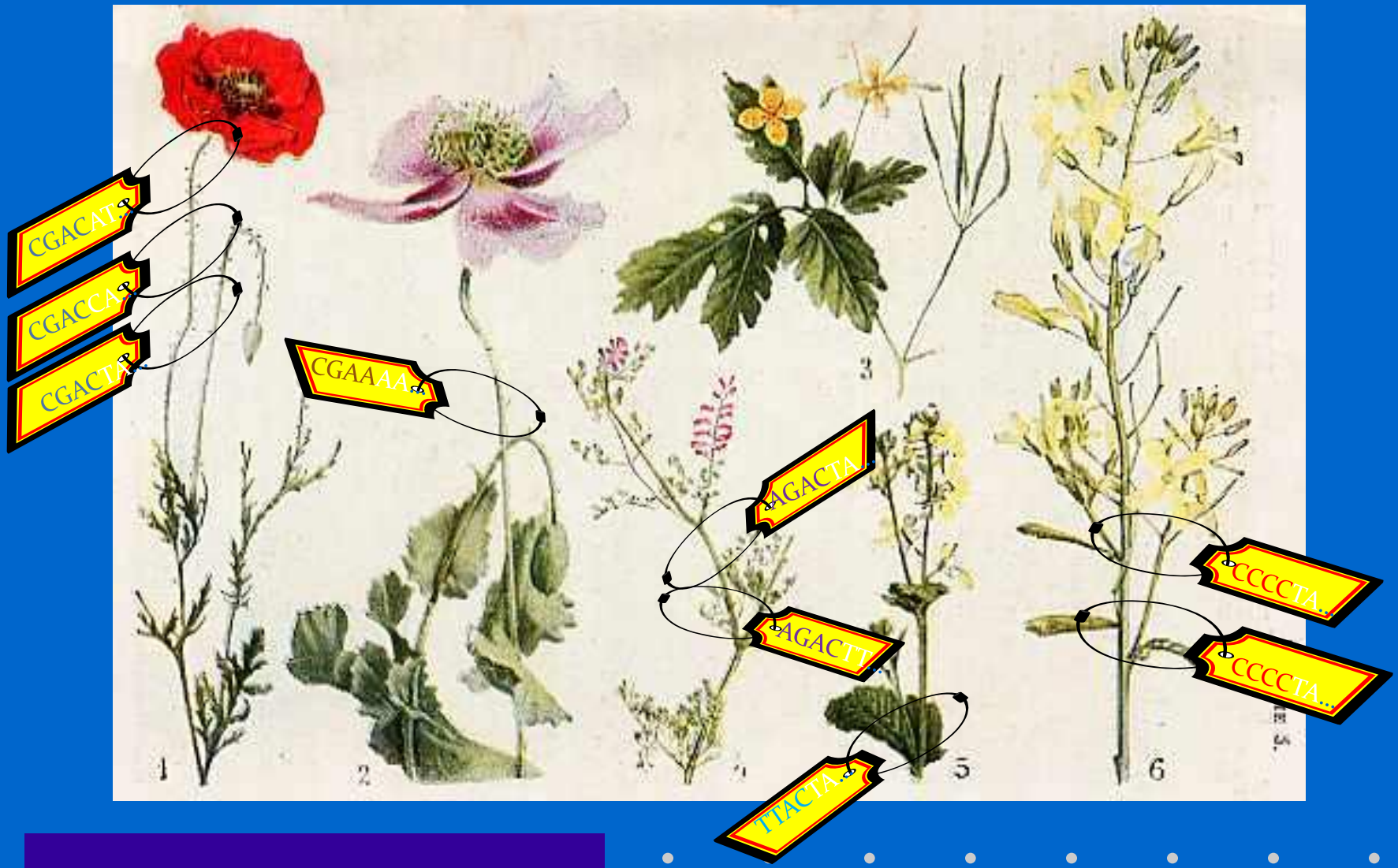
5'AGAGCCTTCGGATCCTCC3'	5'GAGACCTTCGGATCCTCC3'
5'ACTCCCTTCGGATCCTCC3'	5'GTGTCCTTCGGATCCTCC3'
5'AGTGCCTTCGGATCCTCC3'	5'GACACCTTCGGATCCTCC3'
5'AAGGCCTTCGGATCCTCC3'	5'GTCTCCTTCGGATCCTCC3'
5'ACACCCTTCGGATCCTCC3'	5'GATCCCTTCGGATCCTCC3'
5'CACACCTTCGGATCCTCC3'	5'TCTCCCTTCGGATCCTCC3'
5'CTCTCCTTCGGATCCTCC3'	5'TGTGCCTTCGGATCCTCC3'
5'CAGACCTTCGGATCCTCC3'	5'TCTGCCTTCGGATCCTCC3'
5'CTGTCCTTCGGATCCTCC3'	5'TCACCTTCGGATCCTCC3'
5'CAGACCTTCGGATCCTCC3'	5'TGAGCCTTCGGATCCTCC3'

The steps:
1/ Viral extraction for each sample
2/ Viral tagged amplification for each sample (random PCR)
3/ Pooling of samples (up to 96 samples)
4/ Sequencing
5/ Deconvolution and assignement of tagged samples
6/ Sequence blasting and identification of virome for each sample

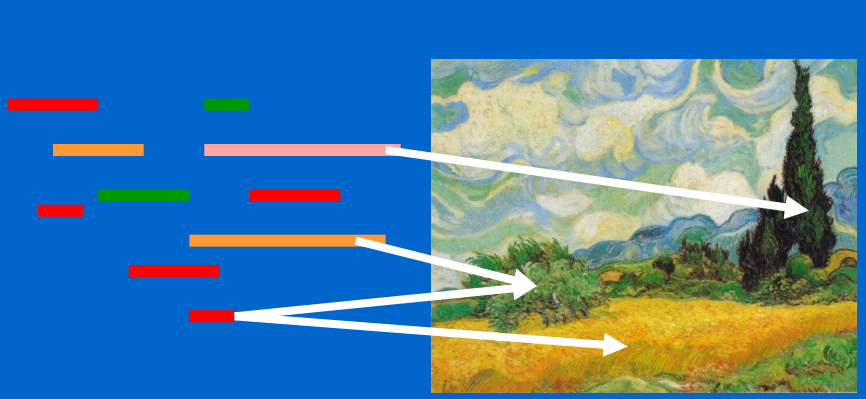


Roossinck *et al*. 2010. *Molecular Ecology*. 19: 81-88

From metagenomics to ecogenomics



From ecogenomics to meta-pathogenomics of sugarcane







- Expected input for the management of sugarcane diseases :

  - In the field: prediction of emerging diseases

  - In Quarantine: simultaneous and sensitive detection of a large variety of pathogens

Conclusion and perspectives

- Genome sequencing resulted in great improvement of our knowledge of host-pathogen interactions, from the pathogen (pathogenicity factors) but also the host side (resistance genes)

- Sequencing data yielded information that allowed implementation of molecular diagnostic assays and better management of sugarcane diseases

- Further improvement still to come, from metagenomic studies to genetic-based control methods (GMO, molecular probes to detect plant resistance genes, etc.)

Conclusion and perspectives

- Phylogeny, diversity and evolution : emergence and re-emergence of diseases (to predict evolution of pathogens by genome comparison: Why can a plant resistance gene be surrounded or not by a pathogen?)

- Genome diversity to further improve molecular diagnostic tools (Rapid and accurate detection of pathogens) and to screen plants for the appropriate race/strain of the pathogen

- To understand the molecular dialogue between the pathogen and the plant (to identify targets for the development of control methods): virulence factors (effectors, etc.) versus resistance mechanisms

ACKNOWLEDGMENTS

Abu Ahmad Y., Rassaby L., Royer M., Borg Z., Braithwaite K.S., Mirkov E., Irey M., Perrier X., Smith G., Rott P., 2006. Yellow leaf of sugarcane is caused by at least three different genotypes of sugarcane yellow leaf virus, one of which predominates on the Island of Réunion. Archives of Virology 151:1355-1371.

Abu Ahmad Y., Costet L., Daugrois J.-H., Nibouche S., Letourmy P., Girad J.-C., Rott P., 2007. Variation in infection capacity and in virulence exists between genotypes of *Sugarcane yellow leaf virus*. Plant Disease 91:253-259.

Daugrois J.-H., Edon-Jock C., Bonoto S., Vaillant J., Rott P., 2011. Spread of *Sugarcane yellow leaf virus* in disease-free sugarcane plots is linked to rainfall and host resistance in the humid tropical environment of Guadeloupe. European Journal of Plant Pathology 129:71-80.

Pieretti I., Royer M., Barbe V., Carrere S., Koebnik R., Cociancich S., Couloux A., Darrasse A., Gouzy J., Jacques M.-A., Lauber E., Manceau C., Mangenot S., Poussier S., Segurens B., Szurek B., Verdier V., Arlat M., Rott P., 2009. The complete genome of *Xanthomonas albilineans* provides new insights into the reductive genome evolution of the xylem-limited *Xanthomonadaceae.* BMC Genomics 10:616.

Rott P., Fleites L., Marlow G., Royer M., Gabriel D.W., 2011. Identification of new candidate pathogenicity factors in the xylem-invading pathogen *Xanthomonas albilineans* by transposon mutagenesis. Molecular Plant-Microbe Interactions 24:594-605.

Rott P., Marguerettaz M., Fleites L., Cociancich S., Girard J.C., Pieretti I., Gabriel D.W., Royer M., 2011. Unravelling pathogenicity of *Xanthomonas albilineans*, the causal agent of sugarcane leaf scald. International Sugar Journal 113(1351):490-496.

And all colleagues and institutes who contributed to this work/talk


THANK YOU