

Regulation of HGT by natural transformation in *Ralstonia solanacearum*

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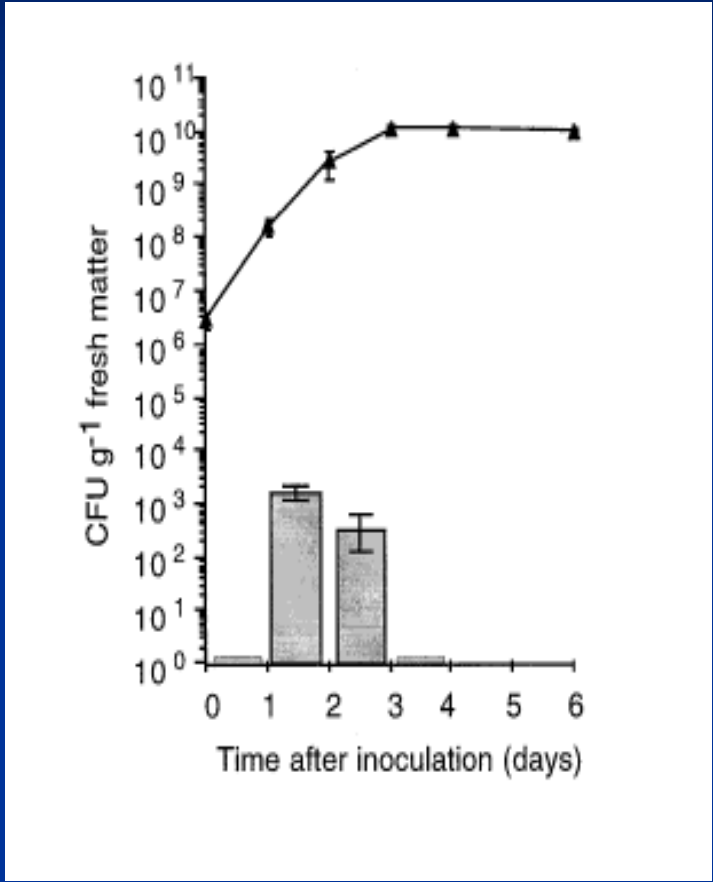
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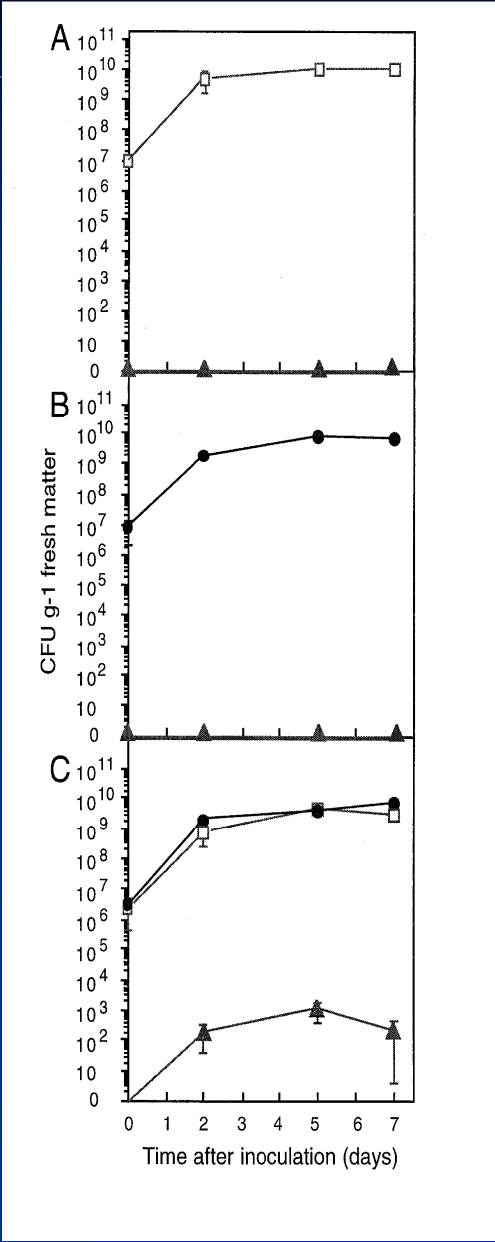
Ralstonia solanacearum

- ❖ A β -proteobacterium, a plant pathogen
- ❖ Unusually wide host range with over 200 host plant species
GMI1000 is a naturally transformable bacteria (Bertolla et al., 1997)
- ❖ Develops competence in plant tissues and transformation *in situ*





In situ Competence



In situ HGT



Horizontal Gene Transfer

Origin of donor DNA ?

Two types of HGT in bacteria

Frequency ?

« Qualitative » regulation ?

« Quantitative » regulation ?

Impact?



Origin of donor DNA ?

- **Foreign genes**

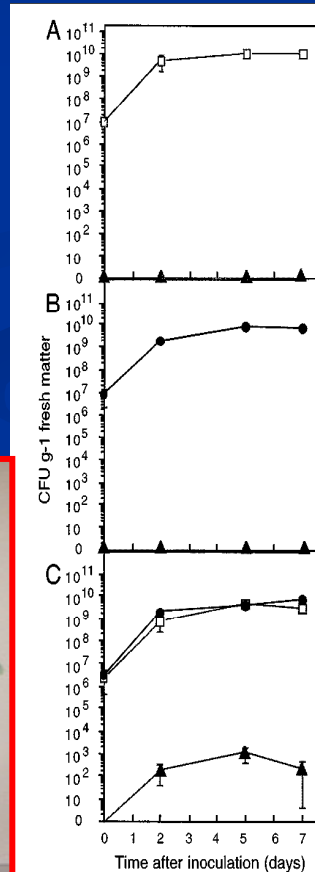
Illegitimate recombination
Low frequency
Strong impact

DNA sequences and bioinformatics tools

- **DNA from more or less closely related cells**

Homologous (homeologous) recombination
High frequency
Impact?

Experimental approaches



I. Detection of foreign genes in *Ralstonia solanacearum* strain GMI1000.

Nature, vol. 415, 2002

articles

Genome sequence of the plant pathogen *Ralstonia solanacearum*

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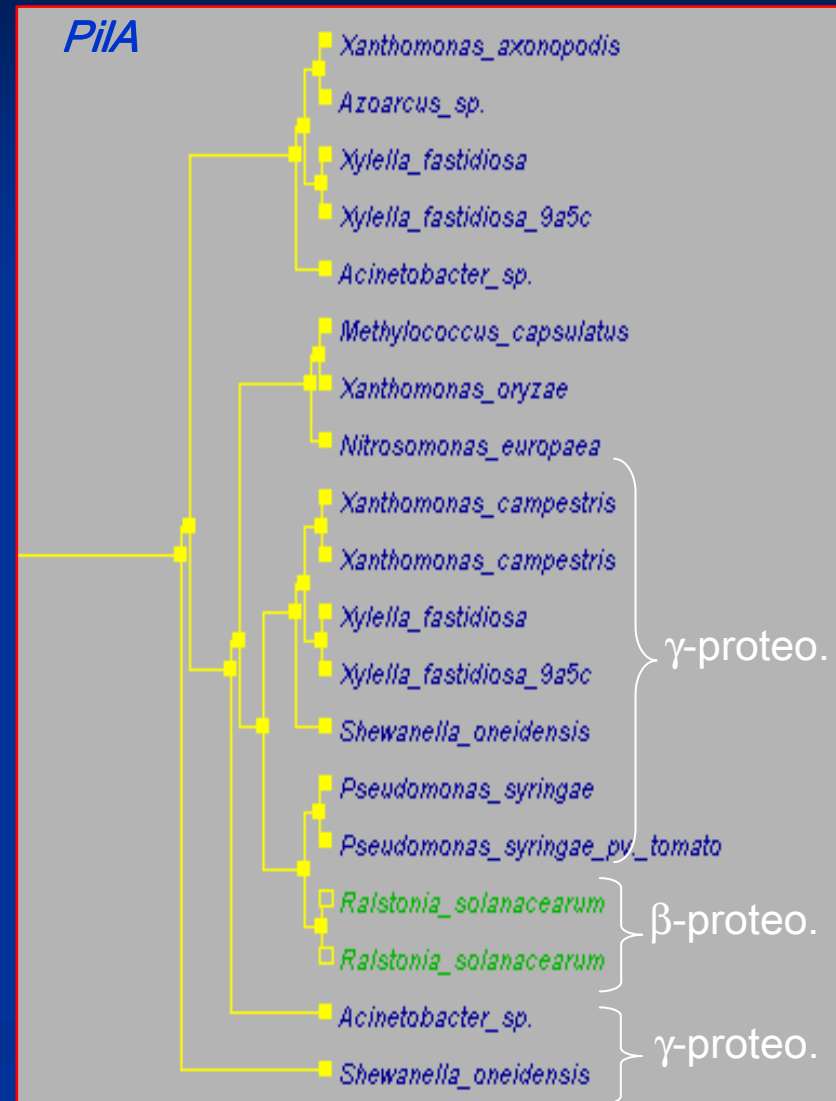
Detection of recently acquired genes

A	Selection of homologous gene families	HOBACGEN (Perriere et al. 2000)	homologous families <i>R. solanacearum</i> β -proteobacterium
B	Alignment and filtration	GBLOCKS (Castresana 2000)	homologous families <i>R. solanacearum</i> β -proteobacterium
C	Neighbor-joining trees	QuickTree (Howe et al. 2002)	
D	Phylogenetic incongruency	Manual check	Identification of donors
E	List of genes completed by two indices of codon usage : the codon adaptation index (CAI) and the χ^2		



Bio-informatics detection of HGT

- About 1139 phylogenetic trees examined
- 151 incongruencies (13.3%) of tested genes
- An example of HGT is represented by the *pilA* gene coding for type 4 fimbrial pilin.



II. The second type of HGT

Ralstonia solanacearum transformed

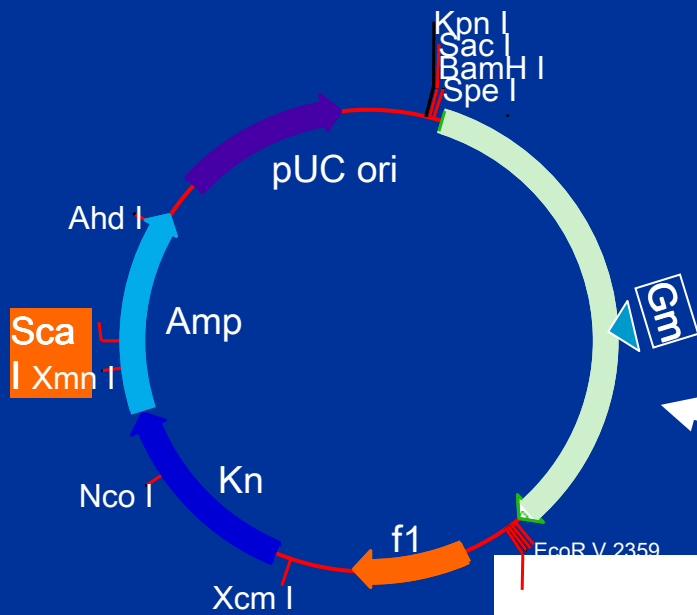
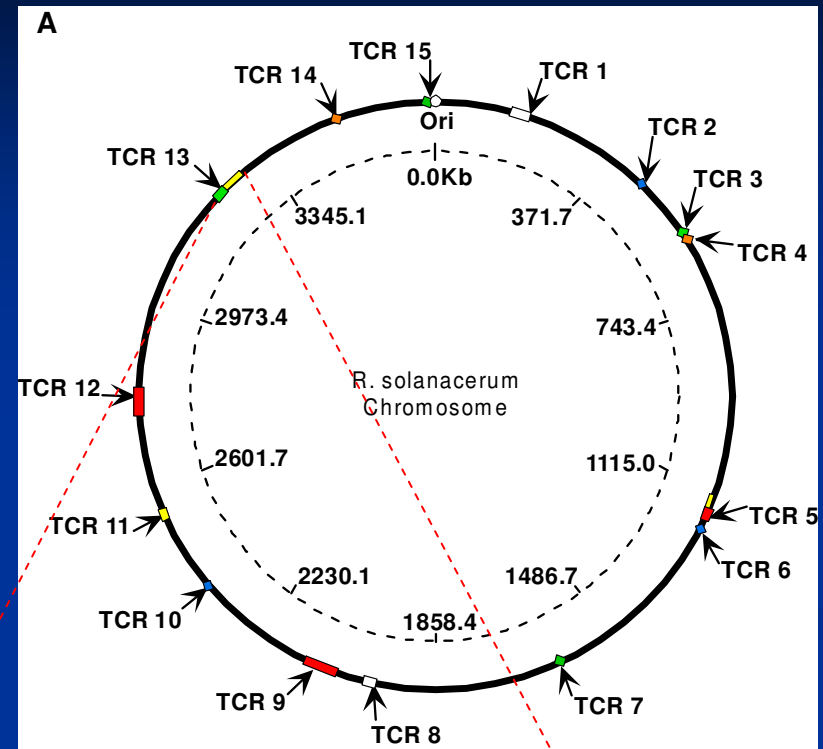
by its own DNA (homologous DNA)

by DNA from more or less closely related strains



Cloning of target chromosomal regions (TCR)

- ❖ 18 regions selected (chromosome and megaplasmid)
- ❖ PCR amplification of a 2Kb fragment
- ❖ Cloning in Topo vector
- ❖ Labeling the 2Kb insert with a Gm cassette



TCR 13



2 kb



Selection of genomic regions according to :

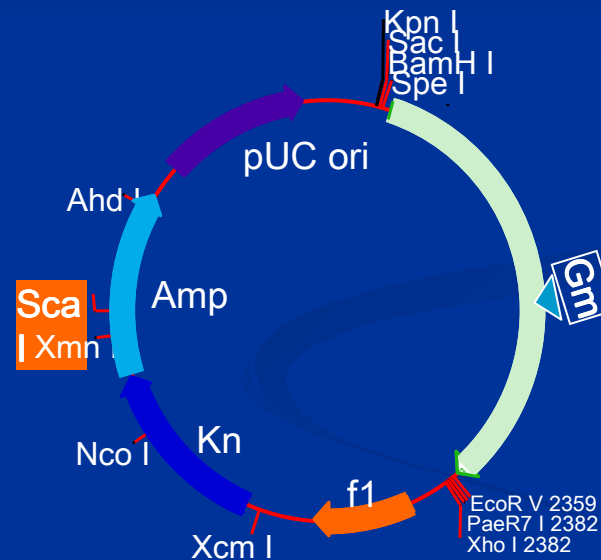
- ❖ Diversity of gene functions :
 - recently acquired genes determined by bioinformatics tools
 - Mobile elements (IS; Tn, phage)
 - house keeping genes
 - genes associated with other cell functions
- ❖ Location in the genome



Transformation tests

- Recipient strain : GMI1000
- Donor DNA

Plasmid DNA



Chromosomal DNA from Gm tagged strains

Gm

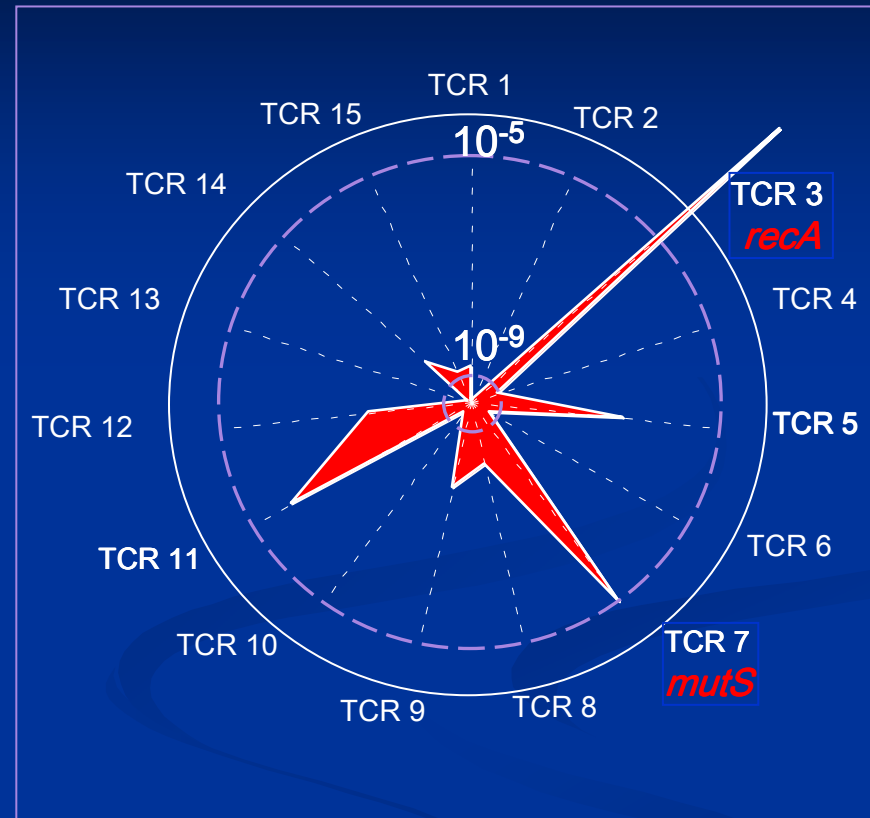


TCR 13



Transformation frequency with plasmid DNA

- ❖ the different genomic regions did not recombine at the same frequency
- ❖ average recombination frequency : 3.9×10^{-6}
- ❖ recombination frequencies of TCR3 and TCR7 were more than 200-fold higher than TCR2 and TCR13
- ❖ TCR3 and TCR7 regions (*recA* and *mutS*) as transformation recombination hot spots.
- ❖ ACURs, IS, Tn and potential genes recently acquired do not exhibit a high frequency recombination rate

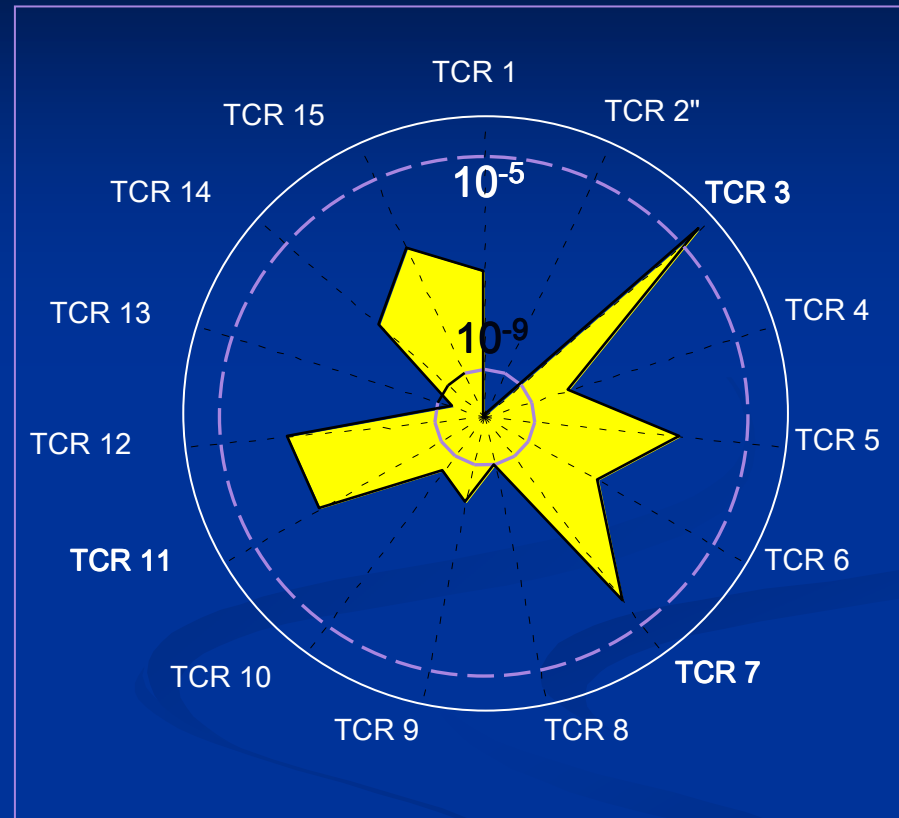


Recombination frequency of the fifteen target regions. Donor DNA: Recombinant plasmids
Recipient DNA: Chromosome of GMI1000



Transformation frequency with genomic DNA

- ❖ The average recombination frequency : 4.8×10^{-6}
- ❖ Difference of one order of magnitude between the lowest (TCR13) and the highest recombination frequency (TCR3)
- ❖ Confirmation that :
the different genomic regions did not recombine at the same frequency



Recombination frequency of selected regions.
Donor DNA: genomic DNA from tagged GMI1000 strains
Recipient strain: GMI1000



Transformation with homeologous or heterologous DNA

Donor DNA: The same 18 tagged DNA (*R. solanacearum* GMI1000)

Recipient bacteria

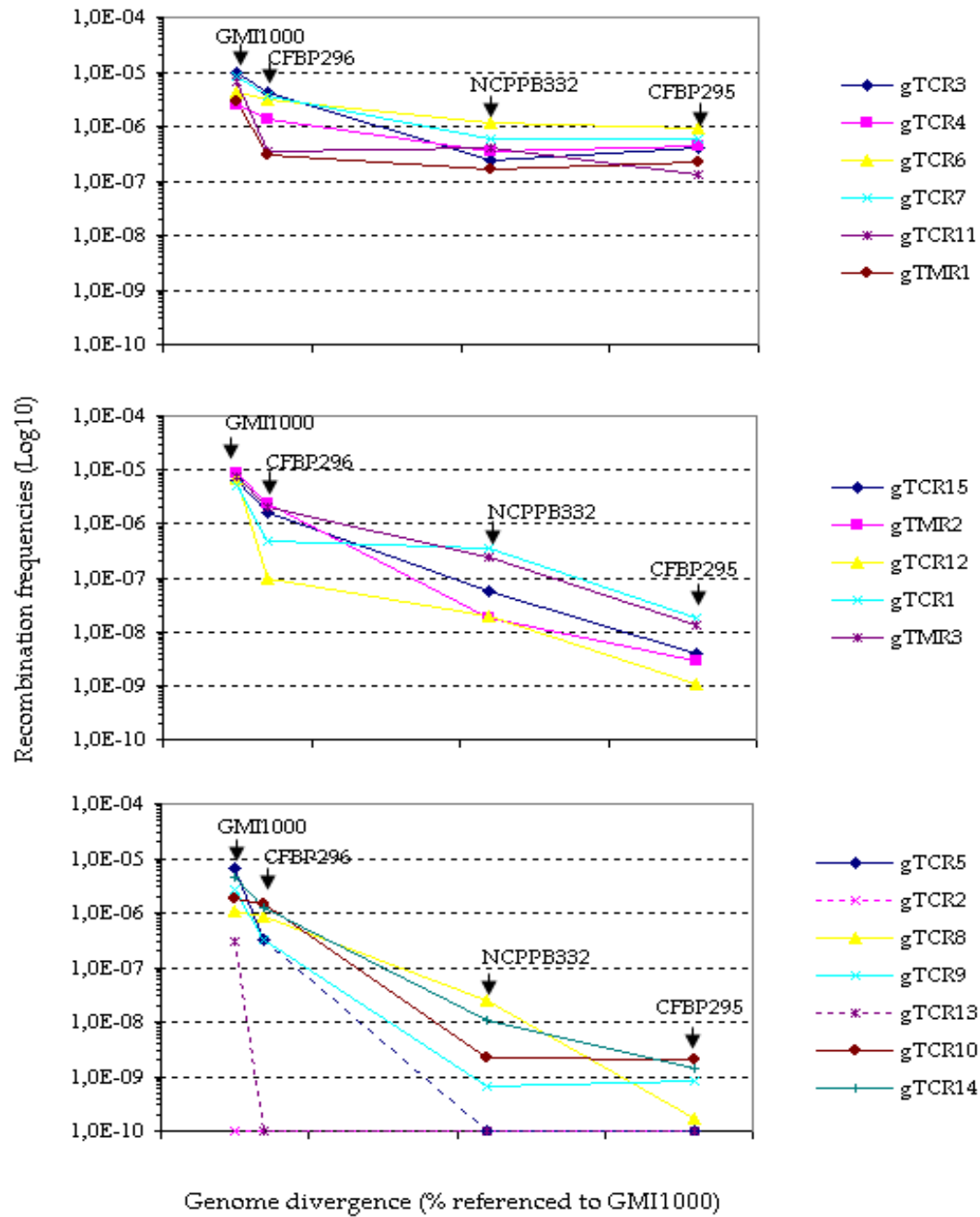
R. solanacearum

CFBP2968, 98%

NCPPB332, 81%

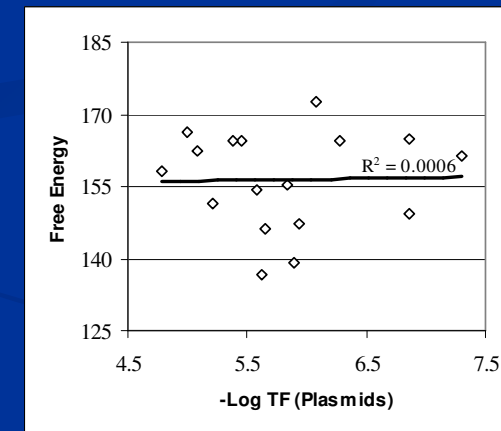
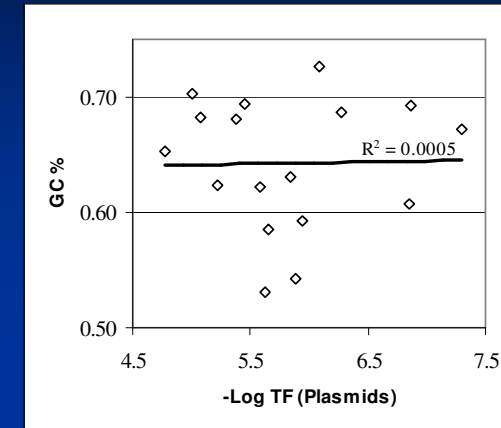
CFBP2957, 69%





Correlation with DNA physical factors

- ❖ Physical parameters : (GC skew, GC%, Free-energy)
- ❖ Lack of linear correlation with physical parameters characterizing the DNA fragments

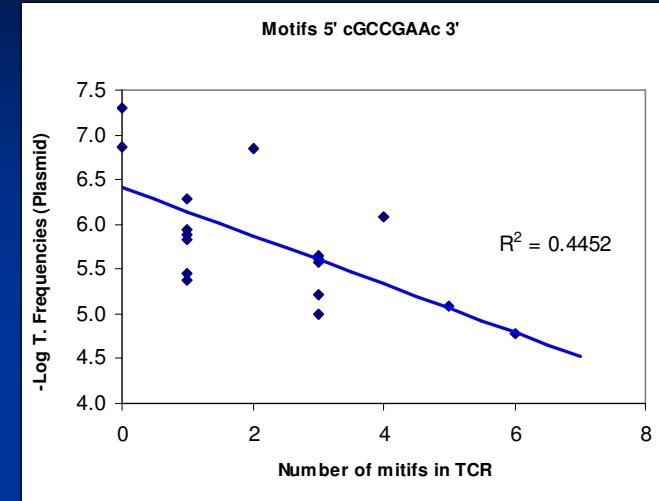


Can the DNA sequence explain variations in transformation frequency ?

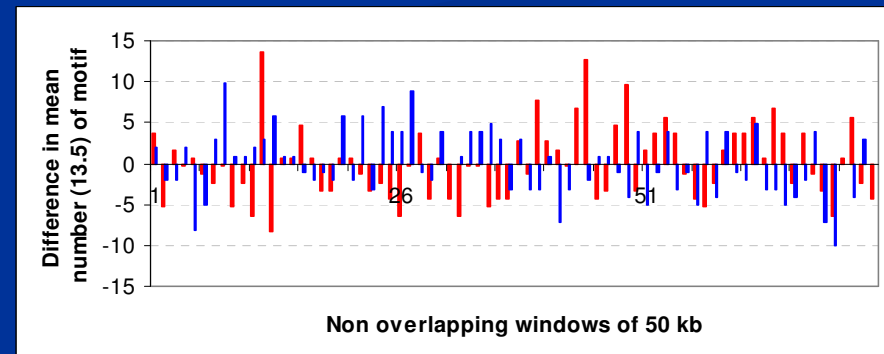


Motifs in DNA fragments

- ❖ Detection of motifs sequence (5' cGCCGAAC 3') in most of DNA fragments
- ❖ Significant correlation between number of motifs and recombination frequency (Spearman correlation)
- ❖ *recA* contained the higher number of motifs (6)



- ❖ Motifs : over-represented in the genome (2027 motifs in two replicons)
- ❖ Shift in the motif orientation bias around the replication origin and terminus



These motifs Chi sequences
role in recombination



Conclusions:

Homologous recombination as the critical step that regulates gene acquisition
(*No need of specific binding sequences for the donor DNA to be processed into the cell*)

Various genomic positions of the genome transformed the recipient strains at markedly different frequencies.

Differences in transfer frequency are only related to the nucleotide sequence of the DNA regions on which homologous recombination occurs.

recA and *mutS* gene-containing positions as natural transformation (and certainly homologous recombination) “hot spots” .

Chi-like (‘5-cGCCGAAc-3’) sequences were detected that might explain differences in homologous and homeologous recombination frequencies.



Conclusions (cont.)

In *E. coli*, Chi sequences are recombinational hotspots at which enzymes bind preferentially to repair damaged DNA

The ends of the broken DNA on double strands are processed by the multi-functional enzyme complex RecBCD, involving successively a helicase activity to split the duplex into its component strands and a nuclease activity to digest them.

At a Chi site, the nuclease activity is attenuated and the RecBCD loads RecA onto the 3' tail of the DNA to initiate recombination.

(*R. solanacearum* possesses *addAB* genes having the same functions as *recBCD* in *E. coli*)

The foreign DNA acquired by HGT could be perceived by recipient cell as damaged DNA and processed by the same enzymes.



Conclusions (cont.)

The genes in a bacterial genome do not exhibit the same *sensu stricto* potential to be transferred even into a new isogenic host.

The Chi-like sequences in *R. solanacearum* could be key components of the adaptation potential by permitting the cell to regulate the gene acquisition process.

Chi-like sequences strongly limit the influence of sequence divergence, which usually decreases recombination efficiency dramatically

DNA exchange frequency for some DNA positions remains very high in spite of a significant overall genomic divergence at a level that led to classifying these strains as two separate genomic species

Species boundaries in bacteria? Strength of biological barriers to regulate DNA exchange?

Difficulty to adapt a bacterial species concept that would be based on genomic coherence between members of a same species sharing an exclusive common gene pool



The fundamental evolutionary question is to know if this property was specifically selected to increase adaptation potential or if it is the side effect of cellular mechanisms in charge of DNA repair.

The « spandrel » effect ?

« Spandrel »

this term refers to the adaptive use of a function selected for another purpose

Gould and Lewontin, 1979



Two complementary HGT-based strategies in *R. solanacearum*

- Acquisition of DNA from more or less closely related cells.
 - Maintaining stability and integrity in some important DNA positions, (DNA homogenization reducing the risk of genetic drift)
 - Spreading beneficial mutations among the population.
 - High frequency and Regulation
- Acquisition of foreign genes
 - Very low frequency
 - Regulation?
 - Result from the combination of several events happening mainly by chance (Ecology of bacteria, hypothetical contact, DNA uptake, integration, rearrangement etc.)

Role in adaptation and evolution ???



Additional questions:

if the natural transformable property is ubiquitously shared among strains belonging to the four *R. solanacearum* phylotypes

the number and size of DNA fragments that can be transferred in *R. solanacearum* during natural transformation.



III. Natural transformation potential in *R. solanacearum* isolates.

Selection of 55 isolates (among the four phylotypes).

Selection of a transformation protocol (limiting growth conditions)

Selection of donor DNA: Replicative plasmids, integrative plasmids, chromosomal fragments.

Transformation-mediated acquisition of the gentamicin resistance phenotype detected in 80% (43/55) of the *R. solanacearum* strains tested.

Detection of transformable strains in each phylotype provides strong evidence that competence is an ubiquitous physiological trait in the *R. solanacearum* species complex.



Transformation frequencies varied over more than four orders of magnitude between strains (from 3.8×10^{-6} to 3.8×10^{-10})

Recombination mediated DNA integration being more efficient than plasmid replication.

- Level of physiological competence highly variable among strains ?
- Experimental biases (transformation protocol)?
- Activity of the methyl mismatch repair (MMR) system (decreased efficiency of the recombination process with donor DNA sequences diverging significantly from those of the recipient genome)?



Number and size of DNA fragments that can be transferred in *R. solanacearum* during natural transformation.

IV. Multiple integration into the *R. solanacearum* genome

- Transformation of the recipient strain with a mixture of two integrative plasmids containing the *recO* and *ftsK* genes distantly separated by 1.5Mb on the GMI1000 chromosome.
- Isolation of transformants resistant to both marker genes indicating that two independent recombination events occurred simultaneously in separate regions in the genome.

Frequency up to three orders of magnitude lower than for one single transfer event by one DNA source.

No triple transformant was detected when a mixture containing three different plasmid or chromosomal DNAs was used.



Number and size of DNA fragments that can be transferred in *R. solanacearum* during natural transformation.

V. Determination of the size of transferred DNA in *R. solanacearum* GMI1000

- Construction of mutant RS28 by successive integrations of four antibiotic resistance cassettes, in the four genes *recO*, RSc1152, *nagl* and *comA*, respectively.
- Transformation tests with RS28 genomic DNA as donor and strain GMI1000 as recipient.
- Selection of transformants resistant to 1, 2, 3 or 4 antibiotics.



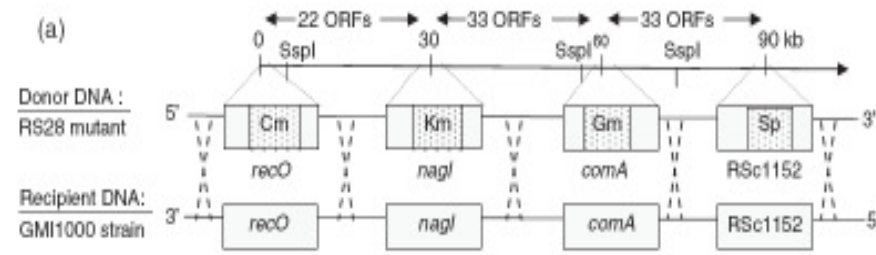
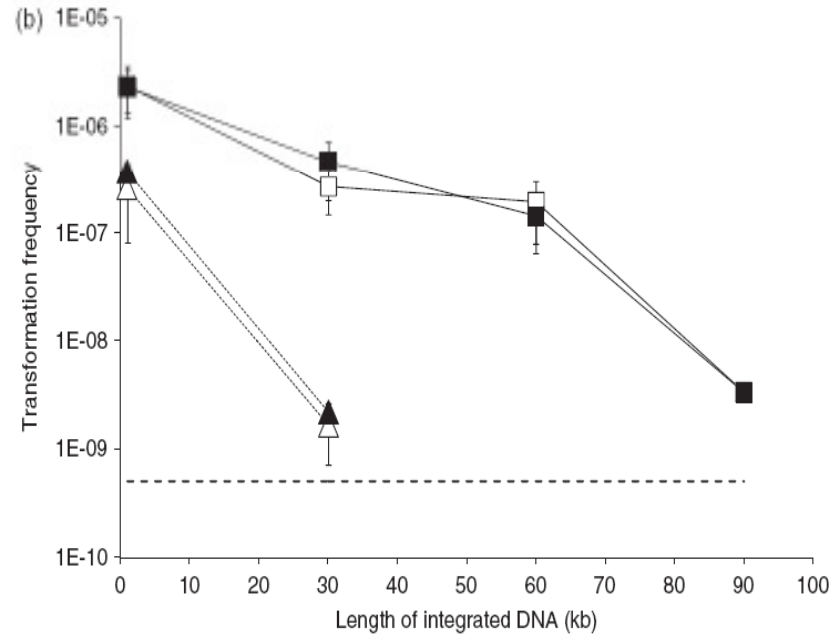


Fig. 1. Relationship between natural transformation frequencies and length of exogenous DNA integrated in the *Ralstonia solanacearum* genome. (a) Locations of possible recombination events between the RS28 mutant and the GMI1000 strain. Crosses represent the possible sites of crossover between the GMI1000 and the RS28 DNA. (b) Correlation between the length of exchanged DNA between the RS28 DNA and the GMI1000 strain and transformation frequencies. Solid and dotted lines represent transformation frequencies obtained with genomic DNA or *SspI*-digested genomic DNA from the RS28 mutant, respectively. Closed and open squares or triangles indicate the combination of selective antibiotics used to select the transformant, 5'-3' or 3'-5' direction, respectively. Horizontal dotted line represents the limit of detection.



At least 90 kb of transforming DNA can be exchanged between the transforming DNA and the GMI1000 genome.



This gene replacement involves up to 88 ORFs or 2% of the recipient genome.

Substitutive recombination might, however, have little effect on the genome plasticity of the *R. solanacearum*, as this gene replacement is more probably gene conversion than acquisition of new properties.

Single nucleotide polymorphisms in converted genes could significantly modify the bacterial phenotype?

Among these 88 ORFs, six and eight genes are implied in metabolism and are essential for *B. subtilis* and *E. coli* growth.

Could facilitate the repair of damaged DNA by the replacement of nonfunctional genes.

The replacement of a nonfunctional *mutS* copy is in agreement with the hypothesis involving HGT as a mechanism for *mutS*-negative mutators to reacquire a functional *mutS* copy to return to a more stable wild-type phenotype (Denamur et al., 2000).



General Conclusions

- Competence is an ubiquitous physiological trait in the *R. solanacearum* species complex.
- Possibility of multiple independent DNA integrations.
- Possibility of acquisition of long DNA fragments (replacement of a significant proportion of the genome).
- R. Solanacearum* uses the 2 types of HGT (foreign genes and its own DNA). Frequency?
- Various genomic positions of the genome transformed the recipient strains at markedly different frequencies. Recombination (hot spots) with a putative role of Chi-like sequences.





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•Coupat B, Chaumeille-Dole F, Fall S, Prior P, Simonet P, Nesme X and Bertolla F Natural transformation in the *Ralstonia solanacearum* species complex: number and size of DNA that can be transferred. FEMS Microbiol Ecol (2008) (in press)

