Tn-phyto: essential genomes of nine bacterial phytopathogens

Julie Baltenneck, Ben Moussa, Loïc Couderc, Areski Flissi, Jacques Pédron, Cindy E. Morris, Marie-Anne Barny, Odile Berge, Hélène Touzet, Erwan Gueguen, et al.

TN-PHYTO: ESSENTIAL GENOMES OF NINE BACTERIAL PHYTOPATHOGENS

Baltenneck J.[1], Ben Moussa H.[2], Couderc L.[3], Flissi A.[5], Pédron J.[2], Morris C.E[4], Barny MA.[2], Berge O.[4], Touzet H.[5], Gueguen E[1*], **Condemine G***[1].

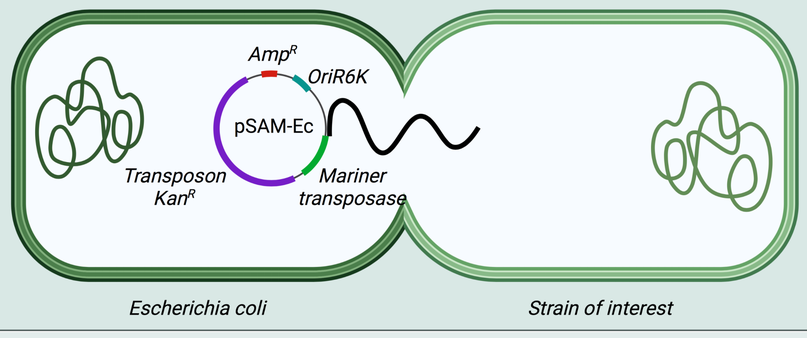
[1] *University of Lyon, Université Claude Bernard Lyon 1, INSA of Lyon, MAP CNRS UMR 5240, 69622 Villeurbanne, France*
[2] *Sorbonne Université, INRAE, Institute of Ecology and Environmental Sciences-Paris, 4 place 7 Jussieu, F-75 252 Paris, France.*
[3] *University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, US 41 - UMS 2014 - PLBS, Lille, France.*
[4] *INRAE, Pathologie Végétale, 84140, Monfavet, France*
[5] *University of Lille, CNRS, Centrale Lille, UMR 9189 - CRIStAL - Centre de Recherche en Informatique Signal et Automatique de Lille, Lille, France.*

The aim of our project is to identify virulence factors of phytopathogenic bacteria isolated from the environment as well as those allowing their survival in water. For this purpose, we use Tn-seq (2), an innovative mutagenesis technique coupled with high throughput sequencing chosen to rapidly identify, on a large panel of strains, genes necessary for growth in a given condition, the plant or water. This study is carried out on strains of *Dickeya*, *Pectobacterium* and on the *Pseudomonas syringae* complex, all of which are capable of growing on a wide range of hosts as well as in water, thus allowing the most complete identification of genes of interest. Another axis of this ANR is the development of a complete bioinformatics pipeline, allowing to process data obtained under multiple conditions and for strains of different species. The results obtained will help us to predict the virulence of strains and/or to develop antibacterial strategies. Overall, this project will pave the way for studies of other environmental pathogens by proposing an original conceptual and technological framework that is extremely promising.

Materials and methods

1

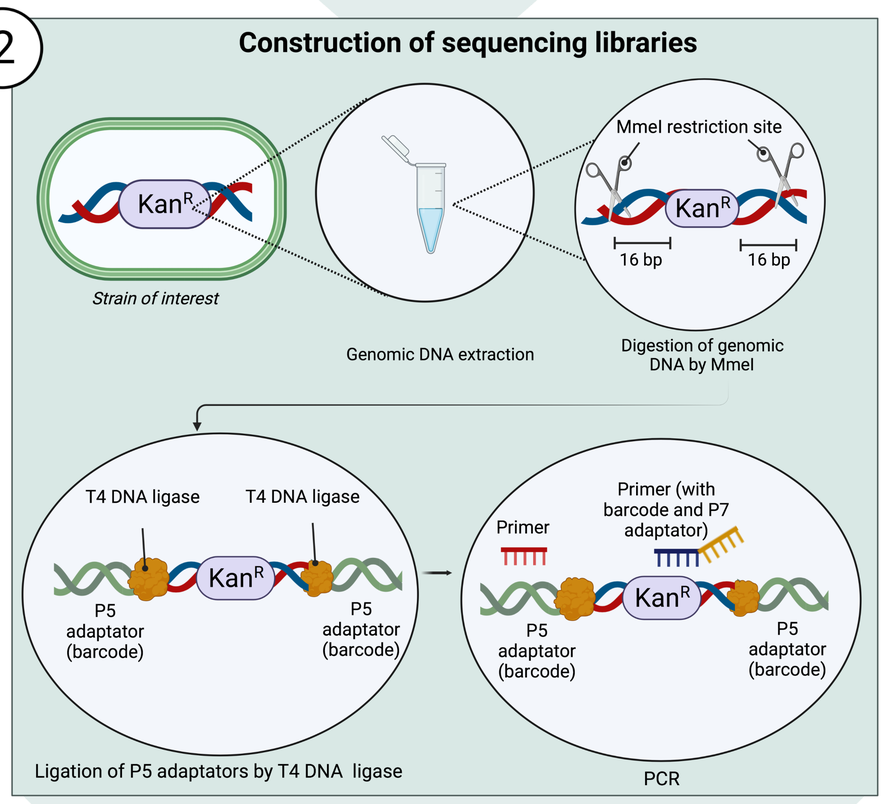
Construction of mutant libraries (conjugation)



Mutant libraries are constructed by conjugation between an *Escherichia coli* MDFpir strain and each of the strains of interest. During this conjugation, the plasmid pSAM-Ec, which contains the mariner Himar9 transposon that confers kanamycin resistance (3), a transposase and an ampicillin resistance gene, is transferred to the strain of interest. It is a suicide plasmid that cannot replicate in the recipient strain. Thus, the transposon will integrate uniquely and randomly into the genome of the strain of interest. The mutants obtained can then be selected on medium containing kanamycin.

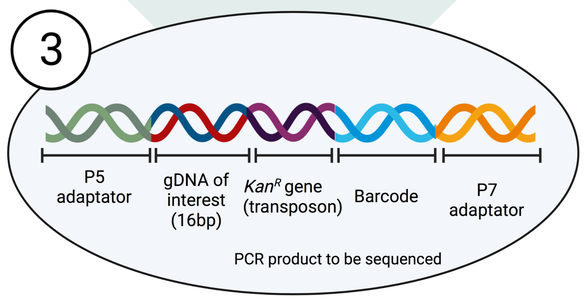
2

Construction of sequencing libraries



Genomic DNA from the mutant libraries is extracted and digested with the enzyme MmeI. Indeed, the transposon has MmeI restriction sites in each of its extremities. The particularity is that the enzyme will cut at 16 bp of its site. This will generate fragments containing the transposon and the adjacent 16 or 17 bp which corresponds to gDNA flanking the transposon. The P5 adapters containing a barcode are ligated to the digested DNA fragments using a T4 DNA ligase. Then, PCRs are used to amplify the fragment which will be sequenced to determine the insertion gene of the transposon.

3



The fragment is sequenced by a Novaseq Illumina sequencer (MGX,

Results

4

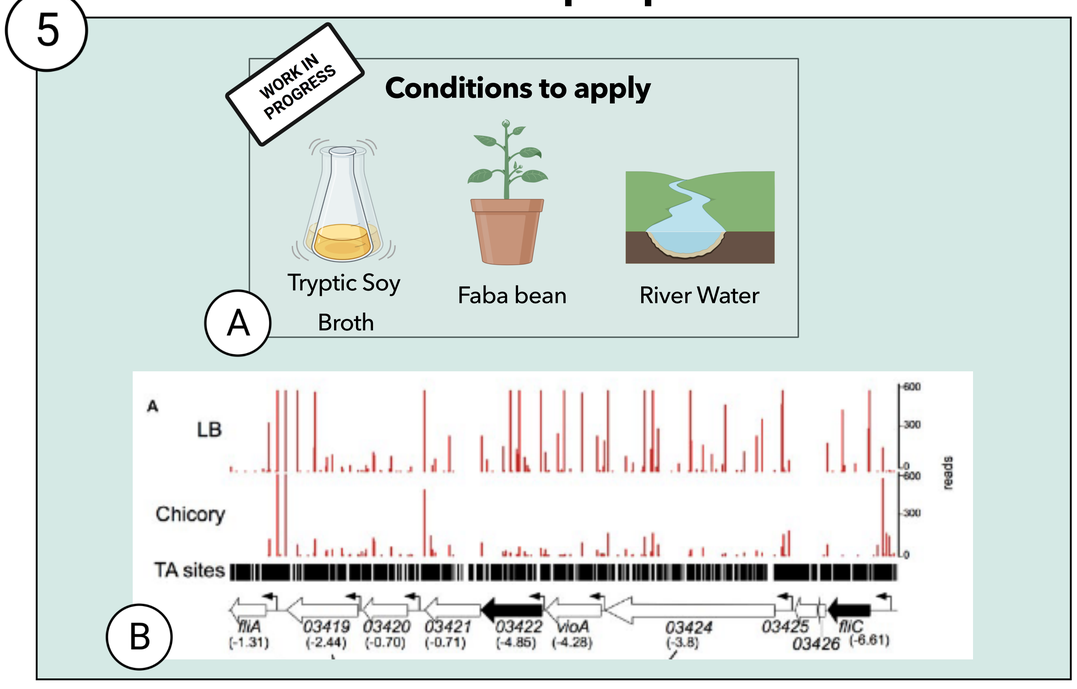
Results of Novaseq sequencing : qualities of mutant libraries

SPECIES	REPLICATE	INITIAL NUMBER OF READS	NUMBER OF UNIQUELY MAPPED READS	TAs	DENSITY	MEAN READ	# ESSENTIAL GENES / TOTAL GENES
Dickeya dadantii 3937	1	11 021 397	10 148 785	171 791	50.1	57.3	435/4687
	2	11 657 925	10 699 150	171 791	50.3	60.4	
Dickeya zeae A586	1	11 420 214	10 996 640	194 008	52.6	56.6	444/4475
	2	11 814 804	11 276 490	194 008	53.0	58.0	
Dickeya fangzhongdai B16	1	14 652 216	13 811 935	166 816	38.1	82.6	447/4530
	2	11 834 554	11 157 191	166 816	37.9	66.7	
Pectobacterium atrosepticum SCRI1043	1	9 100 581	8 638 648	232 639	45.9	35.4	441/4733
	2	10 454 137	9 843 266	232 639	46.5	40.3	
Pectobacterium versatile A73	1	11 354 590	10 579 502	219 900	55.5	55.1	407/5468
	2	10 215 789	9 512 400	219 900	55.3	49.6	
Pectobacterium aquaticum A212	1	12 091 457	10 283 920	199 301	62.7	49.0	480/4344
	2	11 184 408	9 460 081	199 301	62.6	45.1	
Pseudomonas syringae CC440	1	15 818 299	14 860 409	132 365	64.1	112.2	429/5206
	2	12 946 819	12 215 317	132 365	63.8	92.2	
Pseudomonas syringae CC1557 chromosome	1	10 445 631	9 718 582	131 221	65.5	74.5	456/5244
	2	10 820 446	10 146 505	131 221	65.6	77.8	
Pseudomonas syringae TA043	1	9 425 329	8 883 550	121 303	70.2	73.1	431/4756
	2	7 475 968	6 963 428	121 303	69.7	57.3	

The quality of the libraries created for each strain was analyzed by Novaseq sequencing (MGX, Montpellier). It allowed to determine the percentage of transposon insertion in the genome of each strain. A good quality library is considered to have an insertion density of more than 30%. The correlation coefficient shows a high reproducibility of the transposition. These two elements attest to the good quality of the mutant libraries constructed.

Conclusions and perspectives

5



(A) Now that the quality of the library is checked, the objective is to apply different conditions to the mutant libraries in order to determine the essential genes for bean infection or bacterial survival in a specific medium (TSB 50%, sterilized river water).

(B) Figure from Royet et al. (2019) (1). The results that will be obtained will provide a read number of the sequenced fragment. The presence of 16 bp adjacent to the transposon will allow to align these reads to the bacterial genome. The number of reads will reflect the importance of the mutated gene in under a certain condition. If the read count is low for a given gene, the mutant is not represented and the gene has an important role in that condition.

References:

(1) Royet et al. 2019. *Molecular Plant Pathology* : 287-306.
(2) van Opijnen. 2009. *Nat Methods* : 767–772.
(3) Wiles TJ. 2013. PLoS Genet 9(8): e1003716.

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