Pseudomonas aeruginosa can transfer antibiotic resistance genes to other pathogens through conjugative mechanism

Gauthier Dangla-Pélissier (gdangla@imm.cnrs.fr)

IMM - LISM - UMR7255, CNRS, Marseille, France

Introduction:
Pseudomonas aeruginosa is worldwide the third responsible of nosocomial infections for an average of 5000 deaths per year in France. It is also the main evolving bacteria in term of antibiotic resistance acquisition. Among its acquisition, the Integrative and Conjugative Element (ICE) PAPI-1 (*Pseudomonas aeruginosa* Pathogenicity Island 1) encodes for 115 genes involved in pathogenicity, antibiotic resistance and Deoxyribonucleic Acid (DNA) mobility. This ICE can be transferred through a type IV Secretion System (T4SS) to other Gram-negative bacteria conferring new virulence phenotypes to recipients.
Materials and methods:
The T4SS biosynthesis regulatory locus was identified performing random mutagenesis screening by *mariner* transposons insertion. The T4SS enhanced colonies were identified using report-fusion quantification and sequenced to find the transposon insertion locus. Using directed mutagenesis three regulators were identified confirmed by gel shift assays on the T4SS promoter region and β-galactosidase assays. Thus, the transcription factor regulon was identified using *Pseudomonas*-dedicated microarray to compare transcriptomes of wild-type strain and T4SS induced strains. This study was complemented doing antibiograms, competitions against other prokaryotes or eukaryotes, cytotoxicity assays and PAPI-1 Gram-negative bacteria transferences assays.
Results:
During my PhD, we deciphered for the first time the ICE PAPI-1 transference regulation in *P. aeruginosa* PA14. The mechanism occurs as follow:
- The conjugative T4SS biosynthesis is inhibited by compaction of promoter region,
- PAPI-1 encodes for both an uncompacting protein (NdpA2) and a transcription factor (TprA) respectively enabling and enhancing the biosynthesis of the T4SS a 70-time fold and PAPI–1 transference 120-time fold. This proposed mechanism was confirmed by gel shift assay, directed mutagenesis and β-galactosidase assays,
- The transcription factor also directly actives genes involved in virulence, antibiotic resistance and DNA mobility of *P. aeruginosa* shaping its antibiotic resistance and virulence,
- Once activated the ICE PAPI-1 can be transferred to other pathogenic Gram-negative bacteria.

In conclusion, we showed that in *P. aeruginosa*, the ICE PAPI-1 virulence and resistance genes transference is regulated by a three partners mechanism. We also found that inter-species transfer is possible among other Gram-negative bacteria. Taken together my results reveal the PAPI-1 contribution to the global bacteria resistance increase threat.

Mots clés : Horizontal transfer - ICE - Pathogenicity - antibiotic resistance - Pseudomonas aeruginosa.

Références :
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