M2 project Proposed by Céline Loot (équipe PGB, D. Mazel)

Antibiotic resistance: study of the integron system

Antibiotic resistance (AMR) is a major public health concern, now regarded as a threat to antibiotic chemotherapy and, consequently, to modern medicine. Antibiotic resistance genes (ARG) can disseminate between human and animal bacterial pathogens, and their associated commensal populations. Integrons are bacterial genetic elements able to stockpile, express and disseminate ARG (*1*). They are considered to be the principal agent involved in the continuous emergence of AMR in Gram-negative bacteria (GNB).

Integrons are composed of three basic features: an integrase, IntI; a primary recombination site, *attI*; and a promoter, Pc. IntI catalyzes the recombination between *attI* and a secondary recombination site, *attC*, located on circular gene cassettes. This recombination reaction leads to the integration of the cassette in the integron platform, downstream of Pc, from which the CDS contained in the cassette will be expressed (1). Although non-mobile by themselves, integrons can be found associated with mobile elements such as conjugative plasmids and integrating conjugative elements. Many bacterial species also contain sedentary integrons embedded in their chromosome. All *Vibrio* species sequenced to date harbor a sedentary chromosomal integron (SCI). The *V. cholerae* SCI, termed superintegron (SI) (2), contains 179 cassettes (3% of the genome) and, in contrast those found in MIs, the cassettes found in the SI are mostly of unknown function. SCIs play a general role in bacterial evolution, as providers of adaptive functions. Moreover, the evolutionary history of integrons suggests that SCIs could constitute a cassette reservoir and that subsequent harvesting of cassettes from various SCI sources leads to contemporary MIs. It's crucial to better understand how bacteria take advantage of SCIs while also reducing the burden generated by the presence on their chromosomes of these "so large" structures, all this, in the context of the primary role of integrons in the antibiotic resistance development. The genetic mechanism(s) that ensure the stability of SCIs are still mostly unknown. The presence of multiple Toxin-Antitoxin cassettes has been correlated with the size of the cassette array (*2*), and likely prevents cassette deletion through chromosomal rearrangements. The aim of this project is to delete the Toxin-antitoxin cassettes contained in the *V. cholerae* SCI and to follow the evolution of this TA deleted strains and particularly the cassette SCI content evolution. We will therefore establish the TA impact on the stability of the cassettes array. These studies will mix classical bacterial genetics and high throughput sequencing developments.

1. C. Loot#, JA. Escudero#, et al. The integron: adaptation on demand. Microbiol spectrum. 2015 Apr; 3(2):MDNA3-0019-2014. Review *# These authors contributed equally to this work.*
2. D. Mazel, et al. A distinctive class of integron in the *Vibrio cholerae* genome. Science. 1998 Apr 24;280(5363):605-8.