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Gen-directed re-sensitization of carbapenem-resistant microorganisms in biofilms

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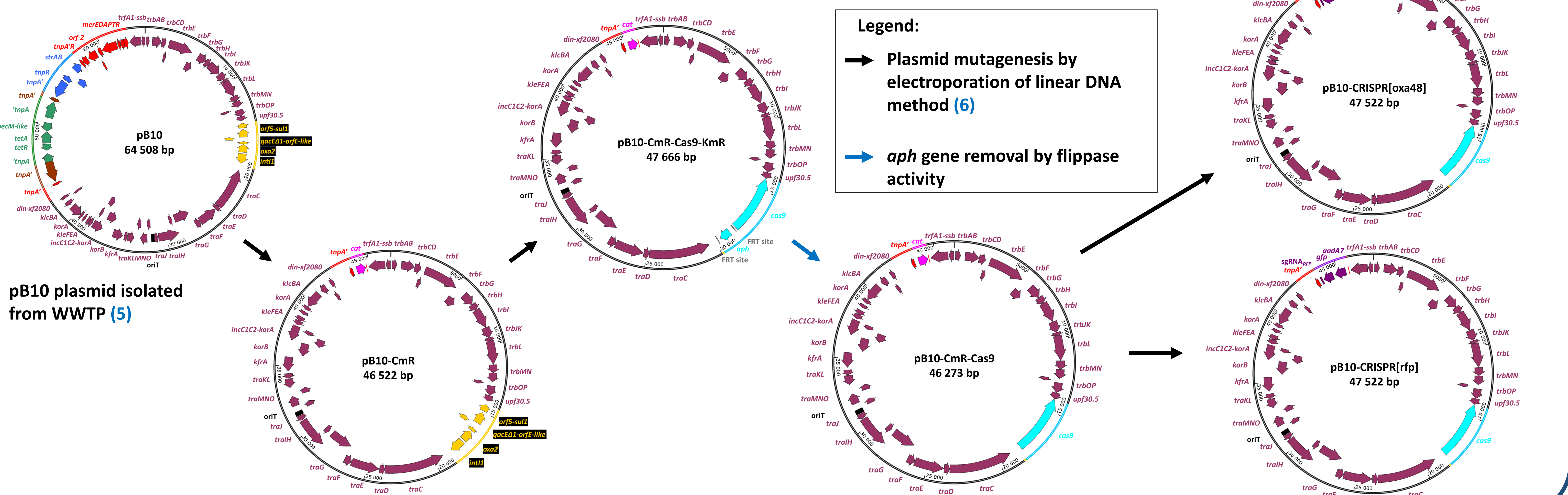
Introduction

Antibiotic resistance is a major concern in public health. In 2019, 1.2M deaths were directly caused by antibiotic resistance (1). It is a necessity to control environmental reservoirs to limit the spread of resistant bacteria and resistance genes. Hospital effluents are considered as hotspots of antibiotic resistant bacteria, primarily within biofilm communities (2). Resistance genes are frequently carried by plasmids, particularly carbapenemase-encoding genes such as OXA48, NDM or KPC (3). CRISPR/Cas9 system can be considered as a new way to fight antibiotic resistance (4) by specifically killing resistant bacteria or re-sensitizing them when the resistance is plasmid mediated.

Goal of this work

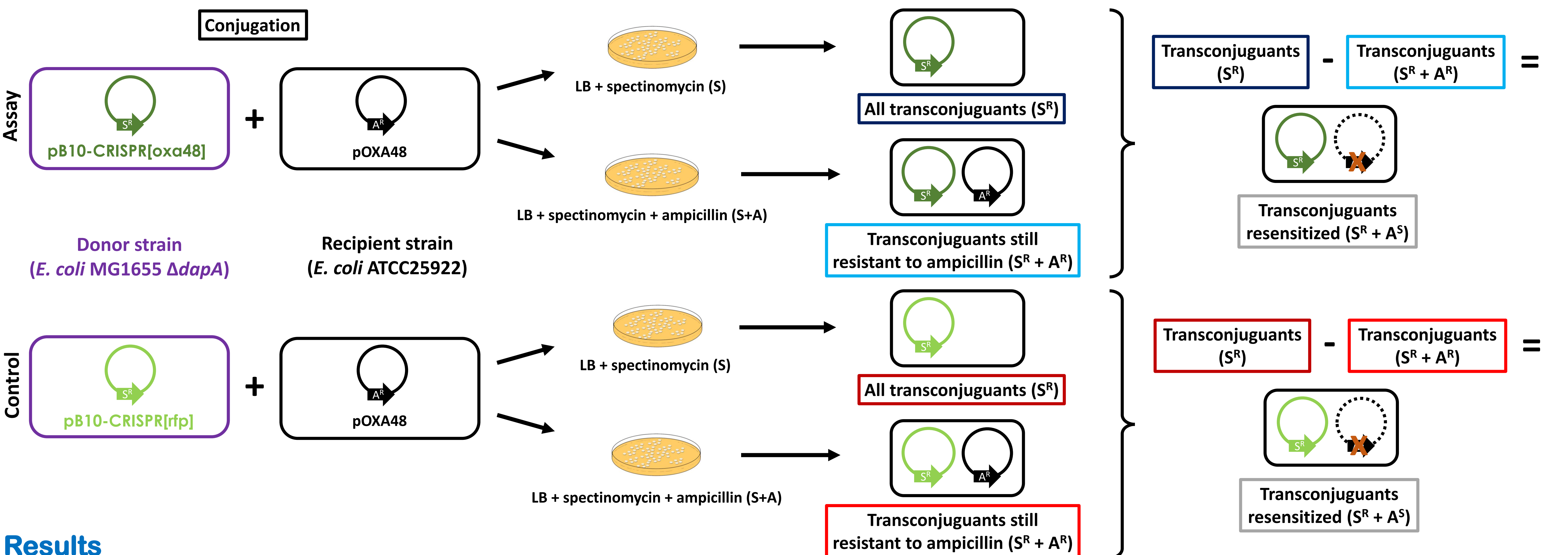
➤ Decrease the burden of carbapenem resistance in complex communities such as hospital effluents, by introducing a conjugative plasmid harboring CRISPR/Cas9 system targeting carbapenemase-encoding genes.

Construction of CRISPR-Cas9 system delivery plasmid



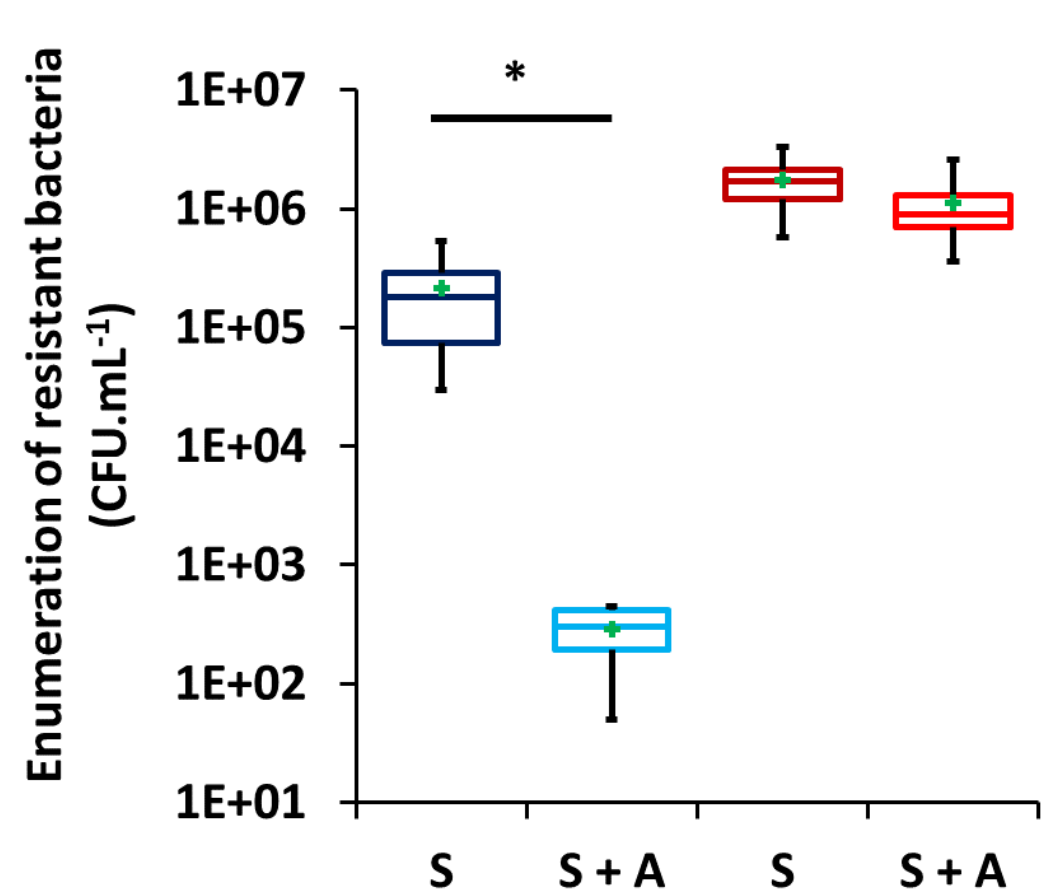
Re-sensitization assay

Method



Results

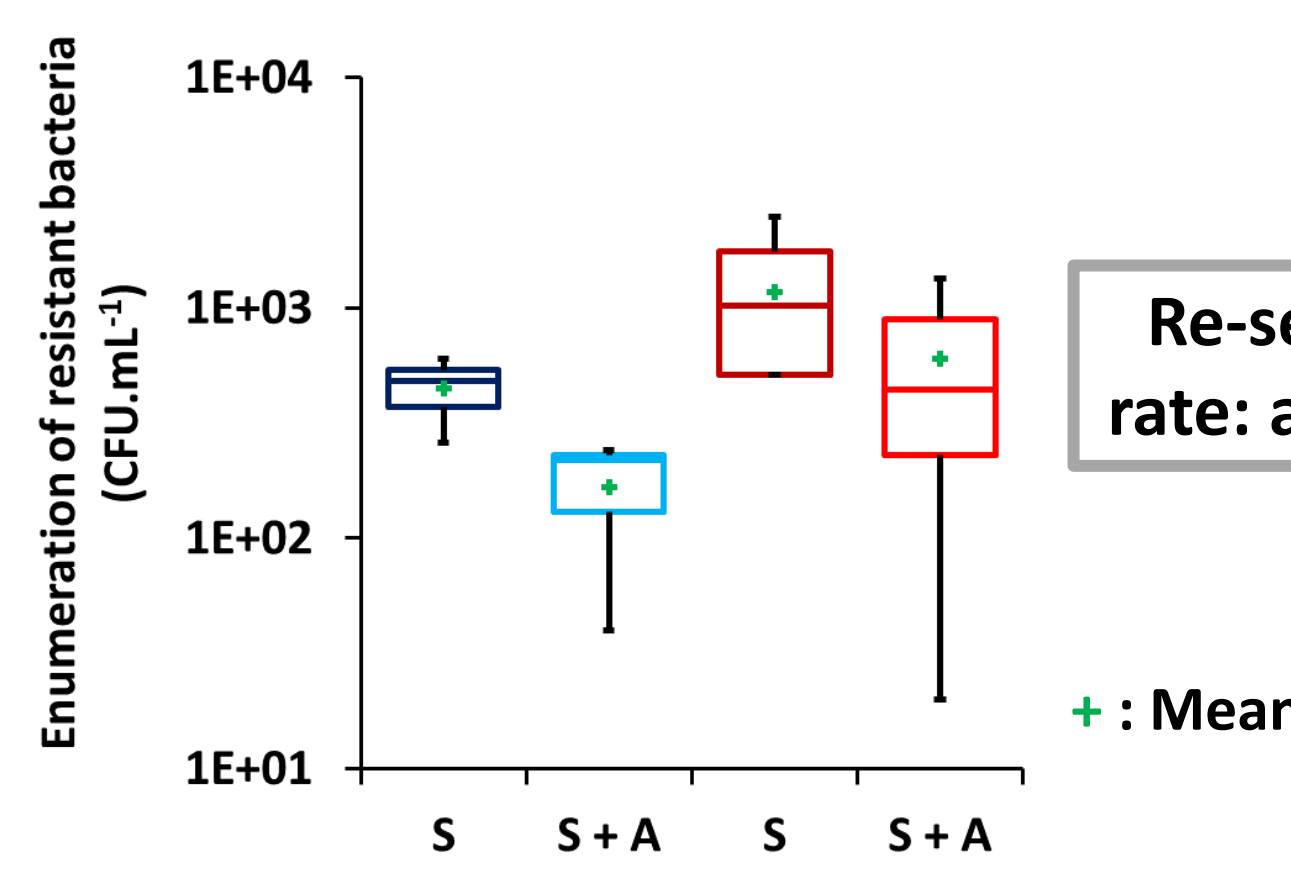
Conjugation performed on LB + DAP plate, by mixing the two liquid cultures



Re-sensitization rate: about 3 log

Conjugation rate: $9.05 \cdot 10^{-4} (\pm 5.21 \cdot 10^{-4})$

Conjugation performed by mixing donor strain from liquid culture and a 24h pre-formed biofilm by recipient strain



Re-sensitization rate: about 0.3 log

Conclusion

- Both engineered plasmids conserve a high conjugation rate
- pB10 carrying CRISPR-Cas9 system is efficient as a re-sensitization system against *oxa48* in liquid media
- ... But the efficiency in pre-formed biofilms conditions and in complex communities remains to be determined

Questions and perspectives

➤ Why some bacteria are not re-sensitized?
 ➤ Plasmids sequencing to investigate the causes

➤ Fine-tuning of assays in monospecies biofilms to investigate the causes of the low re-sensitization observed within pre-formed biofilms, by using different conjugation conditions (temperature, length of contact between bacteria...)

➤ Introduction of a gene encoding a fluorescent protein in the recipient strain to visualize the diffusion of the plasmid in the biofilms

- Can the pB10-CRISPR[oxa48] easily propagate within biofilms?
- Does the donor strain properly deliver pB10-CRISPR[oxa48] into biofilms?

References

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