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Use of the CRISPR/Cas9 gene editing technology to study patho-adaptive mutations of Escherichia coli in a cystic fibrosis patient

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Background: Through a first analysis of 5 clonal *E. coli* isolates recovered from a same cystic fibrosis (CF) patient, we showed that *E. coli*, like *P. aeruginosa,* could evolve under the pressure of natural selection encountered within the CF airways. This bacterial adaptability was promoted by a hypermutable phenotype, and survival in the CF lung was ensured through a persister phenotype (slow growth, biofilm formation, alteration of the lipopolysaccharide structure, nutritional deficiencies, loss of virulence...). The whole-genome sequencing analysis of the 5 isolates allowed the detection of 170 intragenic non-synonymous substitutions (158 SNPs and 12 short/large deletions) associated with persistence in the CF airways. Here, we aimed to explore 5 of the 158 SNPs (observed in candidate fitness genes: *mutL*, *rcsB*, *relA*, *spoT*, *dksA*), by reproducing them *in vitro*, with the CRISPR/cas9 gene editing tool.

Material/methods: A two-plasmid system was used for gene editing: pCas9cr4 with a tight control of *cas9* expression, and pKDsgRNA with the λ-Red recombinase system and a sgRNA (Reisch and Prather, Sci. Rep. 2015 Oct 14). *E. coli* DH5α was used for plasmid constructions, and genomic modifications were performed in *E. coli* 536. The latter and its mutants were characterized in terms of biofilm formation with a Cristal Violet staining method, Congo Red binding, virulence in the *Galleria mellonella* model, or hypermutability on rifampicin agar plates.

Results: The CRISPR/cas9 method enabled in less than 10 days, the selection of the desired substitutions (R95L in MutL, A108V in RcsB, H108R in RelA, R24W in SpoT, and A150T in DksA) with

a high efficiency (17-92% of positives). The R95L substitution in the conserved N-terminal sequence of the DNA mismatch repair protein MutL, resulted in an elevated mutation rate ($\sim 5\text{x}10^{-7}$ *vs* $\sim 6\text{x}10^{-8}$ for *E. coli* 536). The other substitutions in four proteins that are known to modulate intracellular responses to environmental stresses (RcsB, RelA, SpoT, and DksA), were not associated with changes in biofilm formation or virulence in our experimental conditions. Nevertheless, we showed that the A108V substitution in the receiver domain of the response regulator RcsB, resulted in a stronger binding of the Congo Red dye, that is used to identify extracellular adhesive fimbriae termed curli.

Conclusions: Understanding the fitness strategies employed by *E. coli* during persistence in the CF lung, could lead to new ways to prevent or treat chronic infections. Here, the CRISPR/cas9 approach provided a simple and particularly effective solution for creating *E. coli* mutants, and thus gain more insight into the selective advantage of substitutions acquired during survival within the host.