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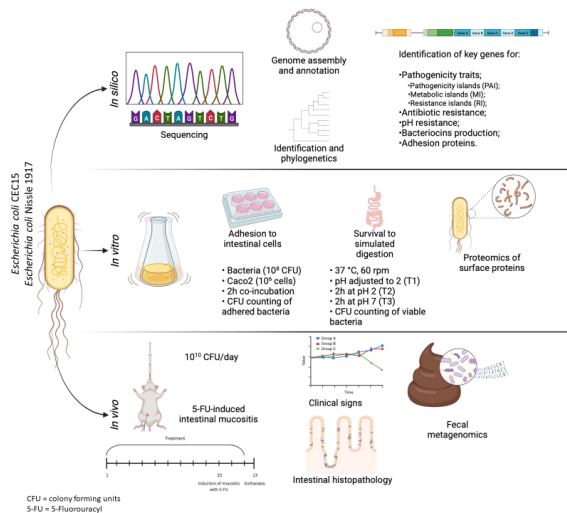
EVALUATION OF COMMENSAL *ESCHERICHIA COLI* CEC15 STRAIN AS A POTENTIAL PROBIOTIC: *IN SILICO*, *IN VITRO*, AND *IN VIVO* ANALYSIS.

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INTRODUCTION

The remarkable potential of probiotics in preventing and treating various illnesses has captured the attention of researchers and consumers alike. However, amidst the enthusiasm, it is crucial to understand the specific effects of each probiotic strain. It is evident that probiotics exhibit immunomodulatory effects, enhance the functionality of the gut barrier, and mitigate inflammation. However, it becomes apparent that a deep comprehension of the unique mechanisms exhibited by individual probiotic strains is imperative for optimizing their therapeutic efficacy. Potential risks are associated with probiotic use, raising the need for careful consideration when employing these interventions. Probiogenomics, which involves high-throughput techniques, can help reveal uncharacterized strains and allow for the rational selection of new probiotics. This study evaluates the potential of the commensal *E. coli* CEC15 strain as a probiotic through *in silico*, *in vitro*, and *in vivo* analyses, compared to the *E. coli* Nissle 1917 reference strain (EcN).

MATERIAL AND METHODS



FINAL CONSIDERATIONS

The CEC15 strain showed safety at the genomic level, with the absence of virulence genes, and *in vivo* maintaining healthy animals safe even in high quantities of administered bacteria. *In vitro* assays suggest that CEC15 arrives at the colon in high amounts and adhere at high rates allowing it to promote its beneficial effects for longer. In general, CEC15 holds promise as a probiotic for modulating the intestinal microbiota, providing anti-inflammatory effects, and reinforcing the intestinal barrier. These findings can have implications for treating gastrointestinal disorders. However, further research is essential to assess the safety and effectiveness of the CEC15 strain in humans.

RESULTS

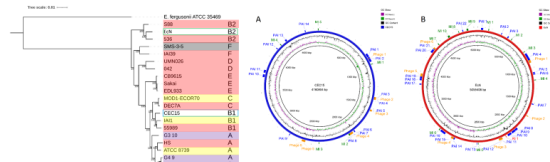


Figure 1. Phylogenomics of *E. coli* strains. CEC15 belongs to the B1 phylogroup, close to commensal bacteria (yellow) while EcN belongs to B2, close to pathogens (red).

Figure 2. CEC15 bears fewer pathogenicity-related genomic islands than the EcN strain. CEC15 presents 14 PAI, 5 MI and 6 prophages while EcN has 22 PAI, 9 MI, 1 PI, and 6 phages. No PAI in CEC15 contain virulence genes and 1 PAI on EcN codes for the genotoxin Collactin. No antibiotic resistance gene was found on PAI or prophage in CEC15.

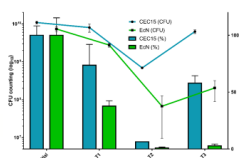


Figure 3. CEC15 strain greatly survives simulated digestion *in vitro*. CEC15 has a 40% loss after being in contact with pH 3 (T1 – 10min) and a 80% loss after being in pH3 for 2h (T2), the viability is recovered to 60% after 2h on intestinal phase (pH7 – T3). EcN on the other hand presented 40%, 2%, and 5% viability for T1, T2, and T3, respectively.

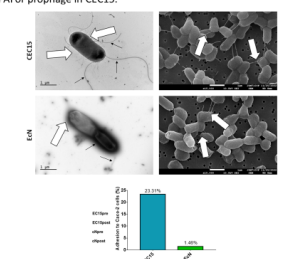


Figure 4. CEC15 presents better adhesion properties. CEC15 adhered to CaCo2 cells 15 times more than EcN. We observed that this could be due to the vast presence of pili, fimbriae, and flagella in CEC15 in comparison to EcN, seen on the micrographies above and confirmed by proteomics. White arrows indicates pili and black arrows indicates flagella.

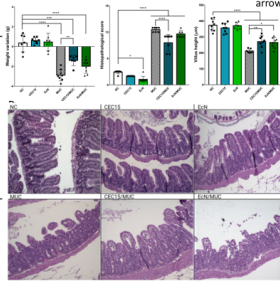


Figure 5. CEC15 ameliorates 5-FU-induced intestinal mucositis. No harm was observed in healthy animals after administration of both strains. When mucositis is induced, CEC15 is able to reduce weight loss and damage to the intestinal mucosa structure (histopathological score), also alleviating the reduction of vilus hight caused by 5-FU administration. HE slides show the structure of the ileum under each condition.

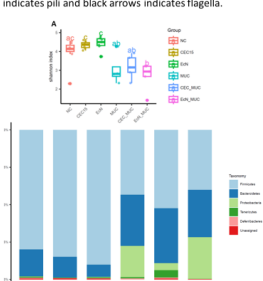


Figure 6. CEC15 shows signs of microbiota modulation. The treatments did not affect richness of, neither on healthy animals or animals with mucositis (shannon index). CEC15, however, seems to alleviate the disturbance of the ratio Firmicutes/Bacteroidetes and the increase of Proteobacteria caused by 5-FU administration