Poster

Evaluation of the efficiency of CRISPR-Cas systems in Gram-negative bacteria

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ABSTRACT

CRISPR-Cas is a genetic tool based on a prokaryotic defense system. This is a technology that involves the simple design of an RNA molecule (crRNA) and its binding to a Cas nuclease, forming a complex capable of breaking DNA. This system, in the presence of a repair system, also allows the introduction of mutations in a targeted manner. CRISPR-Cas has been widely used in eukaryotic cell genome editing, but not so much in prokaryotes [1]. The absence of efficient repair systems in bacteria could be the main reason, but this could be supplemented by the exogenous introduction of a bacterial non-homologous repair system, or NHEJ. Most bacterial NHEJs have only two proteins: Ku, which binds to and protects the broken ends; and LigD, which ligates them [2]. In this work a CRISPR-Cas system has been implemented and studied in Pseudomonas putida, a Gram-negative bacterium of great biotechnological interest, which lacks efficient NHEJ repair systems [3]. For this, two plasmids for the expression of Cas12a and NHEJ from Sphingopyxis granuli TFA have been introduced, in addition to a reporter plasmid that includes the necessary spacer and the target. The efficacy of Cas12 and the NHEJ in double-strand break repair in P. putida has been verified by fluorescence, PCR, and sequencing. However, when the repaired plasmids were analyzed, an unexpected homologous recombination repair (HDR) mechanism was found. The characterization of this phenomenon is currently being carried out.

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