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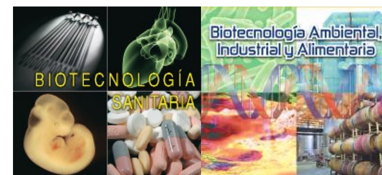

CRISPR/Cas9 system optimization in Pseudomona syringae using a heterologous repair system.

Sanz Martí, Estela
Área de Microbiología y Centro Andaluz de Biología del Desarrollo
Universidad Pablo de Olavide
Carretera de Utrera, Km. 1
41013 Sevilla, Spain
Tutor académico: López Sánchez, Aroa y Govantes Romero, Fernando

Keywords: CRISPR/Cas9; syringae; mutagenesis

ABSTRACT

Motivation: The methods of genetic manipulation in most Pseudomonas species, such as Pseudomonas syringae, remain a laborious endeavor. However, the application of the CRISPR-Cas9 system in this bacterium would allow a means of fast and directed genetic edition. The CRISPR-Cas9 system produces a doubles-stranded DNA break by the Cas9 protein. This break can be repaired by the NHEJ machinery in a very low frecuence either by the homologous repair system, which is widely spread amoung prokaryotes. The bacteria Pseudomona syringae pv. phaseolicola and pv. tomato are phytopathogenic causing diseases that affect crops of high economic value. Therefore, the optimization of a genetic modification system for these bacteria is very important.

Methods: In this study we report the development of the CRISPR Cas9 technology in Pseudomonas syringae pv tomato and pv. phaseolicola by using through the transformation of this bacteria with standardized pSEVA plasmids. After incorporating, the plasmids expressing the Cas9 nuclease and the guide RNA (gRNA), the Cas9 protein is complexed with the gRNA designed to direct a double-strand break to the pyrF gene. This lesion might be repaired by the bacterium intrinsic repair systems, but with a very low frequency. In order to solve this problem, a plasmid family has been used for expression in Pseudomonas syringae of three bacterial NHEJ systems from Sphingopyxis granuli, Mycobacterium tuberculosis and Mycobacterium smegmatis.

Results: The results shown in this study provide a step ahead towards the genetic modification in Gram negative bacteria by using the CRISPR Cas9 system and a non homologos end joining system together. Nevertheless it should be emphasised that the Cas9 protein can be toxic in this bacteria producing non-targeted mutations, because of that is very important keep the nuclease cas9 as little time as possible in the bacterium.

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