

## Talk

# CRISPR/Cas9 system optimization in *Pseudomonas syringae* using a heterologous repair system.



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## 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 double-stranded DNA break by the Cas9 protein. This break can be repaired by the NHEJ machinery in a very low frequency either by the homologous repair system, which is widely spread among prokaryotes. The bacteria *Pseudomonas 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 homologous end joining system together. Nevertheless it should be emphasized 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.

## REFERENCES

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