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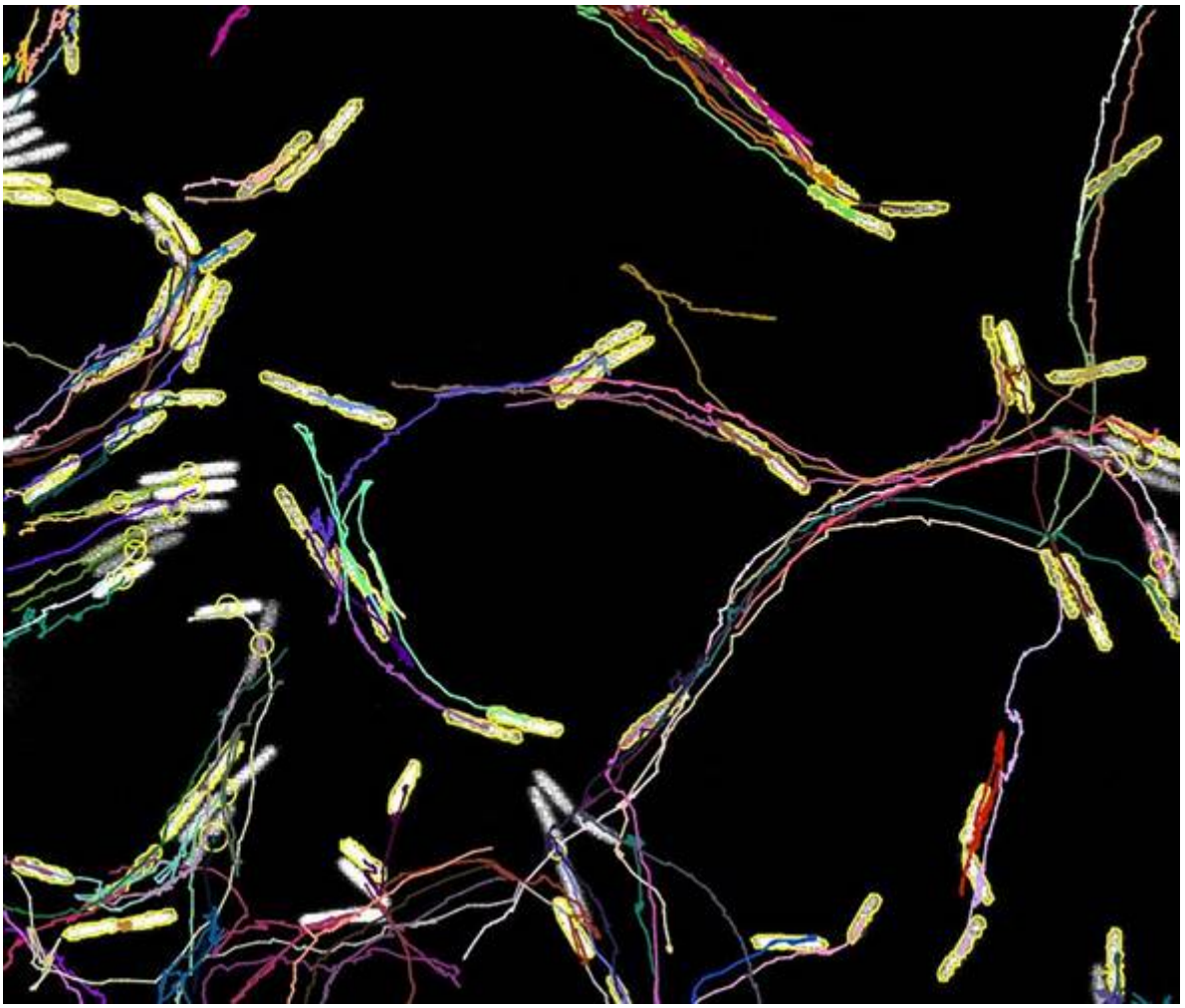

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CRISPR-BASED TOOLS DEVELOPMENT FOR OLEAGINOUS STRAINS OF THE Rhodococcus GENUS

Duhalde, Martín[1]-Martino Román[2]-Smania Andrea[2]-Hernández Martín[1]

1) Universidad Nacional de la Patagonia San Juan Bosco, INBIOP-UNPSJB-CONICET
2) Universidad Nacional de Córdoba, CIQUIBIC-UNC-CONICET
Contacto: tinchohernandez2002@yahoo.com.ar

Some species of the *Rhodococcus* genus, such as *R. opacus*, *R. wratislaviensis* and *R. jostii*, are able to accumulate high levels of triacylglycerol (TAG). For this reason, oleaginous rhodococci are promising microbial cell factories for the production of these lipids as main raw material for the industry of biofuels and oleochemicals. In accordance with their oleaginous phenotypes, these species have shown a huge repertoire of genes coding for enzymes, transporters, regulators and structural proteins associated with lipid metabolism. Some of those genes occurs as several copies, as is the case of the atf coding for DGAT enzymes. Whereas these properties make these strains robust models for TAG production, the basic study of the contribution of each gene on lipid metabolism, individually or in join with others genes, can be a big challenge. One of the most appropriate strategies to evaluate the functionality of one or more genes, consists of their mutation to evaluate their participation in a certain biological process. However, conventional mutagenesis techniques have shown be inefficient in this type of bacteria and therefore, there is an urgent need to find new tools to optimize these processes. In this study, we analyzed different tools based on the CRISPR technology of second (CRISPR-cas9) and third (Base editor; BE) generation to optimize and adapt them to oleaginous *Rhodococcus* strains. Bioinformatics analysis permitted us to analyze the presence of key elements into the predesigned vectors, including promoters, replication origins, antibiotic resistance cassettes, the occurrence of the original and modified Cas9 gene versions and the sgRNA cloning systems compatibility. Based on the collected information, we constructed new plasmid versions carrying the main genetic elements for gene edition in *Rhodococcus* cells. On the other hand, we looked and developed two reporter gene systems to test the functionality of both, original vectors and modified constructions in *Rhodococcus* strains. Our results showed that inducible tipA promoter and constitutive rpsL promoter work well for Cas-9 and BE expression based on growth profile, RT-PCR and Western blot assays. Further, constitutive gapdh, thcA, hsp60, J23119 promoters allowed the sgRNA expression. On the other hand, mcherry and lacZ genes constituted good candidates in mutation screening assays with both CRISPR-Cas9 and BE edition systems. The prototypes of the present work constitute new tools for designing different mutation strategies. They will allow us to evaluate the contribution of specific genes of lipid metabolism, such as the atf genes, in oleaginous bacteria

of the *Rhodococcus* genus in future works.

Palabras clave: RHODOCOCCUS-CRISPR-LIPIDS