Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

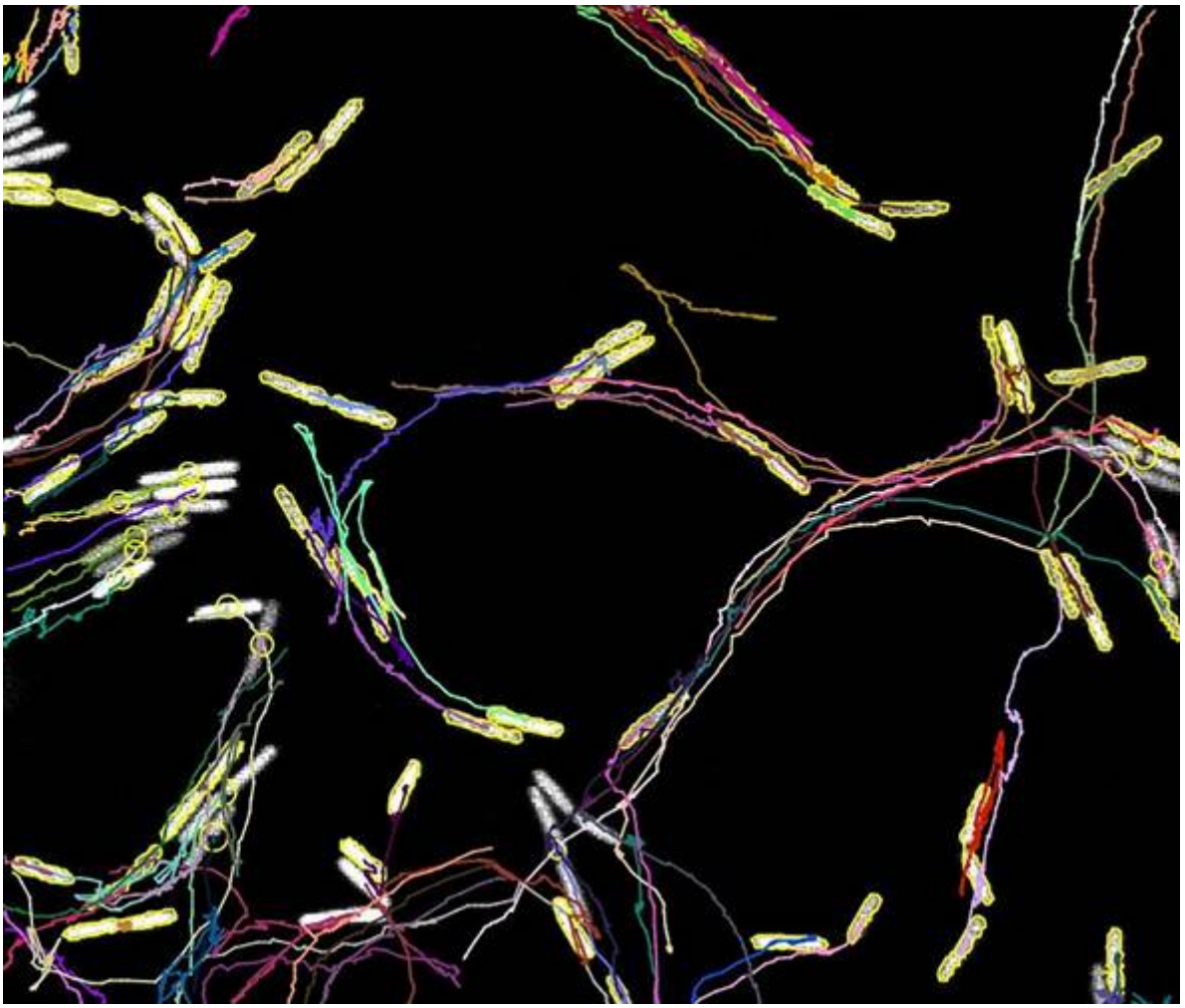

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

DESIGN OF NEW CONSTITUTIVE EXPRESSION VECTORS FOR OLEAGINOUS STRAINS OF THE *Rhodococcus* GENUS

Duhalde, Martín Andrés - Hernández, Martín Alejandro

Universidad Nacional de la Patagonia San Juan Bosco, INBIOP-UNPSJB-CONICET-Comodoro Rivadavia-Chubut-Argentina
Contacto: tinchohernandez2002@yahoo.com.ar

Some species of the *Rhodococcus* genus, such as *R. opacus*, *R. wratislaviensis* and *R. jostii*, are able to accumulate triacylglycerols (TAG) up to 60% or more of their cellular dry weight from pure carbon sources. 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 agree with their oleaginous phenotype, those species with the greatest capacity for TAG synthesis, have shown a huge repertoire of genes coding for enzymes, transporters, regulators and structural proteins associated with lipid metabolism. Although these properties make these strains robust models for lipid production under optimal conditions, in order to improve TAG accumulation in cell cultures grown from non-conventional carbon sources (e.g., industrial waste) or in cultures grown in non-optimal conditions (e.g., stress conditions), the overexpression of native genes or the heterologous expression of genes from other biological sources, constitute important alternatives to be considered. Unfortunately, only a few tools are available for gene expression in rhodococci and most of them are based on inducible plasmid systems. This not only constitutes a limitation for a good gene expression rate, but also makes scaling up the process more expensive. In this study, we present different tools for constitutive gene expression in oleaginous *Rhodococcus* strains. Bioinformatics analysis permitted us to select the presence of key elements into predesigned vectors, including promoters, replication origins, antibiotic resistance, cassettes and cloning systems. Based on the collected information, we designed new plasmids versions carrying the main genetic elements for gene overexpression in *Rhodococcus* cells under a constitutive *lac*Z promoter from *R. opacus* PD630: A replicative plasmid ($pQC2_{LacZPD630}$) and an integrative plasmid ($pMV_{LacZPD630}$). Our results showed that constitutive $lacZ_{PD630}$ promoter works well for the gene reporter *m-cherry* expression based on fluorescence assay. On the other hand, we expressed a native *atf* gene of the Kennedy pathway and observed an improved TAG accumulation in comparison with control strain. The prototypes made in the present work constitute new genetic tools of great utility when designing overexpression strategies. They will allow us to overexpress several specific genes of lipid metabolism in oleaginous bacteria of the *Rhodococcus* genus in future works for biotechnology applications.

Palabras clave: Rhodococcus - LIPIDS - OVEREXPRESSION - VECTORS