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27 - Biotecnología y Bioprocesos

## 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 lacZ promoter from R. opacus PD630: A replicative plasmid (pQC2<sub>LacZPD630</sub>) and an integrative plasmid (pMV<sub>LacZPD630</sub>) ). Our results showed that constitutive *lacZ*<sub>PD630</sub> 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