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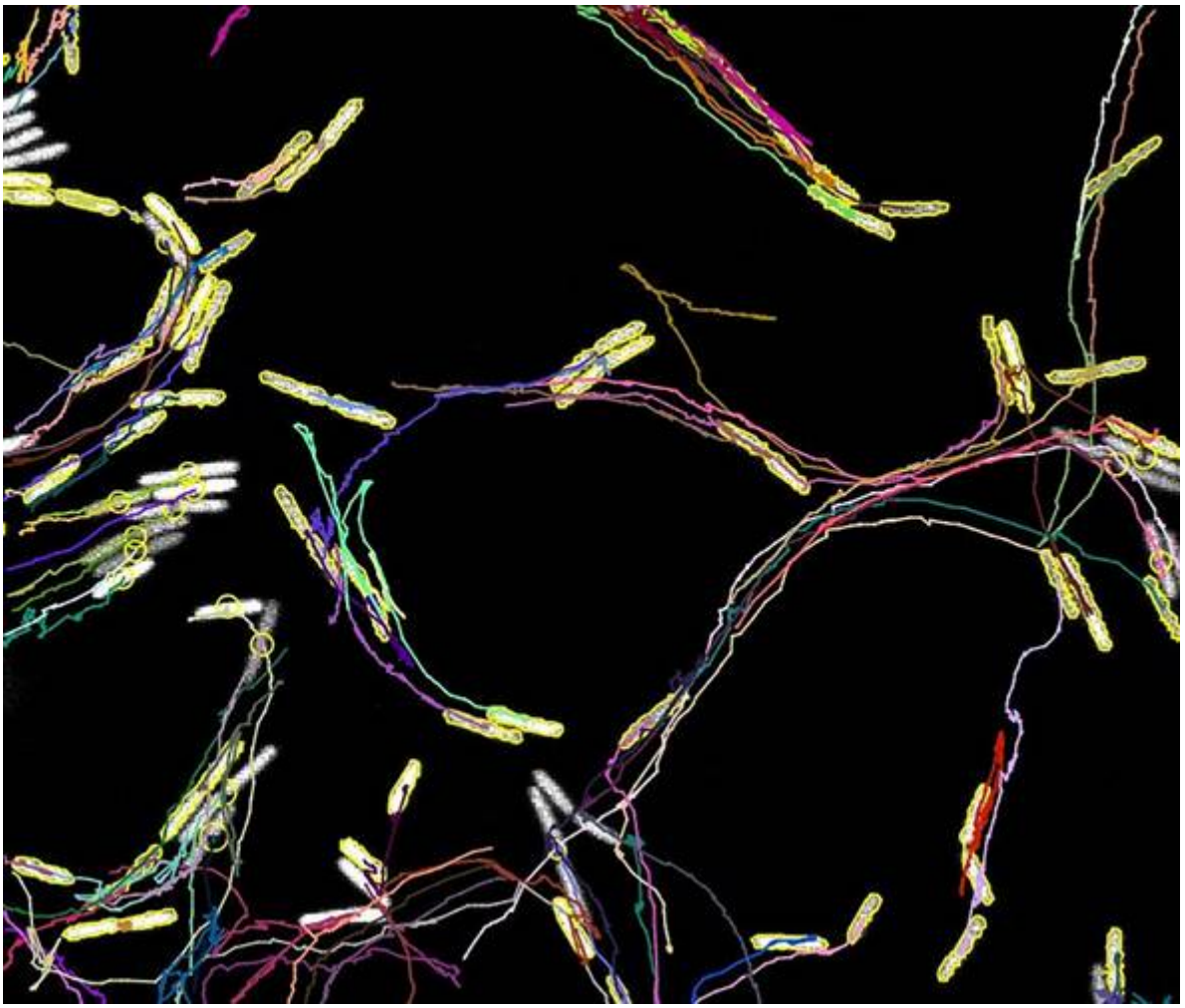

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

9 - Microbiología Molecular y Fisiología

METABOLIC ANALYSES TO DECIPHER LIPID ACCUMULATION IN Rhodococcus jostii RHA1 AT MOLECULAR LEVEL

Gallegos, Andrea L[1] - Silva, Roxana[1] - Escobar, Mariana[2] - Iglesias, Alberto[2] - Asención Diez, Matías[2] - Alvarez, Héctor[1]

1) Instituto de Biociencias de la Patagonia (INBIOP) - UNPSJB-CONICET - Comodoro Rivadavia - Chubut - Argentina
2) Laboratorio de Enzimología Molecular - Instituto de Agrobiotecnología del Litoral - CONICET - Santa Fe - Argentina
Contacto: gallegosandrealaura@gmail.com

Rhodococcus jostii RHA1 is an oleaginous bacterium with the ability to accumulate high amounts of triacylglycerols (TAG) (more than 50% CDW) in the form of inclusion bodies. In this study, we employed untargeted proton nuclear magnetic resonance based-metabolomics to identify changes in metabolites and metabolic pathways in cells during two different culture conditions: (a) cell cultivation under nitrogen-rich conditions that promote cell growth, and (b) cell cultivation under nitrogen-limiting conditions that lead to lipogenesis and lipid accumulation. To better understand RHA1 metabolism, we correlated metabolomic data with transcriptomic and proteomic analyses performed under the same culture conditions, in addition to the results of enzymatic analysis. For metabolomic analysis, RHA1 was grown in mineral salts medium with sodium gluconate (1%, w/w) as carbon source under N-excess (N-E) (growth) and N-limited (N-L) (lipid accumulation) conditions, respectively. A total of 12 cell samples were collected at the exponential growth phase for analysis, including 6 experimental replicates of each condition. Functional omic analyses showed significant perturbations in metabolites and pathways of central-, amino acids, and sugar-P metabolisms. Thirty one metabolites were identified in the 1H-NMR spectrum with a minimum cutoff of 1.2-fold change, P value of ?0.05. High levels of osmolytes were observed in both conditions, including betaine under N-E, and trehalose under N-L conditions. Besides protecting cells from ionic imbalances, osmolites are also associated to the adaptation to various stresses. We observed abundant levels of valine, leucine lysine, and glutamate in N-E cells, and high levels of metabolites associated with lipogenesis, such as pyruvate, succinate, and glycerol-3-P, in N-L cells. Transcriptomic and proteomic data correlated with the up-regulation of genes/enzymes involved in the formation of these metabolites in RHA1, in each condition. Cells grown in N-E conditions showed high levels of glucose-6P, whereas those cultivated under N-L conditions presented a high abundance of glucose-1P. The balance of both sugar-P metabolites allosterically regulates the activity of key enzymes in RHA1, such as ADP-glucose pyrophosphorylase (ADP-Glc PPase) involved in the synthesis of glycogen as a temporal reserve that provides a pool of carbon able of be re-routed to produce storage of lipids under N-L conditions. The dynamics of

glycogen in RHA1, which is preferentially produced during cell growth and not during lipid accumulation, correlated with the high abundance of glucose-6P (activator of ADP-Glc PPase) under N-E conditions, and the high levels of pyruvate (inhibitor of ADP-Glc PPase) under N-L conditions. The combination of results obtained in these studies allowed us to propose a metabolic landscape for R. jostii RHA1 to explain the extraordinary ability of this bacterium to synthesize and accumulate TAG.

Palabras clave: Rhodococcus - Triacylglycerols - Metabolomics - Glycogen-