Assessing carbon source-dependent phenotypic variability in Pseudomonas putida

The soil bacterium Pseudomonas putida is rapidly becoming a platform of choice for applications that require a microbial host highly resistant to different types of stresses and elevated rates of reducing power regeneration. P. putida is capable of growing in a wide variety of carbon sources that range from simple sugars to complex substrates such as aromatic compounds. Interestingly, the growth of the reference strain KT2440 on glycerol as the sole carbon source is characterized by a prolonged lag phase, not observed with other carbon substrates. This macroscopic phenomenon has been shown to be connected with the stochastic expression of the glp genes, which encode the enzymes needed for glycerol processing. In this protocol, we propose a general procedure to examine bacterial growth in small-scale cultures while monitoring the metabolic activity of individual cells. Assessing the metabolic capacity of single bacteria by means of fluorescence microscopy and flow cytometry, in combination with the analysis of the temporal takeoff of growth in single-cell cultures, is a simple and easy-to-implement approach. It can help to understand the link between macroscopic phenotypes (e.g., microbial growth in batch cultures) and stochastic phenomena at the genetic level. The implementation of these methodologies revealed that the adoption of a glycerol-metabolizing regime by P. putida KT2440 is not the result of a gradual change in the whole population, but it rather reflects a time-dependent bimodal switch between metabolically inactive (i.e., not growing) to fully active (i.e., growing) bacteria.

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