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^{13}C-Metabolic flux ratio analysis of Pseudomonas aeruginosa simultaneously producing polyhydroxyalkanoates and rhamnolipids

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Abstract

Pseudomonas aeruginosa is an important platform for simultaneous production of polyhydroxyalkanoates (PHA) and rhamnolipids (RHL). Advances in the understanding of the biosynthesis metabolism of these biocompounds are crucial for increasing yield. 13C-Metabolic Flux Ratio Analysis (13C-MFA) is a technique to estimate in vivo metabolic fluxes ratios. PHA and RHL are essentially non-growth associated products of biotechnological interest and both contain hydroxyalkanoates (HAs), whose labeling patterns could be accessed by GC-MS. In this study, to reveal the relative contributions of the Entner-Doudoroff (ED) pathway and the non-oxidative Pentose Phosphate (PP) pathway to PHA and RHL production, 13C-MFA was performed in Pseudomonas aeruginosa LFM634 when supplied with labeled glucose. This strain lacks both functional EMP and the oxidative PP branch. Labeling patterns in HAs were measured. Experiments with [U-13C] glucose indicated a low flux trough PP pathway. An optimal design of labeling experiment showed that [6-13C] glucose would be the best substrate to enable an estimation of the ED flux with high accuracy. Results of experiments performed with this isotope indicated that about two-thirds of glyceraldehyde 3-phosphate is recycled through a cyclic ED architecture, suggesting that P. aeruginosa utilizes that cycle to regulate the NADPH/Acetyl-CoA ratio for PHA and RHL biosynthesis.

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