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GROW OR STORE? EXPLORING METABOLIC DECISION MAKING UNDER FEAST/FAMINE CONDITIONS USING DYNAMIC ¹³C FLUX ANALYSIS

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Natural habitats of microorganisms are dynamic environments with non-continuous supply of carbon and energy sources, in which intermediate storage of substrates can increase competitiveness. *Plasticum* acidivorans are polyhydroxybutyrate (PHB) accumulating bacteria enriched from activated sludge using carbon feast-famine cycles as selective pressure. Despite growing slowly, *P. acidivorans* outcompetes other bacteria by quickly taking up acetate and storing it intracellularly as PHB to later use it for growth. As soon as acetate is depleted, these bacteria immediately 'switch' their metabolism from PHB production to consumption entailing a very interesting regulatory challenge as parallel activity could lead to significant losses (futile cycling). While the stoichiometry for both feast and famine phases has been extensively described in literature, the switch regulation is not yet fully understood.

To elucidate the responsible regulatory processes, an enrichment of *P. acidivorans* was studied using targeted intracellular metabolite analysis over time, with emphasis on the feast to famine switch. In combination with extracellular rates, the measured intracellular metabolite pools are used to design a labelling experiment to obtain actual intracellular fluxes (dynamic ¹³C flux analysis). Here the challenge is to create an isotopically non-stationary state (usually mediated by changing the substrate's isotopic composition) to study the metabolic response in the transition from presence-to-absence of substrate.

In this way, we aim to unravel the responsible regulatory mechanism governing the metabolic switch from storage-to-consumption and use this knowledge not only to understand its ecological relevance, but to also propose novel metabolic strategies for microbial cell factory design.