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Uncovering the Molecular Basis of Carbon and Nitrogen Metabolic Integration via the Glutamate Pathway in Yarrowia lipolytica

In the face of increasingly serious global challenges, such as growing food shortages, limited energy resources, and problematic waste that is difficult to recycle, the world, through scientific efforts, is urgently seeking new and sustainable solutions. One particularly promising direction is the use of microorganisms in a circular economy. The goal of this model is to minimize waste by valorizing it- converting it into valuable compounds using microorganisms. A microorganism of special interest is the yeast *Yarrowia lipolytica*. This species possesses a unique ability to convert industrial waste- such hydrolyzed lignocellulose biomass, diesel fuel, and even plastic- into valuable compounds. Among the many useful products it can generate are fats, proteins, and sugar alcohols, which serve as sugar substitutes for diabetics.

Although *Y. lipolytica* is already widely used in industry, our understanding of how its metabolism works, particularly how it processes nutrients, remains incomplete. Nitrogen, a chemical element fundamental to all living organisms and a building block of DNA, plays a key regulatory role in this process. Depending on its availability, nitrogen can drive yeast cells to either grow and build biomass or to synthesize valuable compounds such as lipids and organic acids. However, the precise mechanisms that govern how *Y. lipolytica* adapts its metabolism to fluctuating environmental conditions remain poorly understood. In many better-studied yeast species, metabolism is regulated through a mechanism known as Nitrogen Catabolite Repression (NCR). This process allows the cell to prioritize the use of preferred nitrogen sources and to conserve energy by downregulating proteins needed to process more complex, less efficient sources. This regulation occurs at the gene expression level and is mediated by specific transcription factors. One of the key pathways responsible for nitrogen processing is the GDH pathway, which is central to amino acid metabolism. Intriguingly, this pathway also forms a direct link between nitrogen and carbon metabolism via α-ketoglutarate, an intermediate in the Krebs cycle. Additionally, *Y. lipolytica* possesses an alternative nitrogen recovery pathway (ANRP), not found in model yeast species, which allows it to recycle nitrogen more effectively in nutrient-poor environments.

This research project aims to understand how *Y. lipolytica* regulates nitrogen metabolism and integrates it with broader cellular metabolism, focusing on the GDH and ANRP pathways. The project will also investigate whether metabolic intermediates from the GDH pathway, such as α-ketoglutarate and glutamine, act as internal signaling molecules that inform the cell about its environment and adjust metabolic processes accordingly. To answer these questions, the project will implement a comprehensive series of experiments. These will include metabolite profiling, gene expression analysis, tracking the intracellular localization of proteins, and studying how yeast responds over time to varying nutrient conditions. State-of-the-art microscopy, analytical instrumentation, and molecular biology tools- including the CRISPR-Cas9 genome editing system- will be employed throughout the study.

The results of this project will help reveal how *Y. lipolytica* adapts to continuous environmental changes. Given the yeast's immense potential in the energy, food, and pharmaceutical industries, as well as in the valorization of industrial waste, a deeper understanding of its metabolism is essential. This knowledge will support the design of more efficient yeast strains and the optimization of biotechnological processes involving *Y. lipolytica*, ultimately enabling its more effective use in a circular economy. The findings will contribute to the advancement of modern, sustainable biotechnology, crucial for building a better, greener future.