

The mysterious hexokinase - protein bridging carbon and nitrogen metabolism

The term "circular economy" is gaining increasing attention around the world. This means using industrial waste as raw materials to produce valuable compounds with potential applications in various industries, from food to the pharmaceutical industry. The implementation of this type of management aims to minimize the amount of waste generated. This is particularly important given the continuing growth of the world population, which leads to further industrial development and increased waste production. The use of yeast as microfactories allows to easily implement a circular economy, because yeast, due to their abilities, are able to use waste substances as a source of carbon and energy. One of such microorganisms is the yeast *Yarrowia lipolytica*, the research object of the proposed project. Currently, they are widely used to develop industrial-scale processes for the production of organic acids, such as citric acid or α -ketoglutaric acid, or to produce sugar substitutes for diabetics, such as erythritol. *Y. lipolytica* yeast are attractive to industry also due to their ability to use waste carbon sources in the form of glycerol or petroleum substances. One of the interesting directions of their use are processes based on waste glycerol, which is a by-product in the production of biodiesel. *Y. lipolytica* prefers glycerol, which means that when it is present in the culture medium in a mixture with glucose, this yeast will use it first. This phenomenon is particularly interesting because for most organisms the preferred source of carbon is glucose.

The greatest challenge when conducting processes using microorganisms are the variable environmental conditions in which cells grow and carry out life processes. During the growth of yeast, the concentrations of substances in the surroundings of the cells constantly change, some of them are used by them and others are produced. Due to the growing interest in the use of *Y. lipolytica* in industry, understanding the mechanisms that allow it to quickly adapt to the environmental conditions by regulating metabolism is crucial to effectively use them in industrial processes.

In the context of metabolism regulation, it is worth referring to two key mechanisms that regulate metabolism in the yeast *Saccharomyces cerevisiae*: Carbon Catabolic Repression (CCR) and Nitrogen Catabolic Repression (NCR). The CCR controls the expression of genes responsible for the breakdown of complex carbon sources, usually in response to the preferred carbon source, glucose. NCR works similarly, but responds to the nitrogen source, usually preferring ammonium ions. Although these mechanisms are well understood in *S. cerevisiae*, many questions remain unanswered in the context of *Y. lipolytica*.

Hexokinase, the enzyme responsible for incorporating sugars into metabolism, plays an important role in the regulation of metabolism, especially in the CCR mechanism. However, its regulatory role in *Y. lipolytica* cells is still under investigation. Available data show that low nitrogen concentration in the culture medium increase the expression of the gene encoding hexokinase. By analyzing the promoter sequence of this gene, the presence of motifs recognized by NCR-related transcription factors was discovered. Furthermore, the structure of hexokinase itself in *Y. lipolytica* contains two unique loops, one longer (37 amino acids) and one shorter (7 amino acids). Their functions have not yet been precisely defined, but research show that the larger loop may influence gene expression. Due to the presence of potential phosphorylation sites in the longer loop, it can be assumed that this structure is crucial for the regulatory function of hexokinase. The research carried out as part of the project will help clarify this hypothesis. These studies will also prove that hexokinase plays an important role by connecting the CCR and NCR mechanisms. Understanding these regulatory mechanisms will allow for more effective use of *Y. lipolytica* in a circular economy in processes based on complex waste raw materials as a source of carbon and nitrogen.