

## Saccharomyces cerevisiae in cell-free protein synthesis

*Ashima Bhatia*

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Producing proteins outside of living cells through methods like cell-free protein synthesis (CFPS) is crucial for overcoming the limitations associated with traditional cellular expression systems. This approach allows for enhanced control, scalability, and versatility in protein production, addressing the growing demand for diverse applications in fields such as medicine, biotechnology, and industry. CFPS is a valuable method for producing proteins outside of living cells, with applications in various fields. While the commonly used bacterial CFPS has limitations, there is a need for robust eukaryotic CFPS platforms capable of producing complex proteins. The researchers from Northwestern University, USA focused on using *Saccharomyces cerevisiae* (yeast) extract for this purpose due to its advantages in genetic manipulation and well-characterised properties. This yeast can be a great resource for developing a practical method to produce proteins outside of living cells. This method involves using a crude extract that captures the yeast's active metabolism at the time of harvest. During CFPS, the yeast extract provides the machinery for protein synthesis but also contains enzymes involved in various cellular activities. Some of these activities can be beneficial for protein synthesis, while others may hinder it. The researchers aimed to improve yeast CFPS by developing a chemically defined medium which consisted of yeast peptone dextrose complex media, synthetic dextrose-defined media, and synthetic complete defined media, allowing them to identify and control metabolic transitions during growth. Understanding the relationship between yeast growth and extract metabolic activity is crucial for optimising CFPS. The study showed that altering the fermentation conditions, such as the composition of the growth medium, significantly impacted CFPS yields. Yeast, sensitive to nutrient changes, exhibited varied metabolic responses during fermentation. By characterising and optimising the fermentation step, the researchers achieved a four-fold increase in productivity compared to conventional methods. The research showcases that adjustments in fermentation conditions can enhance the efficiency of yeast CFPS for better protein synthesis.

*Keywords: Yeast, Protein synthesis, Fermentation, Metabolism, Cell-free protein*

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Citation:

Ashima Bhatia . *Saccharomyces cerevisiae* in cell-free protein synthesis. The Torch. 2022. 3(39). Available from:

<https://www.styvalley.com/pub/magazines/torch/read/saccharomyces-cerevisiae-in-cell-free-protein-synthesis>.