

## COUPLING ENGINEERING OF *SACCHAROMYCES CEREVISIAE* WITH MEDIUM OPTIMIZATION FOR THE PRODUCTION OF ERGOTHIONEINE

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**Key Words:** Ergothioneine, metabolic engineering, medium optimization, *Saccharomyces cerevisiae*, nutraceutical

Ergothioneine (ERG) is a naturally occurring, exogenous antioxidant that is nonetheless abundant in the human body. It has been shown both to reduce oxidative damage and to be involved in several diseases *in vivo*<sup>1,2</sup>. Therefore, ergothioneine is poised to take a place in the dietary supplement industry. Here we describe the engineering of the yeast *Saccharomyces cerevisiae* and subsequent medium optimization to produce ergothioneine by fermentation. After integrating combinations of biosynthetic pathways from different organisms, we screened yeast strains for their production of ERG. Next, the highest producing strain was engineered with ergothioneine transporters, and its amino acid metabolism was altered by knock-out of Tor1 or Yih1. The bottleneck for ergothioneine production was determined by integration of a second copy of the pathway enzymes. We also optimized the media composition for production of ergothioneine using yeast *S. cerevisiae*. Following these manipulations, we obtained a titer of 630 mg/l in fed-batch cultivation in bioreactors. This work shows that with further engineering of the strain, current chemical synthesis of ergothioneine could be replaced with a sustainable alternative.

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