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Wed. Jul 1, 2026 12:10 PM - 1:00 PM JST | Wed. Jul 1, 2026 3:10 AM - 4:00 AM UTC | Poster 1(4FFoyer+401+402)
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12:10 PM - 1:00 PM JST | 3:10 AM - 4:00 AM UTC

[P03-355] Introduction of point and structural mutations in engineered yeast *Saccharomyces cerevisiae* increases carotenoid production

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Keywords：Saccharomyces cerevisiae、Carotenoid、Point and structral mutageneses、Metabolic engineering、Hydrogen peroxide

Carotenoids are natural compounds with strong antioxidant activity and are widely used in food, pharmaceutical, and cosmetics. Among the various carotenoids, β-carotene is one of the most commercially valuable compounds. The production of these compounds primarily relies on plant extraction and chemical synthesis methods, however, production using the yeast *Saccharomyces cerevisiae*, which is easily cultivated on an industrial scale, is highly anticipated as an alternative approach. Since β-carotene has a considerable antioxidant effect, yeasts that accumulate large amounts of intracellular β-carotene are highly resistant to oxidative stress. Exploiting this characteristic, it has been reported that long-term adaptive laboratory evolution of β-carotene–producing yeast under hydrogen peroxide–induced oxidative stress led to the isolation of *S. cerevisiae* strains with improved β-carotene productivity. However, because adaptive evolution requires a considerable amount of time, there is a strong demand for strategies that enable the rapid and efficient acquisition of beneficial mutants through artificial mutagenesis. Previous studies have developed a genome engineering technique that enables the simultaneous introduction of point and structural mutations into the yeast genome, and it has been reported that this approach allows the generation of diverse mutant strains. Using this mutagenesis technology, a protein overproduction mutant strain, YPH499Mu10G39, was constructed. This mutant strain has been suggested to be a highly advantageous host for production of useful chemicals. In this study, we aimed to construct yeast strains with enhanced carotenoid productivity by simultaneously introducing DNA point and structural mutations into recombinant carotenoid-producing yeast, followed by enrichment culture under oxidative stress conditions.

The carotenoid production plasmid pEU20-Beta3 and the plasmid pEWPMSM_Co, designed for introducing point and structural mutations, were transformed into YPH499Mu10G39 to generate the strain YPH499Mu10G39/pEU20-Beta3/pEWPMSM_Co. This was followed by enrichment cultivation under hydrogen peroxide–induced oxidative stress. As a result, we successfully obtained mutants tolerant to 200 mM hydrogen peroxide. Screening of 360 candidates identified strain 10G39HP200_308 as a high carotenoid-producing strain. This strain achieved the highest β-carotene titer (7.25 mg/L), which was 1.41-fold higher than that of the parental strain, YPH499Mu10G39/pEU20-Beta3 (5.14 mg/L).

In conclusion, we successfully constructed a yeast strain with enhanced carotenoid production by simultaneously introducing point and structural mutations into the protein high-expression mutant YPH499Mu10G39/pEU20-Beta3, which had been engineered for carotenoid biosynthesis, followed by adaptive enrichment cultivation under hydrogen peroxide stress.

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