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Genetic improvement of *Saccharomyces cerevisiae* and process optimization for enhanced squalene production

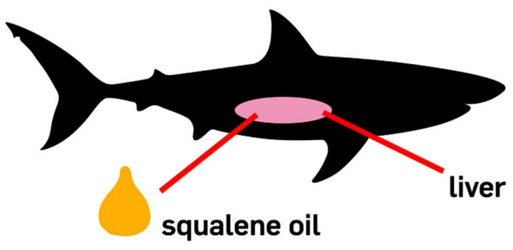
Onanong Inthong[a], Sornsiri Pattanakittivorakul[b], Mamoru Yamada[b,c], Noppon Lertwattanasakul[a,d,*]

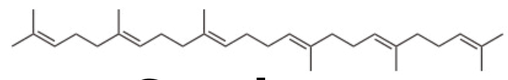
[a]Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
[b]Graduate School of Sciences and Technology for Innovation, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan
[c]Research Center for Thermotolerant Microbial Resources, Yamaguchi University, Yamaguchi 753-8515, Japan
[d]Biodiversity Center, Kasetsart University, Bangkok 10900, Thailand.
*Corresponding author: fscinple@ku.ac.th

1. Abstract

Squalene is a non-polar triterpenic hydrocarbon ($C_{30}H_{50}$) and the first committed precursor of ergosterol in the isoprenoid pathway. Due to the limited availability of natural sources and increasing extraction costs, microbial production of squalene has gained significant interest. This study aimed to enhance squalene production in *Saccharomyces cerevisiae* through UV mutagenesis, and to evaluate the effects of cultivation parameters on squalene accumulation. *S. cerevisiae* TISTR 5606 was cultivated in YPD medium at 30°C and 100 rpm, producing 49.26±0.78 mg/L squalene. UV mutagenesis generated the mutant strain ONI-UV 24(2), which showed increased squalene production of 88.83±0.65 mg/L, representing a 1.8-fold improvement over the wild-type strain. The effects of oxygen availability were further investigated by varying shaking speeds. The highest squalene accumulation was observed at 150 rpm after 60 h, reaching 154.66±1.05 mg/L, equivalent to a 3.1-fold increase relative to the wild type. These results demonstrate that UV-induced genetic modification combined with optimized aeration conditions is an effective strategy for enhancing squalene production in *S. cerevisiae*.

2. Introduction



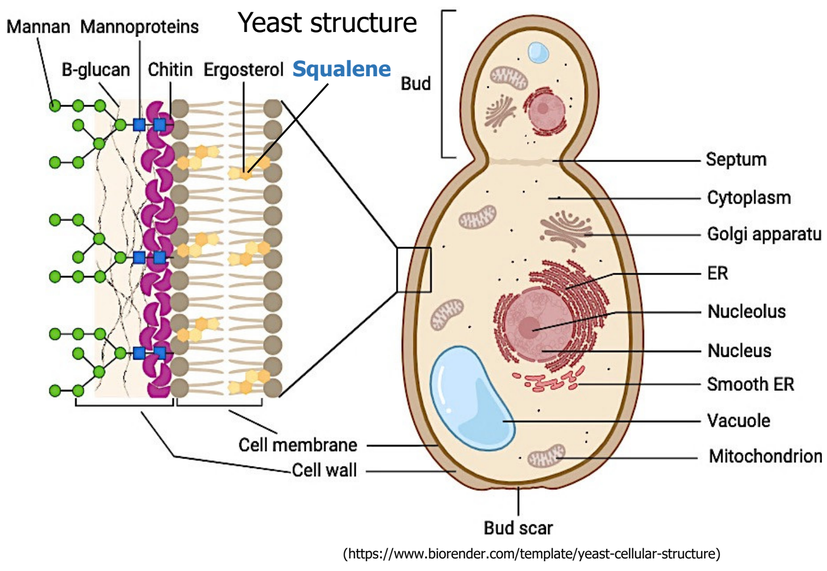


Squalene

Squalene was first discovered in the liver oil of deep-sea sharks.

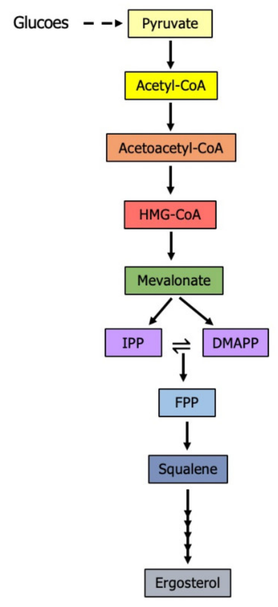
Squalene derived from microorganisms may be an interesting alternative.

Saccharomyces cerevisiae



(https://www.biorender.com/template/yeast-cellular-structure)

Squalene biosythetic pathway

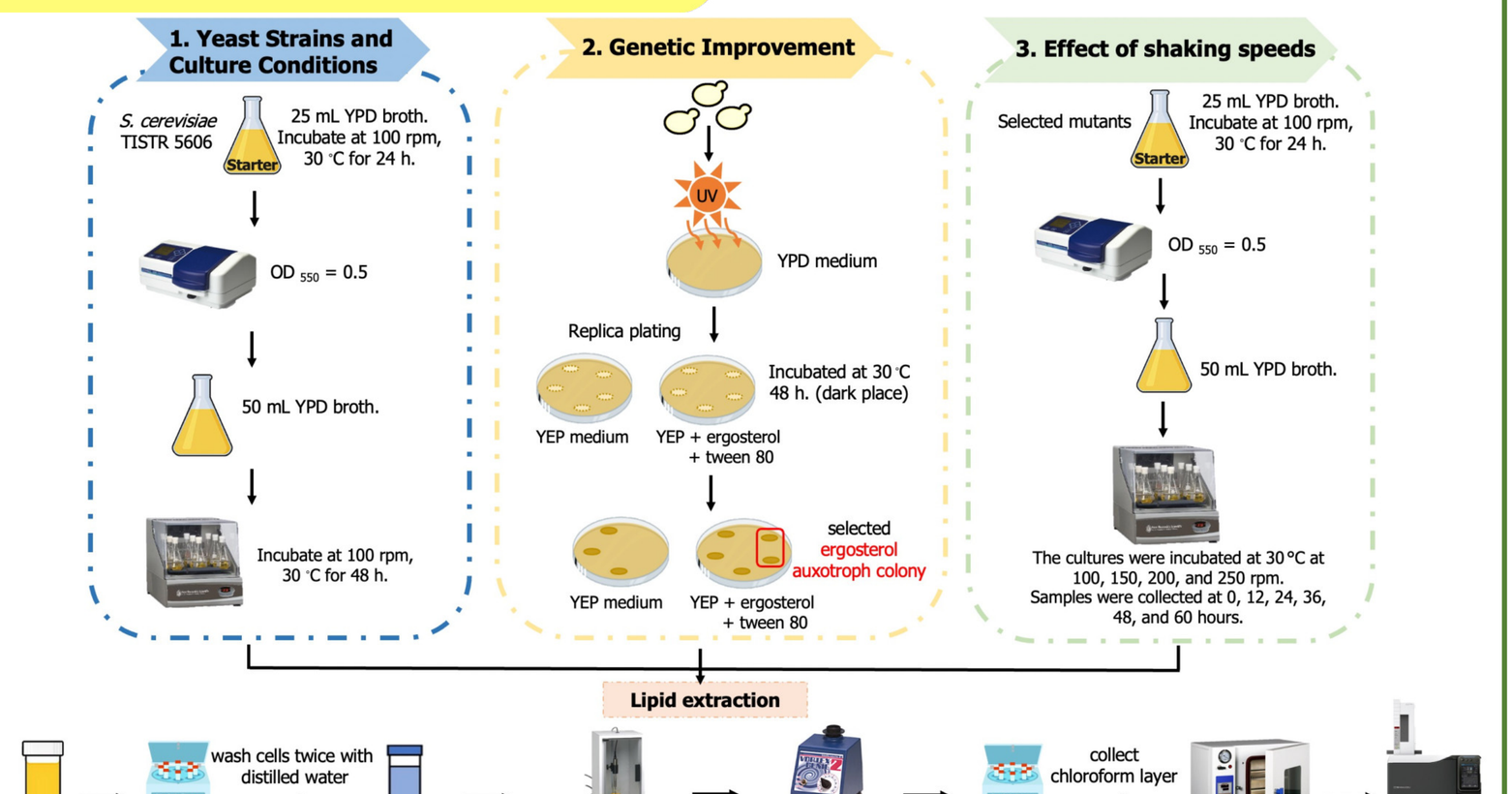


S. cerevisiae has a strong ability to accumulate lipids and can convert them into acetyl-CoA, a key precursor in squalene synthesis.

3. Objectives

This study focuses on enhancing squalene production in *S. cerevisiae* TISTR 5606 through UV mutagenesis and evaluating the effects of shaking speed and incubation time on squalene accumulation. The findings aim to contribute to the development of efficient and sustainable microbial platform for squalene production.

4. Materials and Methods



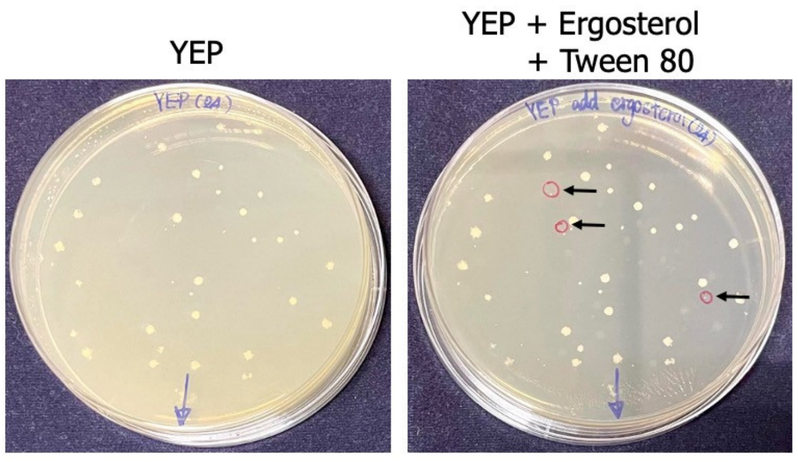


Fig. 1 Screening of UV-mutagenized *S. cerevisiae* for enhanced squalene accumulation. Colonies grown on YEP medium (control) and colonies grown on YEP medium supplemented with ergosterol, used to identify mutants with altered sterol biosynthesis. Colonies showing impaired ergosterol synthesis and potential squalene overaccumulation are indicated by arrows.

5. Results and Discussion

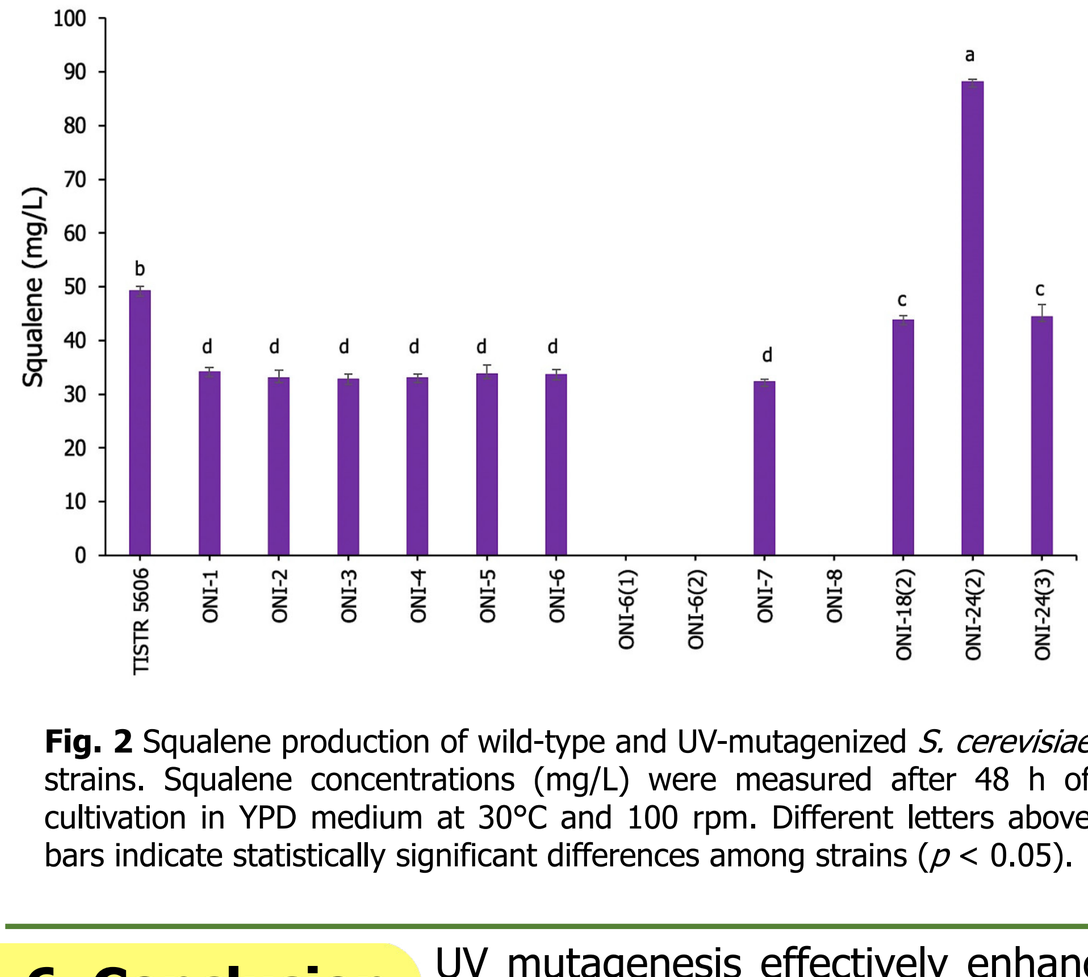


Fig. 2 Squalene production of wild-type and UV-mutagenized *S. cerevisiae* strains. Squalene concentrations (mg/L) were measured after 48 h of cultivation in YPD medium at 30°C and 100 rpm. Different letters above bars indicate statistically significant differences among strains ($p < 0.05$).

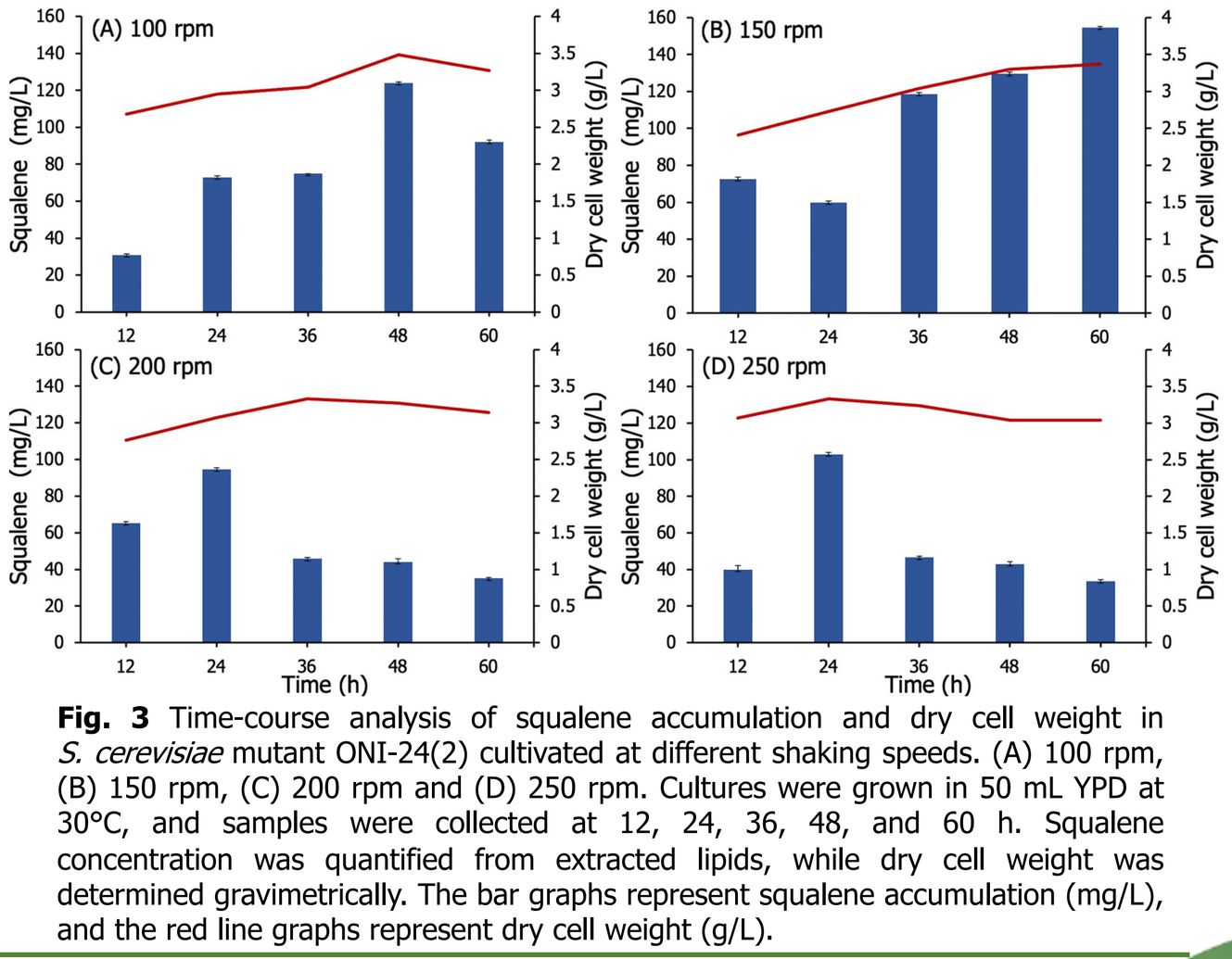


Fig. 3 Time-course analysis of squalene accumulation and dry cell weight in *S. cerevisiae* mutant ONI-24(2) cultivated at different shaking speeds. (A) 100 rpm, (B) 150 rpm, (C) 200 rpm and (D) 250 rpm. Cultures were grown in 50 mL YPD at 30°C, and samples were collected at 12, 24, 36, 48, and 60 h. Squalene concentration was quantified from extracted lipids, while dry cell weight was determined gravimetrically. The bar graphs represent squalene accumulation (mg/L), and the red line graphs represent dry cell weight (g/L).

6. Conclusion

UV mutagenesis effectively enhanced squalene production in *S. cerevisiae* TISTR 5606, and process optimization revealed that moderate aeration (150 rpm) and extended cultivation time maximized squalene accumulation. This study highlights the combined importance of genetic improvement and cultivation control for developing efficient microbial squalene production systems.

Acknowledgments

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