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PROSPECTIVE DIRECTIONS IN BIOTECHNOLOGY OF SQUALENE PRODUCTION USING *SACCHAROMYCES CEREVISIAE*

***D. Usov*[1]**, *O. Bliznjuk*[2], *N. Masalitina*[3]

[1] *Master's student, Department of Biotechnology, Biophysics and Analytical Chemistry, NTU "KhPI", Kharkiv, Ukraine*

[2] *Full Professor, Department of Biotechnology, Biophysics and Analytical Chemistry, Doctor of Technical Sciences, NTU "KhPI", Kharkiv, Ukraine*

[3] *Associate Professor, Department of Biotechnology, Biophysics and Analytical Chemistry, PhD in Technical Sciences, NTU "KhPI", Kharkiv, Ukraine*

usovd137@gmail.com

Squalene is a natural triterpenoid belonging to the class of isoprenoid compounds and serves as an intermediate metabolite in the biosynthesis of sterols — cholesterol in animals and ergosterol in yeasts. Due to its unique properties, including antioxidant, anticancer, anti-inflammatory, and immunostimulatory activities, squalene is widely used in the pharmaceutical industry as an adjuvant in vaccines, in cosmetology as a component of anti-aging products, and in the food industry as a nutraceutical. Traditionally, the main source of squalene has been the liver oil of deep-sea sharks, which may contain up to 40–70% of total lipids. However, this extraction method is environmentally hazardous, unethical, and economically unsustainable, which determines the need for alternative biotechnological production methods from renewable sources.

One of the most promising microbial producers of squalene is the yeast Saccharomyces cerevisiae. This species has GRAS (Generally Recognized As Safe) status, a well-studied metabolic regulation system, and a wide range of available genetic tools and industrial-scale cultivation technologies.

In yeast, squalene biosynthesis proceeds through the mevalonate pathway, where acetyl-CoA is converted to isopentenyl pyrophosphate, then to farnesyl pyrophosphate, and finally to squalene by the enzyme squalene synthase (ERG9). Under normal conditions, most squalene is further oxidized by squalene epoxidase (ERG1) to 2,3-oxidosqualene — a precursor of ergosterol. Therefore, one of the main tasks of modern biotechnology is to redirect the metabolic flux toward squalene accumulation while simultaneously suppressing its further oxidation.

Several effective strategies for enhancing squalene biosynthesis in S. cerevisiae have been developed. The first is genetic modification through overexpression of enzymes that limit the rate of the mevalonate cycle. The most significant is the HMG1 gene encoding HMG-CoA reductase — the key rate-limiting enzyme of mevalonic acid synthesis. Overexpression of a mutant, membrane-independent form of this enzyme results in a several-fold increase in intracellular squalene levels.

Another approach involves the downregulation of ERG1, responsible for converting squalene into oxidosqualene, which effectively reduces the metabolic flux toward sterol synthesis.

A second promising direction is the use of precise promoter engineering within the mevalonate pathway genes. This allows regulation of enzyme expression not only through overexpression but also via fine-tuned balancing of enzyme activities, preventing cell homeostasis disruption. Successful examples include promoter editing of ERG20, ERG9, and IDI1, which led to a 3–5-fold increase in squalene yield compared with wild-type strains.

A third important factor is the optimization of cultivation conditions. It is known that squalene accumulation is closely associated with cellular stress, particularly under oxygen limitation or oxidative stress. The addition of certain inducers, such as ethanol or medium-chain fatty acids, can stimulate enhanced accumulation of lipophilic compounds. The choice of carbon source also plays a crucial role: glucose, glycerol, and ethanol differentially affect the flux through the mevalonate pathway. Glycerol, as a sole carbon source, promotes higher squalene content due to increased cytosolic acetyl-CoA availability.

Equally important is lipid particle engineering, as squalene is stored in cells as a neutral lipid. Modifying the composition of lipid droplets and overexpressing proteins responsible for their formation enables the creation of additional reservoirs for squalene storage, reducing its cytotoxicity. Another promising approach involves developing hybrid yeasts or co-culture systems in which one strain produces precursor metabolites, while another accumulates the target compound.

Intensive studies are also being conducted in the field of bioreactor technologies. At an industrial scale, the most effective processes combine controlled aeration, stable temperature maintenance at 28–30 °C, pH around 6.0, and moderate substrate feeding under fed-batch mode. Under these conditions, squalene concentrations exceeding 200 mg/g dry cell weight can be achieved, which is competitive for pharmaceutical applications. Moreover, the use of nanofiltration and two-phase extraction systems facilitates squalene recovery without damaging cell integrity.

Future perspectives include the search for novel metabolic regulators capable of directing flux toward triterpenoid synthesis, as well as the co-expression of squalene synthase with proteins stabilizing membrane-associated enzymes. Computational modeling and systems biology approaches are being increasingly employed to construct in silico metabolic network models and predict optimal intervention points in yeast metabolism.

Thus, modern biotechnology of squalene production using Saccharomyces cerevisiae integrates genetic, metabolic, and process engineering approaches. These strategies enable high-yield, sustainable production without reliance on animal-derived raw materials, making this field highly promising for the sustainable development of pharmaceutical, cosmetic, and food industries.

Further studies should focus on integrating synthetic promoters, constructing biosynthetic cassettes, and developing universal platform strains for efficient large-scale industrial production of squalene.

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