Table S1. Research progress in the biosynthesis of squalene in yeast.

Strain	Strategies	Yield		References
		Shaking flask	Fermentation	
S. cerevisiae	1. Enhancing precursor supply: Simultaneous knockdown of *PAH1* and *OPI1* improved ER function. 2. Knockdown both of *HRD1* and *OPI1* increased the storage capacity of lipid droplets. 3. The combined deletion of *UTH1* and *ECM33* enhanced the robustness of the engineered strain.	/	55.82 g/L (5 L bioreactor)	[1]
S. cerevisiae	1. Employing computer-aided design: It provides a framework for prediction and mining of terpenoid exporters, which can be used to identify exporters of other terpenoids. 2. Extracellular secretion: the utilization of ATP-binding cassette transporter Pdr5 and oxysterol-binding homology protein Osh3 could promote squalene efflux. Overexpression of *Pdr5* and *Osh3* increased the secretion of squalene by 141.1-fold, and the total amount of squalene reached 3242.33 mg/L in a 3 L bioreactor.		3242.33 mg/L (3 L bioreactor)	[2]

S. cerevisiae	1. Enhancing precursor supply: 1.1 Overexpression and multi-copy integration of *tHMG1* resulted in the highest yield of squalene of 550.89 mg/L. 1.2 By overexpressing *TGL3*, *TGL4*, FAA_1, and FAA_4, the supply of acetyl-CoA was improved and squalene synthesis was promoted. 2. Alleviating consumption pathways: N-degron labeling technology was used to reduce the activity of Erg1 protein by controlled degradation while maintaining normal cell growth.	1024.88 mg/L	6.53 g/L (5 L bioreactor)	[3]
S. cerevisiae	1. Enhancing precursor supply: 1.1 Tagging MLS to the N-terminus of the gene introduced the entire MVA pathway into mitochondria and improved acetyl-CoA flux, then the key genes such as *ERG20* and *ERG9* were further overexpressed. 1.2 Organelle engineering: overexpression of key genes such as *ERG19* and *IDI1* reduced metabolic toxicity in mitochondria. Finally, cytoplasmic and mitochondrial engineering were combined to overexpress *tHMG1* to enhance the effect of the MVA pathway.	3000.63 ± 75.3 mg/L	21.1 g/L (5 L bioreactor)	[4]
S. cerevisiae	1. Enhancing precursor supply: 1.1 Organelle engineering: Enhance the expression of *ERG10*, *ERG13*, *tHMG1*, *NADH-HMGR*, *ERG12*, *ERG8*, *MVD1*, *IDI1*, *ERG20*, *ERG9*, *ANT1* in the	1698.02 mg/L	11.00 g/L (5 L bioreactor)	[5]

	peroxisome and dual cytoplasmic-peroxisomal engineering to overproduce squalene. 2. Cofactor engineering: Introduce heterologous efficient enzyme to relieve cofactor pressure by enhancing the expression of NADH-HMGR from *Silicabacter pomeroyi*. And overexpression of *IDP2* and *IDP3* provided sufficient NADPH to the squalene synthesis pathway in the middle compartment of the peroxisome. 3. Alleviating consumption pathways: The excessive consumption of squalene was reduced by replacing the *pERG1* with *pHXT1*.			
Y. lipolytica	1. Extracellular secretion: 1.1 A series of ABC transporters and oxosterol-binding proteins (OSH) were screened, among which the combination of SNQ2 and OSH3 showed the most significant secretion of squalene. 1.2 A carrier protein-mediated metabolite transport system was designed by fusing the binding domain of OSH3 with a secretory signal peptide. 2. Enhancing precursor supply: overexpression of *AtoB*, *HMGS*, *HMGR*, *ERG12*, *ERG8*, *ERG19*, *IDI*, *ERG20*, *ACL1*, *ACL2*, *YHM2*, *AMPD*, *EfMvaE*, *EfMvaS*^{A110G}, the yield of squalene was 160-fold higher than that of Po1f.	/	12.61 g/L (3.43 g/L extracellular secretion) (3 L bioreactor)	[6]
Y. lipolytica	1. Enhancing precursor supply:	/	32.8 g/L (3 L bioreactor)	[7]

Y. lipolytica	1.1 Overexpression of *ERG10*, *ERG13*, *tHMGR*, *ERG12*, *ERG8*, *ERG19*, *IDI*, *ERG20*, *ERG9* and introduced *MvaE* and *MvaS* genes to enhance metabolic flux in the upstream pathway. 1.2 Squalene compartmentalization in peroxisomes.	2549.1mg/L	51.2 g/L (5 L bioreactor)	[8]
Y. lipolytica	1. Enhancing precursor supply: 1.1. Key gene screening and multicopy integration in the MVA pathway: *tHMG1*, *IDI1*, and *ERG9*. 1.2 Organelle engineering: the entire MVA pathway has been integrated into the peroxisomal and cytoplasm-peroxisome engineering (peroxisomal signal peptide screening) for overexpression and co-expression of *POT1*, *PXA1*/2, *and POX1*/2 to prevent excessive lipid synthesis from consuming carbon flux in the MVA pathway. 1. The remodeling of homologous recombination efficiency results in a 10-fold increase in the rate of homologous recombination.	2.4 g/L	35 g/L (5 L bioreactor)	[9]
	2. Enhancing precursor supply: 2.1 Organelle engineering: the entire MVA pathway has been integrated into the chromosome to enhance squalene production. 2.2 *ACL*, *MvaE* and *tHMG1* were overexpressed, and ACS^{L641P} was integrated to enhance acetyl-CoA utilization by the MVA pathway.			

	3. Terbinafine was added at a certain concentration.			
Y. lipolytica	1. Enhancing precursor supply: 1.1 The efficiency of acetyl-CoA synthesis is improved by adjusting the carbon flux of pyruvate dehydrogenation to promote the binding of acetate to CoA or the cleavage of citrate. 1.2 Overexpression of *Salmonella enterica (acs*)* or *ylACL1* increased intracellular acetyl- CoA levels by more than 50%. 1.3 By iterative chromosome integration of *ylHMG1, acs** and *ylACL1,* squalene production was increased by 16.4-fold.	10 mg/g DCW	/	[10]

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