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Review Article

Metabolic Engineering of *Saccharomyces cerevisiae* for Enhanced Steroid Biosynthesis: Advances, Strategies, and Industrial Implications

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ABSTRACT

Saccharomyces cerevisiae, an extensively studied model yeast has received significant attention largely due to its well-characterised genomic materials, ease of genetic manipulation, and usefulness in the production of steroidal compounds.. Steroids are essential molecules used extensively in pharmaceuticals, cosmetics, and agricultural industries. However, chemical synthesis and extraction techniques for steroids are often inefficient and environmentally taxing. Advances in metabolic engineering and synthetic biology have facilitated the reprogramming of *Saccharomyces cerevisiae* to enhance the biosynthesis of both steroid precursors and specific steroid hormones. This review aimed to explore and consolidate advances in the metabolic engineering of *S. cerevisiae* for enhanced steroid synthesis. The content of this article also provides an in-depth review that focuses on enzyme overexpression, gene knockouts, heterologous pathway integration, and utilisation of synthetic biology devices such as CRISPR-Cas9. Metabolic flux analysis and omics integration were also assessed for their role in improving biosynthetic efficiency. In conclusion, *S. cerevisiae* is a promising biofactory for steroid production; however, to achieve commercial viability, it will require further work on strain optimisation, process scale-up, and regulatory compliance. Future research should focus on refining synthetic pathways, reducing metabolic burdens, and aligning biotechnological innovations with industrial and environmental standards.

Keywords: Ergosterol; Metabolic engineering; *Saccharomyces cerevisiae*; Steroid biosynthesis; Synthetic biology

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1.0 INTRODUCTION

Saccharomyces cerevisiae possesses numerous advantageous traits, including a well-characterised genome, robust growth, and a versatile metabolism, making it an ideal microorganism for metabolic engineering and biotechnological applications (Jimoh *et al*., 2012a; Lacerda *et al.*, 2020). Steroids are a class of natural compounds with diverse physiological functions and commercial applications which have gained substantial interest due to their pharmaceutical, agricultural, and industrial significance (Dembitsky, 2023). Traditionally, steroids are obtained through natural synthesis or chemical extraction, which can be costly and inefficient (Almazrouei *et al.,* 2023). Consequently, there is an urgent need for alternative methods to produce steroids sustainably and economically.

The potential of *S. cerevisiae* as a microbial platform for steroid biosynthesis has been explored due to its well-established genetic manipulation techniques and versatile metabolic pathways (Nevoigt, 2008; Parapouli *et al.,* 2020). Leveraging on the inherent metabolic activities of *S. cerevisiae* and engineering its pathways offer the possibility of developing efficient and sustainable processes for steroid production (Hande Tekarslan-Sahin, 2021). Advances in metabolic engineering and synthetic biology have permitted the manipulation of *S. cerevisiae* metabolic pathways,

allowing the redirection of carbon flux toward desired products (Jimoh *et al.*, 2013a; Jimoh *et al.*, 2013b; Chen *et al.,* 2020), thus engineering natural pathways involved in the steroid precursors' biosynthesis and the introduction of heterologous enzymes or pathways increases steroid production (Batth *et al.,* 2020).

Despite the progress achieved in the metabolic engineering of *S. cerevisiae* for steroid production, challenges such as fine-tuning the metabolic flux, ensuring proper regulation of enzyme activities, addressing substrate availability, and optimising fermentation conditions for efficient steroid production still remain (Nevoigt, 2008; Jimoh *et al.,* 2012a; Jimoh *et al.,* 2012b; Hande Tekarslan-Sahin, 2021). By modifying the expression levels of main enzymes and regulating the metabolic flux, researchers have successfully engineered *S. cerevisiae* strains capable of producing specific steroid compounds (Chu *et al,.*2020; Riley and Guss, 2021). This article intends to review the metabolic activities of *S. cerevisiae* in steroid production and the genetic engineering strategies employed to enhance steroid biosynthesis. Furthermore, it also discusses the prospects and challenges associated with harnessing *S. cerevisiae* for sustainable and economically viable steroid production.

2.0 GENETIC ENGINEERING APPROACHES FOR STEROID BIOSYNTHESIS BY *Saccharomyces cerevisiae*

Saccharomyces cerevisiae can be engineered to enhance steroid biosynthesis through various strategies of genetic manipulation. These approaches involve modifying indigenous pathways, introducing heterologous genes, optimising metabolic flux, and integrating omics technologies during steroid synthesis.

2.1 Manipulation of Indigenous Pathways

Manipulating native pathways in *S. cerevisiae* is an important strategy for enhancing steroid biosynthesis. By overexpression vital enzymes or knocking out/downregulating competitive pathways, it is possible to redirect metabolic flux towards steroid production.

a. Overexpression of Essential Enzymes

Overexpression of core enzymes involved in steroid biosynthesis is a common strategy to boost steroid synthesis in *S. cerevisiae*. These core enzymes are:

i. 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGR or HMG-CoA reductase): Overexpression of HMG-CoA reductase, a rate-limiting enzyme in the mevalonate pathway, increases the synthesis of sterols and steroid precursors in *S. cerevisiae* (Nora *et al.,* 2019). Yeast cells have two (2) HMGRs encoded by paralogous genes (namely, HMG1 and HMG2). Although substituting for one another in generating sufficient HMGR activity when the other gene is deleted is possible, their regulation varies significantly (Jordá and Puig, 2020). A stable protein (Hmg1p) is controlled at the transcriptional and translational levels, whereas Hmg2p is regulated at the post-translational level. Overexpression of HMG1 and HMG2 genes in *S. cerevisiae* yields a significant improvement in the production of amorpha-4,11-diene, , a precursor of the antimalarial drug artemisinin compared to the control strain (Westfall *et al.,* 2012; Karaca *et al.,* 2024); while overexpression of HMG1 gene enhanced the production of ergosterol (Hu *et al.,* 2017).

ii. Ergosterol Biosynthesis Genes: The overexpression of genes involved in the ergosterol biosynthesis pathway can enhance the production of sterols and provide more precursors for steroid biosynthesis (Ruan *et al.,* 2017). The expression of the ERG9 gene, which encodes squalene synthase, occurs at the first dedicated step after farnesyl diphosphate in ergosterol biosynthesis. This contrasts with the ERG20 gene, which encodes farnesyl pyrophosphate synthetase (FPP synthase), a cytosolic enzyme that possesses geranyl transferase and dimethylallyltransferase activities (Parks *et al.,* 1995; Jordá and Puig, 2020). The FPP synthase catalyses the sequential 1-4 coupling of isopentenyl diphosphate (IPP) with geranyl diphosphate and dimethylallyl diphosphate, forming C15 farnesyl pyrophosphate units for sterol and isoprenoid biosynthesis in yeast, whereas ergosterol is the product of the sterol biosynthetic pathway (Bhattacharya *et al.,* 2018). Overexpression of the ERG9 and ERG20 genes in *S. cerevisiae* increased intracellular steroid yields (Callari *et al.,* 2018) and sterol synthesis, such as ergosterol. Additionally, the overexpression of ERG9 resulted in a significant rise in steroid titer compared to the control strain (Ma *et al.,* 2018).

b. Knockout or Downregulation of Competitive Pathways

Knocking out or downregulating competing pathways boosts steroid biosynthesis in *S. cerevisiae* by reducing the activity of competing metabolic pathways, thus directing the flux towards steroid production. Knockout or downregulation of the ergosterol biosynthesis pathway increases the availability of sterol precursors for steroid production through the use of:

i. Knockout of Ergosterol Biosynthesis Genes: Inhibiting or reducing the activity of the ergosterol biosynthesis pathway redirects metabolic flux towards steroid production (Bhattacharya *et al.,* 2018). The ERG3 is the structural gene in *S.*

cerevisiae for sterol delta-5-desaturase, which generates the C5 = C6 unsaturation in ergosterol synthesis (Smith and Parks, 1993). Knocking out of *S. cerevisiae* ERG3 and ERG5 genes increases the availability of sterol precursors, where knockout strains exhibit improved squalene synthesis, with a maximum squalene concentration than the control strain (Xia *et al.,* (2022); while knocking out of ERG20 and ERG9 genes also increases the availability of sterol precursors compared to the control strain (Lv *et al.,* 2016; Hu *et al.,* 2017). A cytochrome P-450 enzyme (C-22 sterol desaturase) encoded by ERG5 catalyses the formation of the C-22(23) double bond in the sterol side chain in ergosterol synthesis.

ii. Downregulation of Competing Pathways: Recent studies have demonstrated the effectiveness of knocking out or downregulating competing pathways, such as the ergosterol biosynthesis pathway and acetyl-CoA synthetase, for enhancing steroid biosynthesis in *S. cerevisiae*, thus reducing the activity of these pathways, increasing precursor availability and improving the production of sterols and steroidal compounds. Downregulation of the expression of the gene encoding acetaldehyde dehydrogenase (ALD6) in *S. cerevisiae* increases the availability of acetyl-CoA and improves steroid synthesis (Ostergaard *et al.,* 2019; Paramasivan and Mutturi, 2022). The downregulation of the acetyl-CoA synthetase gene (ACS1) results in a 2.3-fold increase in steroid synthesis (Hou *et al.,* 2017), while the downregulation of the ACS1 gene led to a 2.4-fold increase in ergosterol synthesis compared to the control strain (Sun *et al.,* 2021).

2.2 Introduction of Heterologous Pathways

Introducing heterologous pathways and incorporating genes from other organisms or constructing hybrid pathways in *S. cerevisiae* are effective strategies for enhancing its metabolic capabilities to improve the production of specific steroid compounds mainly through: .

i. Heterologous expression of pathway genes: Introducing genes from other organisms involved in steroid biosynthesis facilitates the production of specific steroid compounds in *S. cerevisiae*. The expression of heterologous genes from *Mycobacterium neoaurum* in *S. cerevisiae* increases the yield of multiple steroidal intermediates, demonstrating the potential of introducing new biosynthetic pathways for steroid production (Fernández-Cabezón *et al.,* 2018; Wang *et al.,* 2022 and Zao *et al.,* 2023).

ii. Hybrid pathway engineering: Construction of a hybrid pathway by integrating genes from *Saccharomyces cerevisiae* and *Yarrowia lipolytica* enhances steroid production with improved titers and yields compared to the indigenous pathway in *S. cerevisiae*, showcasing the potential of combining heterologous elements for enhanced steroid biosynthesis (Chae *et al.,* 2017). Also, a hybrid pathway combining elements from *S. cerevisiae* and plants has been constructed to produce specific steroids (Zhang *et al.,* 2022).

2.3 Optimisation of Metabolic Flux

Optimising metabolic flux is crucial for improving steroid biosynthesis in *S. cerevisiae*. Integrating omics technologies, including metabolomics, transcriptomics, and proteomics, can provide significant insights into cellular responses and guide metabolic engineering strategies (Mende *et al*., 2017). Balancing the supply of precursor metabolites [(acetyl-CoA and isopentenyl pyrophosphate (IPP))]increases precursor availability achieved through the manipulation of relevant pathways, such as increasing carbon flux towards acetyl-CoA production (Jimoh *et al.,* 2022). Furthermore, modulating the expression levels of transcription factors, regulatory elements, and promoters involved in steroid biosynthesis can fine-tune metabolic flux and improve steroid production. These regulatory elements were also optimised through promoter engineering or synthetic biology approaches (Ham *et al.,* 2008).

2.4 Integration of Omics Technologies in Strain Engineering

Metabolic engineering through Omics-guided approaches requires integrating transcriptomics, proteomics, and metabolomics data, enabling a comprehensive understanding of cellular responses and identifying targets for strain engineering. Omics-guided approaches aid in identifying gene targets for overexpression, knockout, or downregulation, leading to improved steroid production (Asadollahi *et al.,* 2008). This can be achieved through:

i. Systems biology and metabolic modelling: Integrating omics data with genome-scale metabolic models aids the prediction and optimisation of metabolic fluxes, guiding metabolic engineering efforts (Sen and Orešič, 2023). A systems biology approach combining transcriptomics, proteomics, and metabolic modelling has been employed to boost steroid synthesis in *S. cerevisiae* by integrating transcriptomic and proteomic data with a genome-scale metabolic model to identify metabolic challenges and engineer key enzymes and pathways (Lopez-Barbera *et al.,* 2024). This integration significantly increased steroid production by redirecting metabolic flux towards the desired products (Ko *et al.,* 2020; Rainha *et al.,* 2020). These studies demonstrated the power of

integrating omics technologies with systems biology and metabolic modelling approaches to optimise metabolic flux and enhance steroid production in *S. cerevisiae*. By leveraging these tools, it becomes possible to gain comprehensive insights into cellular responses, identify key targets for strain engineering, and improve the production of steroids.

ii. Omics-guided target identification: Integration of transcriptomics, proteomics, and metabolomics data helps identify potential targets for strain engineering to enhance metabolite production (Fu *et al.,* 2024). Also, Guo *et al.* (2018) integrated multi-omics data to identify the main regulatory genes and metabolic pathways involved in *S. cerevisiae* steroid biosynthesis, thus successfully engineering the identified targets, significantly improving steroid production (Chen *et al.,* 2020). Combination of transcriptomics and metabolomics analyses in investigation of metabolic response of *S. cerevisiae* showed improved steroid production (Lopez-Barbera *et al.,* 2024); thus, the omics-guided approach enabled the identification of main metabolic nodes, which led to the engineering of strain-specific modifications, resulting in the significant improvement in steroid production (Mendes *et al.,*2017).

iii. Metabolic modelling and omics integration: Integrating omics data with genome-scale metabolic models permits the prediction and optimisation of metabolic fluxes, aiding strain engineering efforts. According to Zhang *et al.,* (2022), transcriptomics and metabolomics data integrated with genome-scale metabolic modelling of *S. cerevisiae* facilitated the identification of key genetic targets and metabolic engineering strategies which enhance steroid titers and yields by the engineered strains compared to the wild strain.

3.0 Future Perspectives and Challenges

The utilisation of *S. cerevisiae* for steroid production holds significant promise but also presents specific future perspectives and challenges. These future perspectives and challenges highlight reasons for continual research and development to unlock the complete potential of *S. cerevisiae* for steroid production. Focus should be on:

a. Advances in Synthetic Biology and Metabolic Engineering

Advancements in synthetic biology have contributed significantly to metabolic engineering and the production of steroids in *S. cerevisiae*. Recent years have witnessed significant advances in synthetic biology and metabolic engineering, which have revolutionised the field of steroid production in *S. cerevisiae*. These include;

i. Development of novel genetic tools: Synthetic biology has led to novel genetic tools that precisely control gene expression and metabolic pathways. For example, the clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) system has revolutionised genome editing in *S. cerevisiae*, allowing for efficient gene knockout, gene integration, and targeted modifications (Jakočiūnas *et al.,* 2015). The use of CRISPR-Cas9 for multiplex genome engineering in *S. cerevisiae* enabled the simultaneous modification of multiple genes involved in steroid biosynthesis, and this approach provided a powerful tool for strain engineering and optimisation of steroid production (Wu *et al*., 2025). Also, Bao *et al.,* (2015) also reported using the CRISPR-Cas9 system for efficient multiplex genome engineering in *S. cerevisiae*, achieving simultaneous modifications of multiple genes involved in steroid biosynthesis, enabling precise control over metabolic pathways.

ii. Design and construction of synthetic pathways: Synthetic biology facilitates the design and construction of synthetic pathways to produce desired compounds. Through the *de novo* design and synthesis of enzyme-encoding genes, novel biosynthetic pathways can be engineered in *S. cerevisiae*. Ro *et al.* (2006) constructed a synthetic pathway for synthesising hydrocortisone, a valuable steroid, in *S. cerevisiae*, thus designing and assembling a pathway consisting of multiple enzymatic reactions that result in the production of hydrocortisone from simple carbon sources. The construction of a synthetic mevalonate pathway in *S. cerevisiae* for enhanced production of isoprenoid intermediates, including steroid precursors, by integrating multiple heterologous genes, also led to a significant increase in precursor production and overall steroid titers (DeLoache *et al.,* 2015; Smith and Chekan, 2023; Kim *et al.,* 2024). These examples highlight synthetic biology's significant contributions to advancing steroid production in *S. cerevisiae*. The development of novel genetic tools and the design, and construction of synthetic pathways offer powerful approaches for strain engineering and the optimisation of steroid biosynthesis.

iii. Systems and synthetic biology approaches: Advances in systems and synthetic biology have provided comprehensive approaches for understanding and engineering metabolic networks. Integration of omics data, computational modelling, and high-throughput screening has enabled the design and optimisation

of metabolic pathways. Systems and synthetic biology methods were employed to engineer *S. cerevisiae* using computational modelling to identify primary genetic targets and optimise metabolic flux, substantially improving steroid production (Zhang *et al.,* 2021). The development of genetic tools, construction of synthetic pathways, and integration of systems biology approaches have paved the way for enhanced control over metabolic networks and improved production of steroids (Wang *et al.,* 2014).

b. Regulatory and Safety Considerations

As the field of steroid production in *S. cerevisiae* progresses, it is essential to address regulatory and safety considerations for successful implementation. Developing sustainable and safe bioprocesses, compliance with regulatory frameworks, and thorough risk assessments are crucial to successfully implement steroid production in an industrial setting (Wang *et al.,* 2014). Implementing regulatory frameworks for synthetic biology is important in metabolic engineering. Synthetic biology applications, including engineered microbial production of valuable compounds such as steroids, must comply with regulatory frameworks to ensure safety and public acceptance. Standardised regulatory frameworks are crucial for the reliable and sustainable deployment of synthetic biology technologies (Bohua *et al.,* 2023). Establishing global regulatory standards and governance mechanisms for synthetic biology justifies the need for risk assessment, biosafety measures, and effective communication between stakeholders to ensure the safe and effective use of synthetic biology in various applications is essential (Rainha *et al.,* 2020).

CONCLUSION

Saccharomyces cerevisiae has emerged as a versatile yeast for steroid production through metabolic engineering and synthetic biology approaches. The metabolic capabilities of *S. cerevisiae* and advancements in genetic manipulation tools and pathway optimisation strategies have enabled the biosynthesis of steroid precursors and specific steroids. Significant improvements in steroid production have been achieved through the overexpression of key enzymes, knockout or downregulation of competitive pathways, and integration of heterologous pathways. Furthermore, optimising metabolic flux and integrating omics technologies have further enhanced the efficiency of steroid biosynthesis in *S. cerevisiae*.

However, several challenges and considerations should be addressed for the productive implementation of *S. cerevisiae* for steroid production. Regulatory frameworks and safety assessments are crucial in ensuring the effective use of genetically modified organisms. Though economic viability is a key factor that requires optimising production processes, efficient feedstock utilisation, and careful scale-up considerations, synthetic biology and metabolic engineering prospects will offer exciting opportunities for further enhancing the production of steroids in *S. cerevisiae.* Advances in genetic tools, synthetic pathway design, and systems biology approaches will contribute to the development of a more economical and sustainable steroid production processes.

REFERENCES

Almazrouei, B., Islayem, D., Alskafi, F., Catacutan, M. K., Amna, R., Nasrat, S., Sizirici, B. and Yildiz, I. (2023). Steroid hormones in wastewater: Sources, treatments, environmental risks, and regulations. *Emerging Contaminants*, *9*(2):100210-100221. https://doi.org/10.1016/j.emcon.2023.100210

Asadollahi, M., Maury, J., Møller, K., Nielsen, K. F., Schalk, M., Clark, A., and Nielsen, J. (2008). Production of plant sesquiterpenes in *Saccharomyces cerevisiae*: Effect of ERG9 repression on sesquiterpene biosynthesis. *Biotechnology and Bioengineering*, 99(3):666-677. https://doi.org/10.1002/bit.21581

Bao, Z., Xiao, H., Liang, J., Zhang, L., Xiong, X., Sun, N., Si, T., and Zhao, H. (2015). Homology-integrated CRISPR-Cas (HI-CRISPR) system for one-step multigene disruption in *Saccharomyces cerevisiae*. *ACS Synthetic Biology*, *4*(5):585-594. https://doi.org/10.1021/sb500255k

Batth, R., Nicolle, C., Cuciurean, I. S., and Simonsen, H. T. (2020). Biosynthesis and industrial production of androsteroids. *Plants*, *9*(9):1144. https://doi.org/10.3390/plants9091144

Bhattacharya, S., Esquivel, B. D. and White, T. C. (2018). Overexpression or deletion of ergosterol biosynthesis genes alters doubling time, response to stress agents, and drug susceptibility in *Saccharomyces cerevisiae*. *mBio*, *9*(4):1291-1309. https://doi.org/10.1128/mBio.01291-18

Bohua, L., Yuexin, W., Yakun, O., Kunlan, Z., Huan, L., and Ruipeng, L. (2023). Ethical framework on risk governance of synthetic biology. *Journal of Biosafety and Biosecurity*, 5 (2): 45-56. https://doi.org/10.1016/j.jobb.2023.03.002

Callari, R., Meier, Y., Ravasio, D., and Heider, H. (2018). Dynamic control of *ERG20* and *ERG9* expression for improved casbene production in *Saccharomyces cerevisiae*. *Frontiers in Bioengineering and*

Biotechnology, *6*:160-170. https://doi.org/10.3389/fbioe.2018.00160

Chae, T.U., Choi, S.Y., Kim, J.W., Ko, Y.S. and Lee, S.Y. (2017). Recent advances in systems metabolic engineering tools and strategies. *Current Opinion in Biotechnology* 47:67-82. https://doi.org/10.1016/j.copbio.2017.06.007

Chen, R., Yang, S., Zhang, L., and Zhou, Y. J. (2020). Advanced strategies for production of natural products in yeast. *iScience*, *23*(3):100879-100902. https://doi.org/10.1016/j.isci.2020.100879

Chu, L. L., Montecillo, J. A. V., and Bae, H. (2020). Recent advances in the metabolic engineering of yeasts for ginsenoside biosynthesis. *Frontiers in Bioengineering and Biotechnology*, 8:139-157. https://doi.org/10.3389/fbioe.2020.00139

DeLoache, W. C., Russ, Z. N., Narcross, L., Gonzales, A. M., Martin, V. J., and Dueber, J. E. (2015). An enzyme-coupled biosensor enables (S)-reticuline production in yeast from glucose. *Nature Chemical Biology*, 11(7):465-471. https://doi.org/10.1038/nchembio.1816

Dembitsky, V. M. (2023). Biological activity and structural diversity of steroids containing aromatic rings, phosphate groups, or halogen atoms. *Molecules*, *28*(14), 5549-5602. https://doi.org/10.3390/molecules28145549

Fernández-Cabezón, L, Galán, B. and García, J.L. (2018) New insights on steroid biotechnology. *Frontiers in Microbiology,* 9:958.https://doi.org/10.3389/fmicb.2018.00958

Fu, S., Wang, L., Li, C., Zhao, Y., Zhang, N., Yan, L., Li, C. M., and Niu, Y. (2024). Integrated transcriptomic, proteomic, and metabolomic analyses revealed molecular mechanism for salt resistance in soybean (*Glycine max* L.) seedlings. *International Journal of Molecular Sciences*, *25*(24):13559-13590. https://doi.org/10.3390/ijms252413559

Guo, X.J., Xiao, W.H., Wang, Y., Yao, M.D., Zeng, B.X., Liu, H., Zhao, G.R and Yuan, Y.J. (2018). Metabolic engineering of *Saccharomyces cerevisiae* for 7-dehydrocholesterol overproduction. *Biotechnology and Biofuels,* 11192-11205. https://doi.org/10.1186/s13068-018-1194-9

Ham, T. S., Lee, S. K., Keasling, J. D., and Arkin, A. P. (2008). Design and construct a double inversion recombination switch for heritable sequential genetic memory. *PloS one*, *3*(7):2815-2825. https://doi.org/10.1371/journal.pone.0002815

Hande Tekarslan-Sahin, S. (2021). Metabolic engineering of *Saccharomyces cerevisiae* for Industrial Biotechnology. IntechOpen. https://doi: 10.5772/intechopen.96030

Hou, J., Qiu, C., Shen, Y., Li, H., and Bao, X. (2017). Engineering of *Saccharomyces cerevisiae* for the efficient co-utilisation of glucose and xylose. *FEMS yeast research*, 17(4),:1-11. https://doi.org/10.1093/femsyr/fox034

Hu, Z., He, B., Ma, L., Sun, Y., Niu, Y., and Zeng, B. (2017). Recent advances in ergosterol biosynthesis and regulation mechanisms in *Saccharomyces cerevisiae*. *Indian Journal of Microbiology*, *57*(3):270-277. https://doi.org/10.1007/s12088-017-0657-1

Jakočiūnas, T., Bonde, I., Herrgård, M., Harrison, S. J., Kristensen, M., Pedersen, L. E., Jensen, M. K., and Keasling, J. D. (2015a). Multiplex metabolic pathway engineering using CRISPR/Cas9 in *Saccharomyces cerevisiae*. *Metabolic Engineering*, *28*:213-222. https://doi.org/10.1016/j.ymben.2015.01.008

Jimoh, S. O., Ado, S. A., Ameh, J. B. and Whong, C. M.Z (2012a). Characteristics and diversity of yeast in locally fermented beverages sold in Nigeria. *World Journal of Engineering and Pure and Applied Science,* 2(2): 40-44.

Jimoh, S. O., Ado, S. A., Ameh, J. B. and Whong, C. M.Z (2012b). Osmotolerance and fermentative pattern of brewer's yeast. *World Journal of Life Science and Medical Research,* 2(2): 11-16.

Jimoh, S. O., Ado, S. A., Ameh, J. B. and Whong, C. M.Z. (2013a). Expression of *ADH1* and *ADH2* genes by ethanol-producing *Saccharomyces cerevisiae* strains using PCR analysis. *International Journal of Research and Advancement in Bioscience,* 3(1):15-23.

Jimoh, S. O., Ado, S. A., Ameh, J. B. and Whong, C. M.Z. (2013b). Heat-shock and ethanol-osmotic effect on fermentable yeast cells. *International Journal of Biology and Biological Sciences,* 2(6): 91-96.

Jimoh, S. O., Sanusi, S. A., Olaogun, F. A., Badmos-Oladapo, R. B. and Asafa-Adedimeji, K. O. (2022). Mevalonate pathway analysis of *Saccharomyces cerevisiae* during bioisoprene synthesis**.** *Novel Research in Microbiology Journal*, 6(6):1821-1835. https://doi.org/10.21608/nrmj.2022.276738

Jordá, T., and Puig, S. (2020). Regulation of Ergosterol Biosynthesis in *Saccharomyces cerevisiae*. *Genes*, *11*(7):795. https://doi.org/10.3390/genes11070795

Karaca, H., Kaya, M., Kapkac, H. A., Levent, S., Ozkay, Y., Ozan, S. D., Nielsen, J., and Krivoruchko, A. (2024). Metabolic engineering of *Saccharomyces cerevisiae* for enhanced taxadiene production. *Microbial Cell Factories*, *23*(1):241-250. https://doi.org/10.1186/s12934-024-02512-z

Kim, H., Lee, Y., Yu, J., Park, J.Y., Lee, J., Kim, S.G. and Hyun, Y. (2024). Production of the antimalarial drug precursor amorphadiene by microbial terpene synthase-like from the moss *Sanionia uncinata*. *Planta* 260:145-158. https://doi.org/10.1007/s00425-024-04558-0

Ko, Y.S., Kim, J.W., Lee, J.A., Han, T., Kim, G.B., Park, J.E., Lee, S.Y. (2020). Tools and strategies of systems metabolic engineering for the development of microbial

cell factories for chemical production. *Chemical Society Reviews*, 21;49(14):4615-4636. https://doi.org/10.1039/d0cs00155d

Lacerda, M. P., Oh, E. J., and Eckert, C. (2020). The model system *Saccharomyces cerevisiae* versus emerging non-model yeasts for the production of biofuels. *Life*, 10(11):299-319. https://doi.org/10.3390/life10110299

Lopez-Barbera, A., Abasolo, N., Torrell, H., Canela, N., and Fernández-Arroyo, S. (2024). Integrative transcriptomic and target metabolite analysis as a new tool for designing metabolic engineering in yeast. *Biomolecules*, 14(12):1536-1553. https://doi.org/10.3390/biom14121536

Lv, X., Wang, F., Zhou, P., Ye, L., Xie, W., Xu, H. and Yu, H (2016). Dual regulation of cytoplasmic and mitochondrial acetyl-CoA utilisation for improved isoprene production in *Saccharomyces cerevisiae*. *Nature Communications,* 7:12851-12863. https://doi.org/10.1038/ncomms12851

Ma, B. X., Ke, X., Tang, X. L., Zheng, R. C., and Zheng, Y. G. (2018). Rate-limiting steps in the *Saccharomyces cerevisiae* ergosterol pathway: towards improved ergosta-5,7-dien-3β-ol accumulation by metabolic engineering. *World Journal of Microbiology and Biotechnology*, 34(4):55. https://doi.org/10.1007/s11274-018-2440-9

Mendes, I., Sanchez, I., Franco-Duarte, R., Camarasa, C., Schuller, D., Dequin, S., and Sousa, M. J. (2017). Integrating transcriptomics and metabolomics for the analysis of the aroma profiles of *Saccharomyces cerevisiae* strains from diverse origins. *BMC genomics*, 18(1):455-468. https://doi.org/10.1186/s12864-017-3816-1

Nevoigt E. (2008). Progress in metabolic engineering of *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews,* 72(3):379–412. https://doi.org/10.1128/MMBR.00025-07

Nora, L. C., Westmann, C. A., Guazzaroni, M. E., Siddaiah, C., Gupta, V. K., and Silva-Rocha, R. (2019). Recent advances in plasmid-based tools for establishing novel microbial chassis. *Biotechnology Advances*, 37(8):107433. https://doi.org/10.1016/j.biotechadv.2019.107433

Ostergaard, S., Olsson, L., and Nielsen, J. (2019). Metabolic engineering of *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews,* 64(1):34–50. https://doi.org/10.1128/MMBR.64.1.34-50.2000

Paramasivan, K., and Mutturi, S. (2022). Recent advances in the microbial production of squalene. *World Journal of Microbiology and Biotechnology*, 38(5):91. https://doi.org/10.1007/s11274-022-03273-w

Parapouli, M., Vasileiadis, A., Afendra, A. S., and Hatziloukas, E. (2020). *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiology*, 6(1):1–31. https://doi.org/10.3934/microbiol.2020001

Parks, L.W., Smith, S.J. and Crowley, J.H. (1995), Biochemical and physiological effects of sterol alterations in yeast-A review. *Lipids*, 30:227-230. https://doi.org/10.1007/BF02537825

Rainha, J., Gomes, D., Rodrigues, L. R., and Rodrigues, J. L. (2020). Synthetic Biology Approaches to Engineer *Saccharomyces cerevisiae* towards the Industrial Production of Valuable Polyphenolic Compounds. *Life*, 10(5):56. https://doi.org/10.3390/life10050056

Riley, L. A., and Guss, A. M. (2021). Approaches to genetic tool development for rapid domestication of non-model microorganisms. *Biotechnology for biofuels*, 14(1), 30. https://doi.org/10.1186/s13068-020-01872-z

Ro, D. K., Paradise, E. M., Ouellet, M., Fisher, K. J., Newman, K. L., Ndungu, J. M., Ho, K. A., Eachus, R. A., Ham, T. S., Kirby, J., Chang, M. C., Withers, S. T., Shiba, Y., Sarpong, R., and Keasling, J. D. (2006). Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature*, 440(7086), 940–943. https://doi.org/10.1038/nature04640

Ruan R, Wang M, Liu X, Sun X, Chung K-R, Li H (2017) Functional analysis of two sterol regulatory element binding proteins in *Penicillium digitatum*. PLoS ONE 12(5): e0176485. https://doi.org/10.1371/journal.pone.0176485

Sen, P., and Orešič, M. (2023). Integrating omics data in genome-scale metabolic modeling: A methodological perspective for precision medicine. *Metabolites*, 13(7):855-874. https://doi.org/10.3390/metabo13070855

Smith, A. B., and Chekan, J. R. (2023). Engineering yeast for industrial-level production of the antimalarial drug artemisinin. *Trends in Biotechnology*, 41(3):267-269. https://doi.org/10.1016/j.tibtech.2022.12.007

Smith, S. J., and Parks, L. W. (1993). The ERG3 gene in *Saccharomyces cerevisiae* is required for the utilisation of respiratory substrates and in heme-deficient cells. *Yeast*, 9(11),:1177-1187. https://doi.org/10.1002/yea.320091104

Sun, Z. J., Lian, J. Z., Zhu, L., Jiang, Y. Q., Li, G. S., Xue, H. L., Wu, M. B., Yang, L. R., and Lin, J. P. (2021). Combined biosynthetic pathway engineering and storage pool expansion for high-level production of ergosterol in industrial *Saccharomyces cerevisiae*. *Frontiers in Bioengineering and Biotechnology*, 9:681666-681675. https://doi.org/10.3389/fbioe.2021.681666

Wang, C., Kim, J.H., and Kim, S.W. (2014). Synthetic biology and metabolic engineering for marine carotenoids: New opportunities and future

prospects. *Marine Drugs*, 12(9):4810-4832. https://doi.org/10.3390/md12094810

Wang, X.X., Ke, X., Liu, Z.Q. and Zheng, Y. (2022). Rational development of mycobacteria cell factory for advancing the steroid biomanufacturing. *World Journal of Microbiology and Biotechnology* 38:191. https://doi.org/10.1007/s11274-022-03369-3

Westfall, P. J., Pitera, D. J., Lenihan, J. R., Eng, D., Woolard, F. X., Regentin, R., Horning, T., Tsuruta, H., Melis, D. J., Owens, A., Fickes, S., Diola, D., Benjamin, K. R., Keasling, J. D., Leavell, M. D., McPhee, D. J., Renninger, N. S., Newman, J. D., and Paddon, C. J. (2012). Production of amorphadiene in yeast, and its conversion to dihydroartemisinic acid, precursor to the antimalarial agent artemisinin. *Proceedings of the National Academy of Sciences of the United States of America*, 109(3):111-118. https://doi.org/10.1073/pnas.1110740109

Wu, X., Wan, X., Yu, H., and Liu, H. (2025). Recent advances in CRISPR-Cas system for *Saccharomyces cerevisiae* engineering. *Biotechnology Advances*, 81:108557. https://doi.org/10.1016/j.biotechadv.2025.108557.

Xia L, Lv Y, Liu S, Yu S, Zeng W and Zhou J (2022). Enhancing Squalene Production in *Saccharomyces cerevisiae* by Metabolic Engineering and Random Mutagenesis. *Frontiers in Chemical Engineering.* 3:790261. https://doi.org/10.3389/fceng.2021.790261

Zhang Y, Guo X, Yang H and Shi S (2022). The studies in constructing yeast cell factories for the production of fatty acid alkyl esters. *Frontiers in Bioengineering and Biotechnology.* 9:799032. https://doi.org/10.3389/fbioe.2021.799032

Zhang, H. W., Lv, C. Zhang, L. J., Guo, X., Shen, Y. W., Nagle, D. G., Zhou, Y. D., Liu, S. H., Zhang, W. D., and Luan, X. (2021). Application of omics- and multi-omics-based techniques for natural product target discovery. *Biomedicine and Pharmacotherapy*: 141:111833. https://doi.org/10.1016/j.biopha.2021.111833

Zhao, M., Li, X., Xiong, L., Liu, K., Liu, Y., Xue, Z., and Han, R. (2023). Green manufacturing of steroids via *Mycolicbacteria*: Current Status and development trends. *Fermentation*, 9(10):890. https://doi.org/10.3390/fermentation9100890.