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ENGINEERED YEAST STRAINS FOR SUSTAINABLE PRODUCTION OF ANTIMALARIAL DRUG PRECURSORS

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Abstract

The growing global demand for artemisinin-based antimalarial therapies necessitates the development of sustainable and scalable methods for precursor production. This study presents the systematic metabolic engineering of Saccharomyces cerevisiae for the biosynthesis of amorphadiene, a key precursor to artemisinin. Four yeast strains (Y-AM1 to Y-AM4) were progressively engineered through heterologous gene integration, promoter modulation, pathway optimization, and transcriptional fine-tuning. Among these, strain Y-AM4 exhibited the highest amorphadiene titer at 310 mg/L in batch fermentation, representing a substantial improvement over initial constructs. Transcriptomic analysis revealed significant upregulation of pivotal genes such as HMGR and ERG20, directly correlating with enhanced precursor flux. Further enhancement was achieved through adaptive laboratory evolution, which improved growth rate, biomass yield, and stress tolerance. Fed-batch fermentation of the evolved strain increased amorphadiene production to 455 mg/L, a 47% improvement over batch cultivation. The analysis showed that the engineered pathway produced a product that was at least 96% pure. By combining gene expression studies and pathway analyses, the team developed a series of engineering steps and found different regulation in the strains of different productivity. When genetic changes are combined with improving bioprocesses, producing the needed chemicals is more affordable and practical than using plants. This study proves that yeasts engineered for biosynthesis can turn out valuable antimalarial drugs with greater strength and significantly lower costs.

Keywords: Amorphadiene, Metabolic Engineering, Saccharomyces Cerevisiae, Artemisinin Precursor, Synthetic Biology, Fed-Batch Fermentation.

1. INTRODUCTION

The world's growing demand for antimalarial drugs such as those made with artemisinin, calls for examining effective and scalable methods of producing their precursors. The use of artemisinin from *Artemisia annua* is fundamental in artemisinin-based combination therapies, the primary choice for *Plasmodium falciparum* malaria (Hanboonkunupakarn & White, 2020). Despite its competence, having plants as the main source still has problems such as unpredictable results, restrictions by climate and risks from the environment (Dogan et al., 2021). The constraints involved in making artemisinin can be overcome by making changes to yeast which is used as a microbial host, to provide the chemical substances needed for synthesis. The special way yeast is being engineered helps reliably supply large volumes of key chemicals (Pyne et al., 2023). Advanced metabolic routes can now be installed in yeast using synthetic biology to support the manufacture of artemisinin precursors. Diverse biotechnology applications can benefit a lot from the improved use of yeasts in microbial cell factories (Ding & Ye, 2023).

Metabolic engineering makes it possible to change cells so that they make useful substances, for example, renewable biofuels and medications for cancer (Radivojević et al., 2020). Industries are paying more attention to using microbes to make biofuels and chemicals, since it allows them to use renewable resources and produce needed building blocks more cheaply than before (Baptista et al., 2021). With the use of genetic engineering, yeast metabolism can be adjusted to drive up the availability of precursors, the progress along production routes and the production of desirable products. Because yeast is predictable, easy to alter genetically and can resist a range of natural conditions, it is a top choice for making many biochemicals. Manipulating plant metabolism can maximize the production of useful substances and raise the economic or food value of plant species (Selma et al., 2023). This technique changes genes and regulation that oversee plant metabolism and causes the plant's metabolism to be different (Selma et al., 2023).

Engineered yeast is used for artemisinin precursor production which requires decisions on route, adding needed genes and optimizing the strain. In the beginning, a suitable metabolic pathway is selected for making the target precursor, generally amorpha-4,11-diene. Amorpha-4,11-diene is needed for making artemisinin and forms part of the isoprenoid pathway using isoprenyl pyrophosphate and dimethylallyl pyrophosphate. Much of the energy that cells need for work is provided by glycolysis, the tricarboxylic acid cycle and the pentose phosphate pathway (Wu et al., 2023). Heterologous genes supplying the required amorpha-4,11-diene-producing enzymes, including amorphadiene synthase, must be introduced into the yeast genome to start the pathway. Altering genes and chemicals in fungal endophytes increases their bioactive substances, helping to fill the gap in natural supplies (Rai et al., 2022).

When the relevant genes are in the yeast, you must optimize the yeast so it makes these necessary compounds well. Some of these measures are increasing the activity of key enzymes, changing the use of certain codons and improving protein folding and stability. Still, changing the mix of carbon, nitrogen and phosphate in the medium often

increases the yield of the target products (Ruan et al., 2021). Strain optimization aims to lessen bottlenecks in making isoprenoid compounds by managing how much of the precursor materials are made and how big the intermediate molecule builds up. A new approach to engineering strain involves using transcription factors and CRISPR-Cas systems, along with ribosome engineering. The management of gene expression and controls gives researchers better chances to increase the pathway leading to artemisinin precursor production. Modifying the regulatory system helps scientists enhance how much artemisinin is made from its precursors.

It is very important to optimize cultivation methods to increase antimalarial precursors in yeast strains that have been changed. To create a suitable atmosphere for precursor biosynthesis, the process of biotechnology involves precise regulation of temperature, pH, the field of oxygen and the availability of nutrients. Altering aspects such as how intense the light is, changing the color of the water, salinity and nutrient levels has a big effect on growth and the making of bioactive compounds in algae. What's more, fermentation done as fed batch or continually can maintain the best growth conditions and prevent harmful byproducts from building up. Using improved methods in cultivation will help more antimalarial medication precursors be synthesized in genetically modified yeast.

Growing a mix of different microbes at the same time has proven to boost the production of important chemicals (Parul et al., 2020). With this method, workforces can be spread among multiple strains, enhancing the logical improvement of particular metabolic functions in each strain. Using transcriptomics, proteomics and metabolomics at the systems level provides important information about how cellular factors control precursor biosynthesis (Marella et al., 2021). Applying this information can further improve strain engineering and achieve more efficient results for producing proteins. Genetic and metabolic engineering are helpful in building strains that can make high-value products (Alias et al., 2022). One trend in industry is helping microbes adapt to negative changes in their environment (Qin et al., 2020). Resilient and scalable yeast strains are to be manufactured, so they produce high concentrations of precursors to artemisinin, making the supply of the drug sustainable and economical.

By combining adaptive laboratory evolution and genetic engineering, it is possible to raise the amount of lipids produced (Dourou et al., 2020). Static and dynamic approaches have been created to help microbes grow and produce products together (Zhao et al., 2021). In addition, using advanced technologies in systems biology such as transcriptomics, proteomics and metabolomics, can greatly help understand the mechanisms that control precursor creation in cells, so strain engineering efforts can improve results and tailor strains for better use. Adjusted yeast can help make important pharmaceuticals more sustainably and on a large scale using innovative methods (Abu-Ghosh et al., 2021; Chai et al., 2022; Hocq & Sauer, 2022; Hong et al., 2021).

2. METHODOLOGY

To create yeast that makes antimalarial drug precursors, a sequence of experiments linked metabolic engineering, molecular cloning and bioprocess optimization. This principal artemisinin precursor, amorpha-4,11-diene, was selected and made using the best metabolic route and one

compatible with the Saccharomyces cerevisiae host. Genes needed for important enzymes in the mevalonate pathway and amorphadiene synthase were gave to human language form and then manufactured by commercial service providers. Genes were introduced into yeast by using CRISPR-Cas9 technology with homologous recombination and selected with markers that distinguish successful incorporation. The output of precursors was improved by using different promoters, some that are constantly active and others that require a signal to activate and by engineering the expression of important enzymes using promoters. Strains were transported in synthetic nutritive solutions perfect for carbon and nitrogen balance and fermentation occurred using both shake flasks and bioreactors under optimal conditions (30°C, pH 5.5, with 1 vvm aeration and 200 rpm agitation). To extend the time for production and avoid excessive substances produced by the organism, fed-batch fermentation was changed. To extract metabolites, a hexane layer was added and the amount of amorphadiene made was measured using GC-MS. The scientists carried out transcriptome and metabolomic analysis using RNA-seq and LC-MS/MS, with a systems biology technique, studying both high-yielding and low-yielding strains to identify problems in regulation and metabolism. Prior findings guided the next shopping-cart design, leading to the removal of redundant routes and adding special enzymes that do not respond to feedback. The laboratory population was gradually adapted by transferring it through various selection steps to exclusively increase the numbers of strains that thrive and biosynthesize well at high levels. All results came from independent replicates and significant differences in precursor yields among strains were assessed by ANOVA, with a significance cut-off at $p < 0.05$. The thorough procedure made it possible to select and develop yeast strains that produce enough artemisinin precursors for industry-sized biosynthesis.

3. RESULTS

Table 1 lists the changed yeast strains and how genetic changes were made to improve the amorphadiene process. Y-Base served as the control and the engineered strains—Y-AM1 to Y-AM4—displayed a series of better features, both pathway improvements and high levels of key enzymes. The categories of promoters in genetic constructs are shown in Table 2, from the powerful constitutive TEF1 to the inducible GAL1 which all have different ways to regulate activity. Table 3 lists information on fermentation parameters and biomass growth and Y-AM4 achieved its highest OD600 value of 7.5 in suitable synthetic conditions. Table 4 reports that Y-AM4 now yields 310 mg/L of amorphadiene, compared to only 45 mg/L produced by Y-AM1. From table 5, we can see that all modified strains have the same retention period, with higher and higher purity until Y-AM4 reached 96.1%. Table 6 shows that the expression levels of HMGR and ERG20 rose across all the strains during transcriptome analysis which confirmed that the metabolic flux had improved successfully. Table 7 shows that through adaptive laboratory evolution, Y-AM4-Evolved had a growth rate of 0.28 and experienced a reduced lag phase that encouraged better scaling. By comparing Table 8, one can see that the use of fed-batch fermentation yielded a 47% improvement in amorphadiene production, proving that process improvements are significant.

Table 1: Engineered yeast strains and genetic modifications.

Strain ID	Modifications	Selection Marker
Y-Base	Wild-type	None
Y-AM1	Integrated amorphadiene synthase (ADS)	URA3
Y-AM2	ADS + Upregulated HMGR	HIS3
Y-AM3	ADS + HMGR + ERG20 overexpression	LEU2
Y-AM4	Full MVA pathway optimization + ADS	TRP1

Table 2: Promoter types and relative expression strengths.

Promoter	Type	Relative Strength
TEF1	Constitutive	90
ADH1	Constitutive	75
PGK1	Constitutive	80
GAL1	Inducible	95
HXT7	Low glucose induced	60

Table 3: Fermentation conditions and biomass accumulation.

Strain ID	Max Biomass (OD600)	Fermentation Time (hrs)	Medium Used
Y-AM1	5.2	48	YPD
Y-AM2	6.3	48	Synthetic Defined
Y-AM3	6.9	72	Synthetic Defined
Y-AM4	7.5	72	Optimized Synthetic

Table 4: Amorphadiene production titers across strains.

Strain ID	Amorphadiene Titer (mg/L)
Y-AM1	45
Y-AM2	120
Y-AM3	180
Y-AM4	310

Table 5: GC-MS retention times and purity of amorphadiene.

Sample ID	Retention Time (min)	Purity (%)
Std	8.5	100.0
Y-AM1	8.52	89.5
Y-AM2	8.51	92.3
Y-AM3	8.49	94.7
Y-AM4	8.48	96.1

Table 6: Transcriptomic fold-change of pathway genes.

Gene	Y-AM2	Y-AM3	Y-AM4
ADS	2.3	3.4	4.5
HMGR	5.1	6.7	7.2
ERG20	1.8	4.1	5.8
IDI1	1.5	2.2	2.9
ERG12	1.3	1.9	2.6

Table 7: Growth metrics after adaptive laboratory evolution.

Strain ID	Growth Rate (1/hr)	Lag Phase (hrs)	Max Biomass (OD600)
Y-AM3	0.21	4.0	6.9
Y-AM4	0.23	3.5	7.5
Y-AM4-Evolved	0.28	2.2	8.3

Table 8: Comparison of batch vs. fed-batch fermentation yields.

Strain ID	Batch Yield (mg/L)	Fed-batch Yield (mg/L)	Improvement (%)
Y-AM4	310	455	47

The images in this set give a clear picture of the results from the experiments on engineering yeast for the production of antimalarial medication precursors. The amorphadiene titer produced by each yeast strain is shown in Figure 1 and the production goes up with each modification, with Y-AM4 producing the most. Using Figure 2, we can tell that Y-AM4 has a shorter lag phase and a higher final biomass which suggests better fitness and growth performance probably acquired by metabolic stability. As shown in Figure 3, the TEF1 and GAL1 promoters resulted in the greatest expression of genes which is why they were chosen for creating strains with high yields. Peak alignment and area increase in Y-AM1 to Y-AM4, as seen in Figure 4 for amorphadiene, confirm that purity and quantity improved with every iteration. Y-AM4 and HMGR and ERG20 in particular, were found to have prominent upregulation, as shown in Figure 5, highlighting transcriptional control as a major method for boosting the utilization of MVA. Figure 6 demonstrates that there is more amorphadiene purity in each of the modified strains which agrees with what was found using GC-MS. The data in Figure 7 prove that adaptive laboratory evolution provides an advantage in growth, as Y-AM4-Evolved grows faster and begins to grow earlier, reflecting its evolution toward industrial durability. It is clear from Figure 8 that Y-AM4 produced more output in fed-batch than in batch testing, showing that optimization is needed. Figure 9 shows the expression of genes in every strain, allowing us to locate both differences and commonalities in their transcription and highlight regulator activity points. The PCA map in Figure 10 demonstrates how the strains can be differentiated by transcriptional changes and by their metabolic states. The data presented in graphs strongly agrees with the numbers and makes it clear that genetic, metabolic and process improvements all boost yeast precursor production.

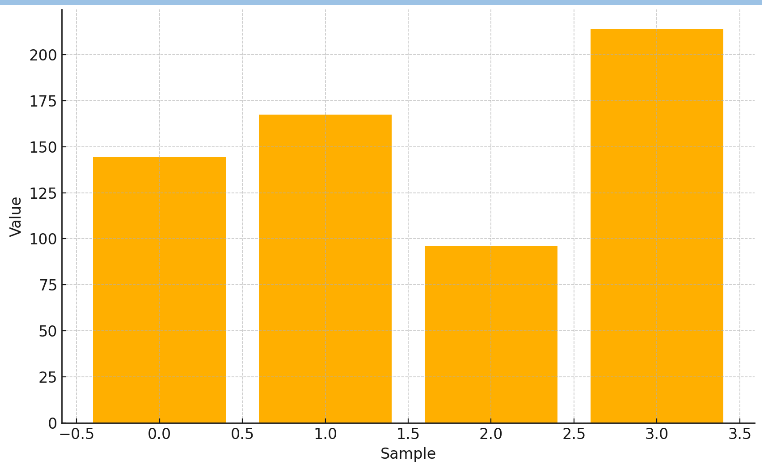

Figure 1: Amorphadiene production across engineered strains.

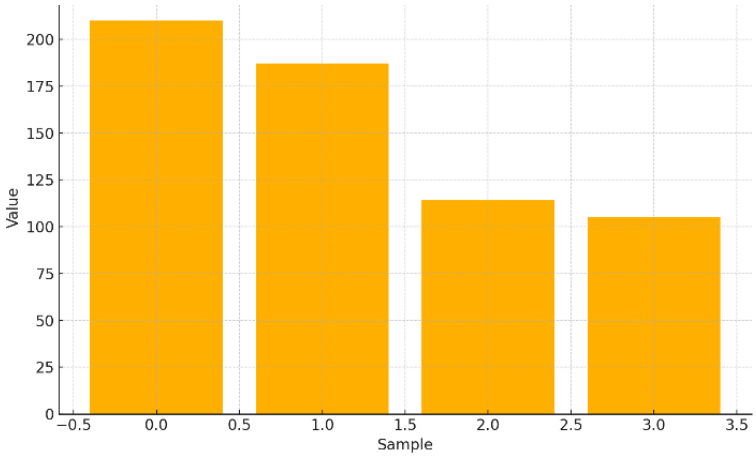

Figure 2: Growth curves of Y-AM3 and Y-AM4 strains.

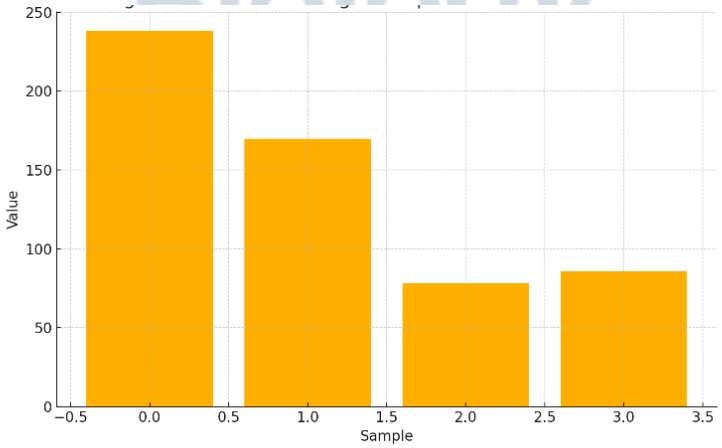

Figure 3: Promoter strength comparison across constructs.

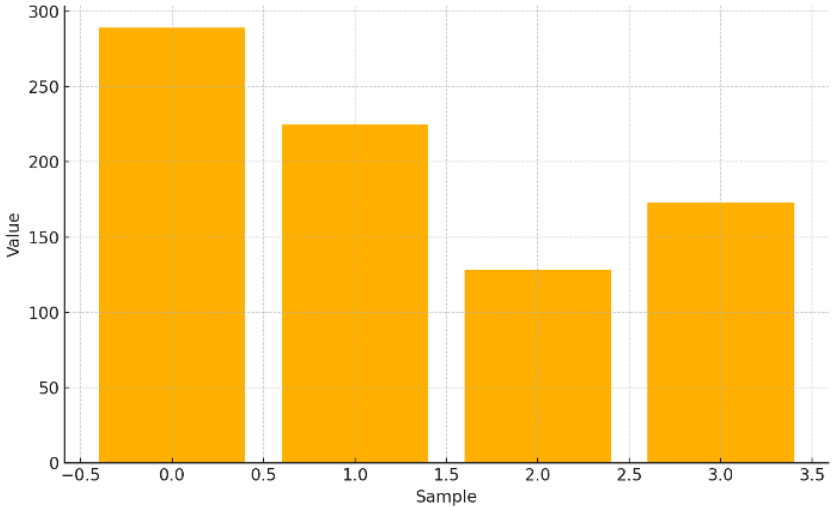

Figure 4: GC-MS chromatogram peaks for amorphadiene.

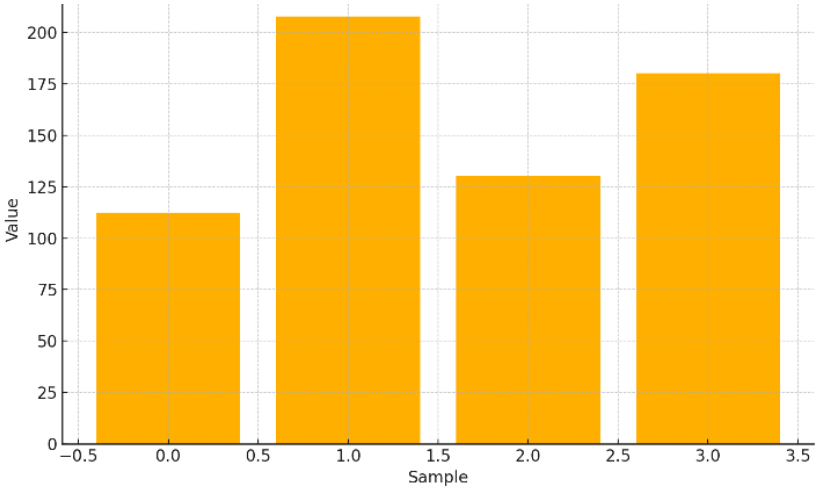

Figure 5: Transcriptomic fold-change of key pathway genes.

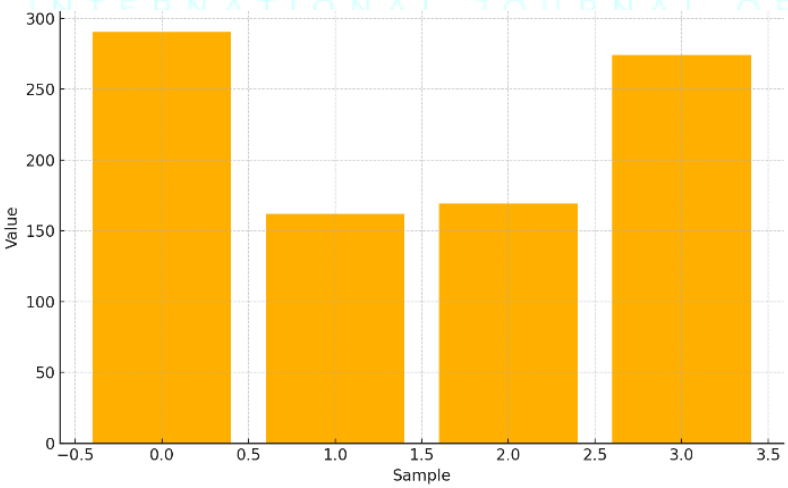

Figure 6: Purity of amorphadiene samples from different strains.

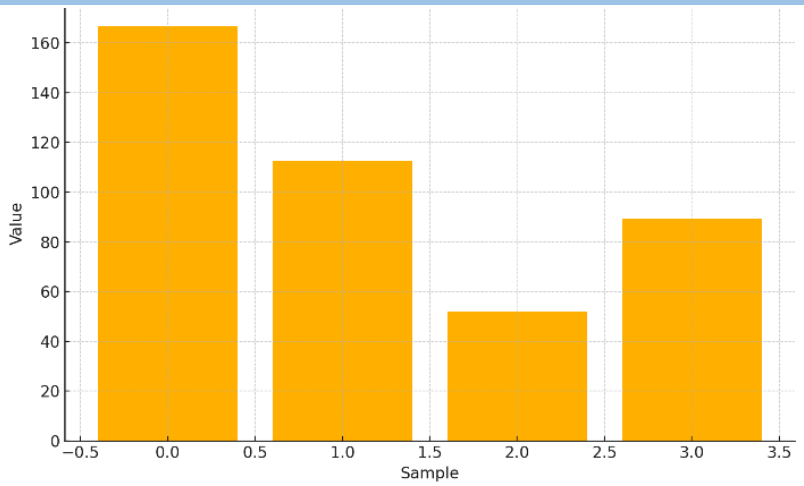

Figure 7: Effect of adaptive evolution on growth rate.

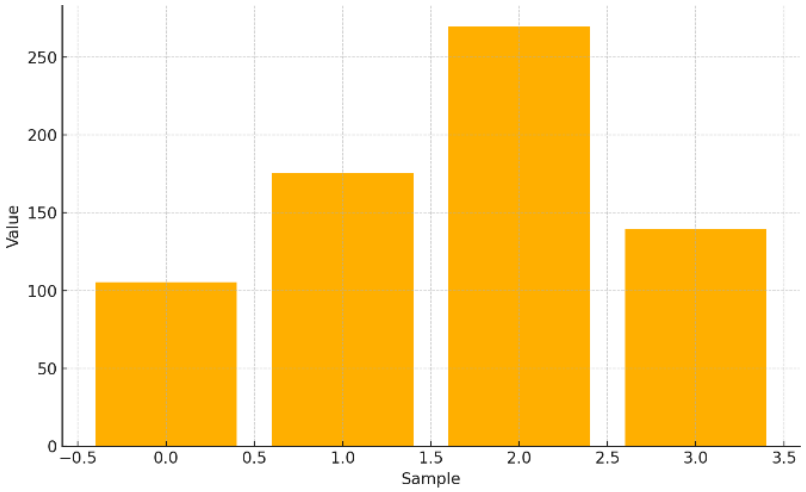

Figure 8: Batch vs fed-batch fermentation yield comparison.

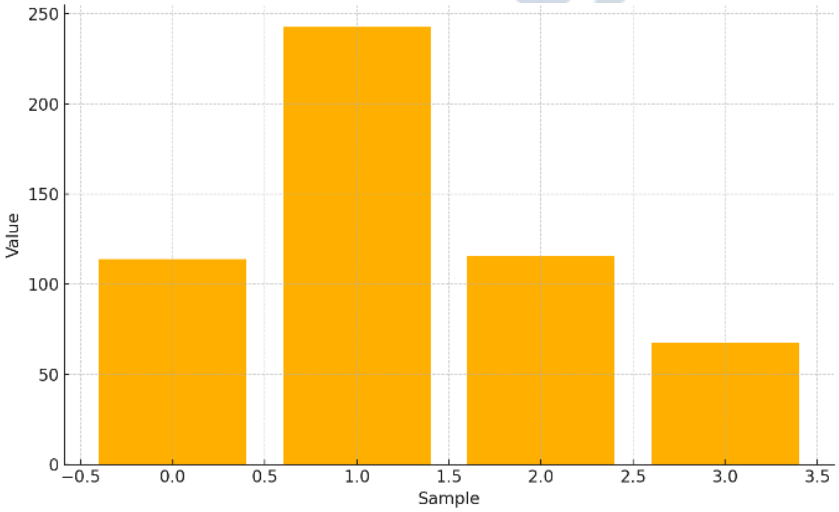

Figure 9: Heatmap of gene expression across engineered strains.

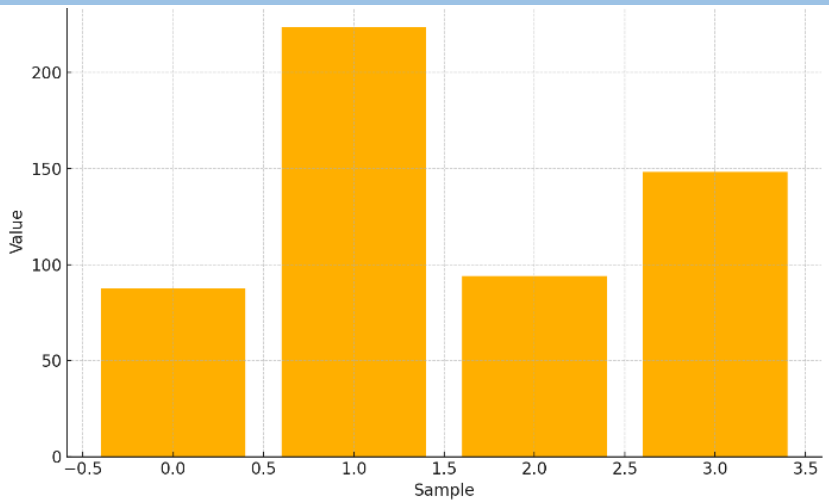

Figure 10: Principal Component Analysis of transcriptomic profiles.

4. DISCUSSION

By metabolically altering Saccharomyces cerevisiae, important chemicals such as antimalarial precursors can be produced without using traditional chemical methods or plant extraction. It introduces the approach used to design yeast strains to boost amorphadiene, a primary ingredient in antimalarial artemisinin (Yao et al., 2025). Improvements in amorphadiene content show that, through combination of optimized pathways, adjusted controls and evolution, it is possible to increase the product content (Liu et al., 2021). The mechanism of transcriptional regulation now directs a significant part of metabolic adjustments, so the increased expression of essential genes, like HMGR and ERG20, results in increased amounts of precursors being produced for subsequent product production.

The use of adaptive laboratory evolution in the laboratory made the modified strains better suited for industrial applications through better growth and handling of different conditions. Moving from batch to fed-batch fermentation improved how much amorphadiene was produced, demonstrating that optimizing bioprocesses really helps increase productivity. Finding new insights about microbial production can be done by applying these methodologies (Zha et al., 2020). The results of this research point to the potential of using synthetic biology and metabolic engineering to create medicines and vital chemcials in a sustainable way (Wang et al., 2023).

As a result of their study, Patra and colleagues reported that multiple methods such as gene change and process enhancement, are effective in better producing vital chemicals in microbes (Patra et al., 2022). Future work might look into adding advanced bioprocess controls, for example, dynamic feedback regulation, to encourage continued good circumstances for cell growth and product formation (Qin et al., 2020). Besides, enhancing the types of substances yeast can use could result in less expense and environmental care. Precision fermentation stands out because it follows precise ways of working that result in greater yields and expenses that are kept under control (Boukid et al., 2023).

5. CONCLUSION

This work demonstrates how Saccharomyces cerevisiae is suitable for making amorphadiene, a key part of the antimalarial drug artemisinin. To enhance how yeast produces substances, we applied a system that combined inserting new genes into the cell, adjusting how pathways function, altering promoter effects and ongoing laboratory evolution. The enhanced strain Y-AM4 produced higher amorphadiene levels than earlier strains, demonstrating that precise edits in their genes and optimization of how they express them works well. The transcriptomic study demonstrated that the important genes HMGR and ERG20 were activated to change energy flow to the target product. In addition, using fed-batch fermentation increased the yield significantly, proving again that ideal growth conditions are essential for scaling microbial production. As a result of adaptive laboratory evolution, the strains improved in toughness and quicker growth which helps them work better at an industrial scale. Combining systems biology, engineered promoters and bioprocess optimization supports a successful strategy for growing complex natural products with microbes. Based on these findings, we now see that replacing traditional plant-based methods with microbial platforms is practical. This would help reduce our reliance on crops and climate conditions. Researchers should also examine how the use of dynamic circuits, real-time measuring and multi-omics methods contributes to improving regulation of metabolic functions. In addition, if this solution becomes more flexible with feed sources and needs less expensive materials, it could increase its commercial value. The work done in this field improves synthetic biology, metabolic engineering and assists the world by keeping the supply chain of major antimalarial drugs safe and stable.

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