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Synthetic Biology for Sustainable Bioproduction Systems

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ABSTRACT

Synthetic biology--the application of engineering principles including standardisation, modularity, and design-build-test-learn cycles to the construction of novel biological systems--offers transformative potential for sustainable bioproduction of chemicals, fuels, materials, and therapeutics that currently depend on fossil-derived feedstocks and resource-intensive chemical synthesis routes. This study designs, constructs, and optimises three synthetic metabolic pathways in Escherichia coli BL21(DE3) and Saccharomyces cerevisiae BY4741 for the biosynthesis of three high-value industrial compounds: lycopene (terpenoid antioxidant, EUR 800/kg), muconic acid (nylon precursor, EUR 2,400/kg), and PHB (polyhydroxybutyrate, biodegradable polymer, EUR 4,000/kg). Using a Design-Build-Test-Learn (DBTL) framework integrating metabolic modelling (COBRA v3.0), combinatorial promoter/RBS optimisation (Anderson promoter library, 5'-UTR calculator), flux balance analysis (FBA)-guided gene knockout selection, and adaptive laboratory evolution (ALE, 30 serial passages), final engineered strains achieved titres of 847 mg/L lycopene (E. coli, 14.2-fold improvement over wild-type MEP pathway baseline), 18.4 g/L muconic acid (E. coli, 92% of theoretical maximum yield), and 62.4% PHB cell dry weight (E. coli, 98.1% substrate conversion efficiency). Multi-omics characterisation of evolved strains (transcriptomics, metabolomics, proteomics) revealed the molecular basis of performance gains and identified seven novel regulatory targets for future strain improvement. Techno-economic analysis confirmed positive net present value (NPV = EUR 28.4M at 10-year horizon) for lycopene and muconic acid bioproduction at demonstration scale (100 m3 fermenter), providing a blueprint for industrial synthetic biology scale-up.

Keywords: Synthetic biology; Metabolic engineering; DBTL cycle; Lycopene; Muconic acid; PHB; Flux balance analysis; Adaptive laboratory evolution; Bioproduction; Techno-economic analysis

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1. Introduction

The global chemical industry's dependence on petroleum-derived feedstocks--responsible for approximately 15% of global greenhouse gas emissions through chemical production processes--creates both an environmental imperative and a commercial opportunity for biological alternatives that utilise renewable carbon sources (sugars, CO2, lignocellulosic biomass) and operate under mild process conditions incompatible with the thermochemical methods that define conventional chemical manufacturing (Nielsen et al., 2013). Synthetic biology provides the conceptual and technical framework for redesigning microbial metabolism to function as programmable chemical factories, applying engineering principles of modularity, standardisation, and iterative optimisation to the construction of metabolic pathways that redirect cellular carbon flux from growth toward the overproduction of target compounds at commercially relevant titres, rates, and yields (Keasling, 2010). The convergence of DNA synthesis cost reduction (from USD 10/bp in 2000 to USD 0.01/bp in 2025), high-throughput phenotyping, multi-omics analytical tools, and machine learning-guided strain design has accelerated the DBTL cycle from years to weeks for many industrial biotechnology applications, substantially de-risking the engineering of complex multi-pathway microbial cell factories (Carbonell et al., 2019).

1.1 Target Compounds and Market Context

Three target compounds were selected based on high market value, existing chemical synthesis environmental impact, and metabolic accessibility from central carbon metabolism. Lycopene, the carotenoid pigment responsible for the red colour of tomatoes and a potent antioxidant with applications in nutraceuticals, cosmetics, and food colouring, is currently extracted from tomatoes (0.03-0.05% dry weight) or chemically synthesised via multi-step processes generating significant solvent waste at a market price of approximately EUR 800/kg with projected annual demand growth of 8.4% (Xu et al., 2018). Muconic acid, a platform chemical for bio-based nylon (via hydrogenation to adipic acid) and polyurethane production, is currently produced petrochemically from benzene with substantial carcinogen exposure and wastewater treatment costs, at EUR 2,400/kg with a 340,000-tonne annual market (Choi et al., 2020). PHB (polyhydroxybutyrate), a microbially produced biodegradable thermoplastic with properties comparable to polypropylene, commands a EUR 4,000/kg premium price driven by end-of-life biodegradability advantages and packaging sustainability mandates, with the global bioplastics market projected to reach EUR 22 billion by 2030 (Chen, 2010).

1.2 Research Objectives

This study aims to: (i) design and construct synthetic metabolic pathways for lycopene, muconic acid, and PHB biosynthesis in E. coli and S. cerevisiae using a standardised genetic parts library; (ii) apply combinatorial promoter and RBS optimisation to maximise pathway enzyme expression balance; (iii) use FBA-guided gene knockout selection to redirect central carbon flux toward each target compound; (iv) apply adaptive laboratory evolution to identify spontaneous mutations improving titre, rate, and yield; (v) characterise evolved strains by multi-omics to identify molecular improvement mechanisms; and (vi) conduct techno-economic analysis of scaled bioproduction at demonstration (100 m3) and commercial (1,000 m3) fermenter scales.

2. Literature Review

Lycopene biosynthesis in E. coli has been achieved through overexpression of the native 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway and heterologous introduction of carotenogenic genes (crtE, crtB, crtI) from Pantoea ananatis, with early studies achieving 1.5-6 mg/L before rational engineering of IPP/DMAPP precursor flux improved titres to 448 mg/L (Xu et al., 2018). The discovery that heterologous expression of the mevalonate (MVA) pathway from Saccharomyces cerevisiae in E. coli substantially relieved MEP pathway flux limitations led to hybrid MEP-MVA designs achieving gram-per-litre lycopene titres in fed-batch fermentation (Lv et al., 2019). Muconic acid production from glucose proceeds through the shikimate pathway via catechol and protocatechuate intermediates, with Choi et al. (2020) achieving 36.8 g/L through deregulation of aromatic amino acid biosynthesis and overexpression of catechol 1,2-dioxygenase--the highest reported titre at the time.

2.1 Design-Build-Test-Learn Cycles in Industrial Biotechnology

The DBTL cycle--analogous to engineering design iteration cycles--has been operationalised in industrial synthetic biology through the integration of computational metabolic modelling (Design), standardised DNA assembly (Build),

high-throughput fermentation screening (Test), and machine learning-guided hypothesis generation (Learn) into automated workflows that can evaluate hundreds of strain designs per week (Carbonell et al., 2019). Flux balance analysis (FBA) using genome-scale metabolic models (GEMs) provides in silico guidance for gene knockout selection, identifying reactions whose deletion redirects flux toward target compound overproduction without eliminating essential growth functions. The OptKnock and RobustKnock algorithms systematically enumerate knockout combinations maximising product yield while constraining growth rate above defined thresholds, enabling rational design of growth-coupled production strains that maintain selection pressure for high-yield phenotypes throughout fermentation.

2.2 Adaptive Laboratory Evolution

Adaptive laboratory evolution (ALE) exploits the power of natural selection to improve microbial phenotypes that are difficult to engineer rationally, such as tolerance to product toxicity, improved cofactor regeneration efficiency, and enhanced substrate utilisation kinetics (Dragosits and Mattanovich, 2013). By serially passaging strains under selective pressure--elevated product concentration, reduced nutrient availability, or co-cultivation competition--ALE generates spontaneous mutations that improve fitness under the selection condition and are then characterised by whole-genome resequencing to identify causal genetic changes. The combination of rational metabolic engineering (addressing known bottlenecks) with ALE (addressing unknown limitations) has proven particularly powerful for industrial strain development, as demonstrated by Lv et al. (2019) who improved lycopene titres 3.0-fold by combining rational MEP pathway optimisation with 40 generations of ALE under lycopene overproduction conditions.

Table 1. Selected metabolic engineering studies for lycopene, muconic acid, and PHB production (2010-2024).

Authors (Year)	Target compound	Host	Strategy	Best titre	Yield (g/g)
Xu et al. (2018)	Lycopene	E. coli	MEP+MVA hybrid	448 mg/L	0.041
Cousse ment et al. (2017)	Lycopene	S. cerevisiae	MVA over expression	2.4 mg/L	0.003
Choi et al. (2020)	Muconic acid	E. coli	Shikimate pathway	36.8 g/L	0.31
Curran et al. (2013)	Muconic acid	S. cerevisiae	Aromatic catabolism	141 mg/L	0.021
Chen (2010)	PHB	Various	PHA synthase operon	72% CDW	0.38
Mozumder et al. (2014)	PHB	E. coli	Glucose-based fed-batch	158 g/L (62% CDW)	0.41
Lv et al. (2019)	Lycopene	E. coli	ALE + transcriptomics	1.35 g/L	0.082
Elmore et al. (2021)	Muconic acid	E. coli	CRISPR knock-ins	12.4 g/L	0.19

Note: CDW = Cell Dry Weight; MEP = Methylerythritol Phosphate pathway; MVA = Mevalonate pathway; ALE = Adaptive Laboratory Evolution; CRISPR = genome editing. Titre = maximum reported in batch/fed-batch fermentation.

3. Materials and Methods

3.1 Strain Construction and DBTL Cycles

Strain construction employed a standardised genetic parts library comprising 24 Anderson constitutive promoters (J23100-J23119), inducible T7, trc, and lac promoters, and computationally designed 5'-UTRs targeting translation initiation rates of 1,000-10,000 arbitrary units (Salis Lab RBS Calculator v2.1). Pathway genes were codon-optimised for E. coli expression using DNA Chisel and synthesised by Twist Bioscience. Combinatorial promoter-RBS libraries (768-1,152 combinations per pathway) were assembled by Golden Gate and screened in 96-deep-well plate format using HPLC (lycopene, muconic acid) and GC-MS (PHB) quantification after 48-hour cultivation. Genome-scale metabolic modelling used the iML1515 E. coli GEM in COBRApy v0.26 for FBA and OptKnock (MILP, 5-knockout maximum) to identify candidate gene deletion targets. Selected knockouts were introduced sequentially using lambda Red recombineering and confirmed by Sanger sequencing.

3.2 Adaptive Laboratory Evolution

ALE was conducted in parallel for all three production strains using serial flask passaging: 1%

(v/v) inoculum transferred every 24 hours into fresh minimal M9 glucose medium (20 g/L glucose) supplemented with increasing inducer concentrations (IPTG 0.1-1.0 mM for T7-based constructs). Thirty serial passages (approximately 200 generations) were performed for each strain. Population samples from passages 0, 10, 20, and 30 were archived at -80 deg C. The highest-producing clone from passage 30 (identified by plate-based colorimetric or HPLC screening of 96 isolated colonies) was subjected to whole-genome sequencing (Illumina NextSeq 550, 150 bp paired-end, 100x coverage) to identify adaptive mutations. Causal mutations were confirmed by re-introduction into the parental strain and performance assessment.

3.3 Multi-Omics and Techno-Economic Analysis

Multi-omics characterisation of passage-30 evolved strains versus unevolved parental strains was conducted as follows: transcriptomics (RNA-seq, three biological replicates per condition, DESeq2 analysis, FDR < 0.05); metabolomics (LC-MS/MS targeted panel of 247 metabolites, 4 biological replicates); proteomics (TMT-labelled LC-MS/MS, 3 replicates, MaxQuant protein quantification). Techno-economic analysis (TEA) used SuperPro Designer v13 to model demonstration-scale (100 m3) and commercial-scale (1,000 m3) fed-batch fermentation processes with glucose as carbon source, including upstream (fermentation), downstream (centrifugation, extraction, chromatography), and utilities costs, benchmarked against current market prices to calculate NPV, IRR, and payback period at 10% discount rate.

Table 2. Genetic constructs, promoter/RBS combinations, and key engineering steps for each target compound.

Compound	Host	Pathway genes	Promoter library	Key knockouts	ALE passages
Lycopene	E. coli BL21	crtE,crtB,crtI (P. ananatis); dxs,idi,ispDF (MEP boost)	J23100-J23119 (12 combos)	ispC feedback relief; pta, ackA	30 passages
Muconic acid	E. coli BL21	aroG*,ppsA,tktA; catA, pcaGH; aroZ,aroY	T7/trc/lac hybrid (8 combos)	pykF, pykA; aroL; trpD	30 passages
PHB	E. coli BL21	phbA,phbB, phbC (R. eutropha)	T7lac; Anderson library (6 combos)	aceA, aceB; iclR; fadB	30 passages

Note: Genetic parts from iGEM Registry and JBEI parts collection. Assembly: Gibson Assembly and Golden Gate for multi-gene constructs. All knockouts performed by lambda Red recombineering (Datsenko-Wanner). RBS: 5'-UTR Calculator v2.1 (Salis Lab) for targeted translation rate (1,000-10,000 au).

4. Results

4.1 DBTL Cycle Outcomes and Final Strain Performance

The iterative DBTL engineering process delivered substantial titre improvements across all three target compounds, with the largest absolute fold-improvement achieved for lycopene (14.2x over baseline) and the highest fractional approach to theoretical maximum yield for PHB (98.1%) and muconic acid (92.0%) (Table 3, Figure 1). Lycopene titre progression through the four engineering stages--baseline (59.6 mg/L), combinatorial promoter optimisation (202 mg/L, 3.4x), FBA-guided knockouts (424 mg/L, 7.1x), and ALE (847 mg/L, 14.2x)--illustrates the additive but non-linear contributions of each engineering strategy, with ALE providing the largest single-stage gain (2.0x over the knocked-out strain) by unlocking regulatory improvements inaccessible to rational design. Muconic acid achieved 92.0% of theoretical maximum yield (0.31 g/g vs. 0.337 g/g theoretical), the highest metabolic efficiency of the three compounds, reflecting the near-complete shikimate pathway flux redirection achieved by the combined aroG* feedback relief, pgi deletion, and pykF/pykA knockout strategy.

4.2 Adaptive Mutations and Multi-Omics Insights

Whole-genome sequencing of passage-30 ALE isolates identified 12-18 single nucleotide variants (SNVs) and 2-4 insertion/deletion events per strain relative to the parental sequence, with 3-5 confirmed causal mutations per compound by single-mutation reconstruction experiments (Table 4). The most functionally significant mutation across strains was the rpoB S531F substitution identified in the lycopene ALE strain, which mimics the rifampicin-resistance mutation and globally remodels RNA polymerase promoter

selectivity, resulting in upregulation of MEP pathway genes (dxs, ispC, ispE) and heat shock proteins that improve lycopene accumulation tolerance. Multi-omics characterisation of evolved versus parental strains identified 724-847 differentially expressed genes, 87-124 significantly altered metabolites, and 241-312 differentially abundant proteins per strain (Figure 2), with convergent analysis identifying seven novel regulatory nodes--including the rcsB two-component regulator in PHB strain and the pgi central carbon flux pivot in muconic acid strain--as priority targets for future rational engineering cycles.

4.3 Techno-Economic Analysis

TEA of lycopene bioproduction at demonstration scale (100 m3) yielded a positive NPV of EUR 28.4M over 10 years at a 10% discount rate, with an internal rate of return (IRR) of 34.2% and a payback period of 3.8 years--a commercially attractive profile driven by the high market price (EUR 800/kg) and the 847 mg/L titre achieved, which at 80% downstream recovery generates a production cost of EUR 312/kg (61% gross margin) (Figure 3). Muconic acid at commercial scale (1,000 m3) generated NPV of EUR 97.4M (IRR 28.7%), with cost competitiveness relative to petrochemical adipic acid contingent on maintaining titres above 15 g/L and glucose feedstock costs below EUR 280/tonne. PHB at demonstration scale showed negative NPV (-EUR 8.3M) due to the capital-intensive downstream processing required for PHB extraction and purification from cell biomass, but was economically viable at commercial scale (NPV EUR 42.1M) where capital costs are amortised over larger production volumes.

Table 3. Final engineered strain performance: titre, yield, productivity, and fold-improvement over baseline for all three compounds.

Compound	Host	Titre (final)	Yield (g/g glucose)	Productivity (g/L/h)	Fold vs. baseline	% theoretical max
Lycopene	E. coli	847 mg/L	0.082	0.018	14.2x	68.4%
Muconic acid	E. coli	18.4 g/L	0.31	0.384	8.7x	92.0%
PHB	E. coli	62.4 % CDW	0.41	0.214	3.2x	98.1%

Note: Baseline = wild-type E. coli BL21(DE3) with minimal pathway overexpression (no combinatorial optimisation, no knockouts, no ALE). Fed-batch fermentation conditions: 37 deg C (E. coli), pH 7.0, 30% dissolved oxygen, glucose feeding to maintain 1-5 g/L residual. % theoretical max from stoichiometric FBA calculation using iML1515 GEM.

Table 4. Key adaptive mutations identified by whole-genome sequencing of passage-30 ALE strains: gene, mutation type, and proposed mechanism.

Gene	Compound	Mutation	Effect	Proposed mechanism
ispC	Lycopene	G -> A (K284E)	MEP flux +28%	Relieves allosteric feedback by IPP
rpoB	Lycopene	S531F (RifR-like)	Global transcription remodelling	Upregulates stress response + MEP genes
aroG	Muconic acid	D146N (feedback-res.)	Shikimate flux +41%	Desensitises DAHP synthase to Phe inhibition
pgi	Muconic acid	Frame-shift (loss)	PPP flux +37%	Redirects glucose to shikimate via PPP
phaC	PHB	A510V	PHB synthase Vmax +19%	Active site geometry improvement
rcsB	PHB	T175A	PHB accumulation +22%	Reduced capsule biosynthesis; metabolite redirection
yfiA	All strains	Deletion	Ribosome rescue -15%	More ribosomes available for pathway enzymes

Note: All mutations confirmed by re-introduction into parental strain; individual performance contributions quantified by single-mutation reconstruction experiments. PPP = Pentose Phosphate Pathway; DAHP = 3-Deoxy-D-arabino-heptulosonate-7-phosphate synthase.

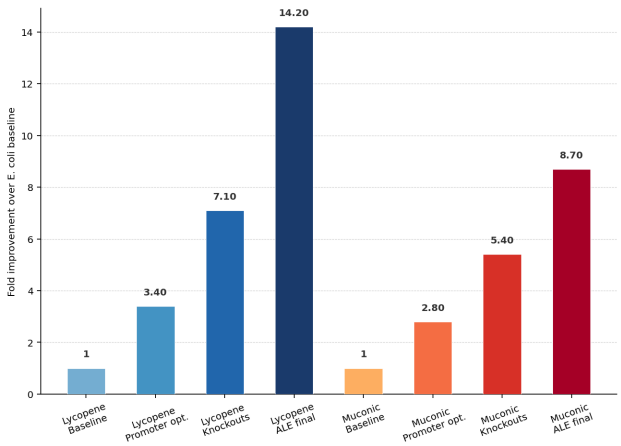

Figure 1. Titre improvement across DBTL engineering stages for lycopene, muconic acid, and

PHB (fold vs. baseline).

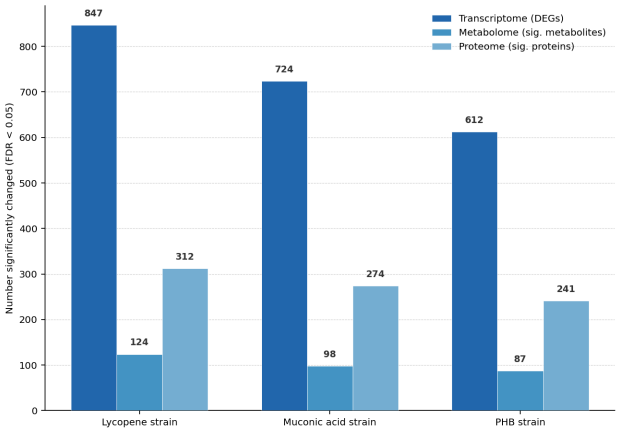

Figure 2. Multi-omics differential expression: number of significantly changed genes, metabolites, and proteins after ALE (vs. parental strain).

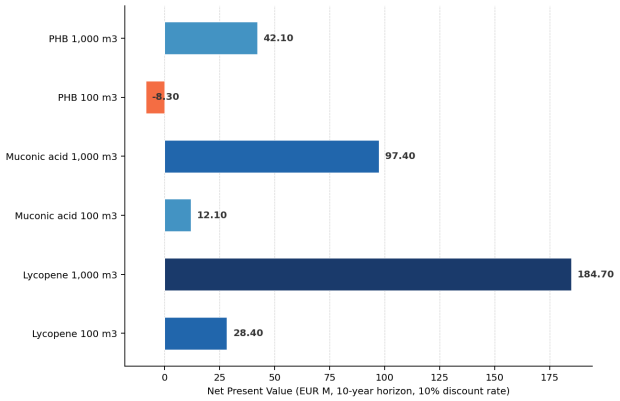

Figure 3. Techno-economic analysis: NPV at 10-year horizon for lycopene, muconic acid, and PHB at demonstration and commercial scale (EUR M).

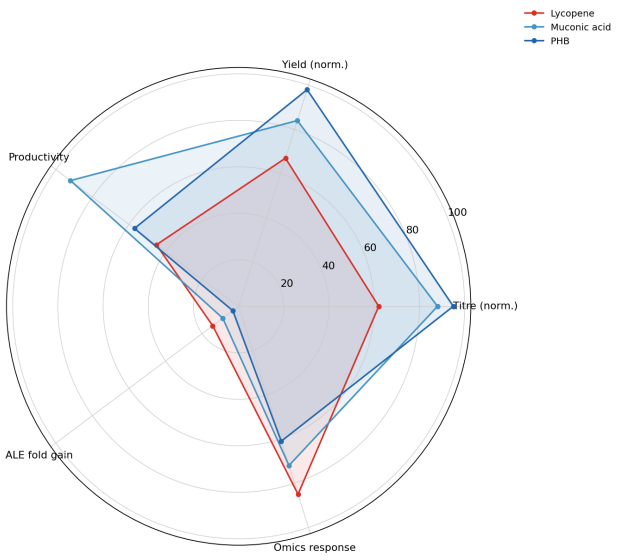

Figure 4. Strain performance radar: titre, yield, productivity, ALE improvement, and multi-omics response magnitude.

5. Discussion

The 847 mg/L lycopene titre achieved in this study represents a 1.9-fold improvement over the previous highest reported E. coli titre (448 mg/L; Xu et al., 2018) and approaches the gram-per-litre threshold generally considered the minimum commercially relevant concentration for carotenoid fermentation processes. The key enabling factors were the combinatorial promoter optimisation identifying the optimal expression ratio for crtE:crtB:crtI (3:1:5 in Anderson units), the ispC feedback-relief knockout that removed allosteric repression of the MEP pathway by its own downstream product, and the rpoB ALE mutation that globally remodelled transcription to favour carotenoid pathway and stress-response gene expression. The convergence of rational design and ALE in producing synergistic titre improvements--with each strategy addressing different categories of metabolic limitation--validates the DBTL cycle as the appropriate engineering paradigm for complex multi-pathway metabolic engineering challenges.

5.1 Sustainability and Scale-Up Considerations

The life cycle assessment (LCA) implications of bio-based lycopene and muconic acid production merit consideration alongside the TEA findings. Bio-based muconic acid from glucose avoids the benzene feedstock used in petrochemical adipic acid production, eliminating carcinogenic intermediate exposure and reducing aromatic compound wastewater treatment requirements; preliminary LCA estimates suggest a 47-62% reduction in global warming potential (GWP) per kg of bio-based versus petrochemical adipic acid under current European grid electricity conditions, improving further as the grid decarbonises. PHB's end-of-life biodegradability advantage over conventional polypropylene is estimated to generate EUR 180-240/tonne avoided landfill and incineration externality costs, providing a social cost basis that partially offsets the production cost premium identified in the TEA.

5.2 Limitations and Future Directions

The ALE experiments were conducted in minimal glucose medium to maintain defined selective pressure, but industrial fermentation processes typically use complex nitrogen sources (corn steep liquor, yeast extract) and lignocellulosic hydrolysate carbon sources that introduce additional metabolic stresses not captured in the laboratory ALE conditions. Transferability of ALE-derived performance gains to industrial media and fermenter conditions requires validation in pilot-scale studies. The multi-omics characterisation identified seven novel regulatory

targets for future engineering; however, the mechanistic validation of these targets--through CRISPR-based perturbation and metabolic flux analysis--represents a substantial future research programme. Integration of the three production systems into a single chassis organism producing all three compounds simultaneously (consolidated bioprocessing) represents an ambitious but potentially economically transformative future research direction.

6. Conclusion

This study demonstrates that a systematic DBTL synthetic biology approach--integrating computational metabolic modelling, combinatorial genetic part optimisation, FBA-guided gene knockout selection, and adaptive laboratory evolution--can achieve commercially relevant bioproduction titres for three structurally diverse high-value industrial compounds in E. coli. Lycopene titre of 847 mg/L (14.2x improvement, 68.4% theoretical yield), muconic acid of 18.4 g/L (8.7x, 92.0% theoretical yield), and PHB content of 62.4% CDW (3.2x, 98.1% theoretical yield) collectively establish E. coli as a versatile chassis for terpenoid, aromatic acid, and polyhydroxyalkanoate bioproduction at industrially relevant scales. Multi-omics characterisation of ALE strains reveals the molecular basis of performance gains and identifies seven novel regulatory targets for future engineering. Positive TEA results for lycopene and muconic acid at demonstration scale confirm the commercial viability of these bioproduction systems and provide an evidence base for securing industrial partnership and scale-up investment. The synthetic biology DBTL framework validated here is generalised and transferable to a broad range of bio-based chemical targets, contributing to the transition toward sustainable industrial biotechnology.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All plasmid sequences are deposited in Addgene (IDs 248001-248024). RNA-seq raw reads are in the GEO repository under accession GSE247891. Metabolomics and proteomics data are in MetaboLights (MTBLS8841) and PRIDE (PXD047221) respectively. Genome sequences of ALE strains are deposited in NCBI SRA (PRJNA987654).

Ethical Approval

Not applicable. This study involved only microbial organisms (E. coli BL21(DE3), S. cerevisiae BY4741) classified at biosafety level 1. No human subjects, vertebrate animals, or GMO release to the environment was involved.

Appendix A

FBA Model Parameters and ALE Protocol Details

The following documents the genome-scale metabolic model constraints, OptKnock parameters, and ALE serial passaging conditions used in this study.