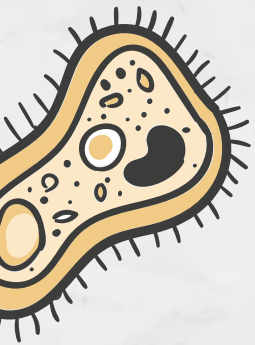
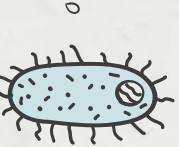
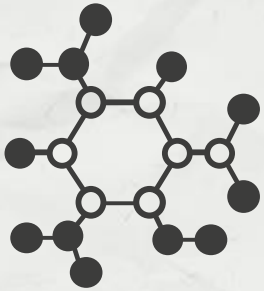
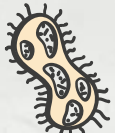


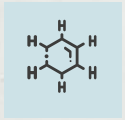



Industrial Microorganism and Metabolic Engineering

BCH 608
Ghadah A. Alamro

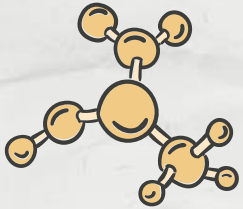
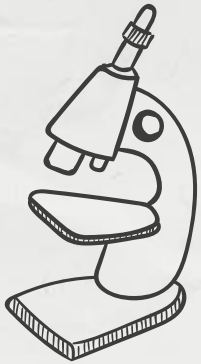
Outline

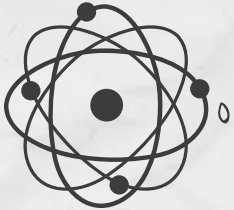
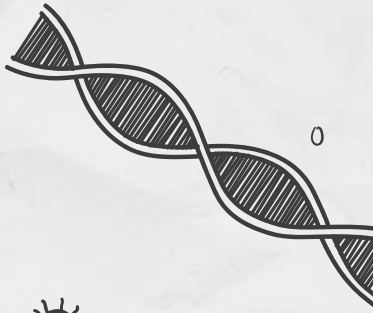


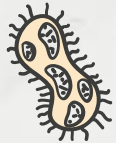
- **Introduction:**
  - What are Industrial Microorganisms?
  - Definition of Metabolic Engineering.
- **Why Use Microorganisms in Industry?**
  - Advantages, Technologies, Applications and examples.
- **Interplay between protein and metabolic engineering:**
  - Role of Protein Engineering within Metabolic Engineering
  - Strategies.
- **Metabolic Engineering and pathway construction.**
  - Key Concepts.
  - Tools and Techniques in Metabolic Engineering.
  - Applications.
- **Challenges, Commercial Limitations, and Future Prospects.**

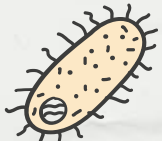
01

Introduction



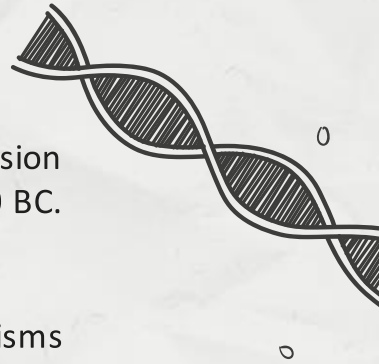
What are Industrial Microorganisms?

- **Definition:** *Industrial Microbiology* is a branch of biotechnology in which microorganisms, such as such as bacteria, algae, and fungi are used for the production of important substances, such as antibiotics, food products, enzymes, amino acids, vaccines, and fine chemicals.

- **Key concept:** Industrial microbiology is achieved for **large-scale** use of microorganisms to synthesize products of commercial value and a wide variety of applications.

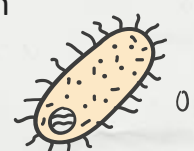
- In the last 25 years, industrial microbes have increasingly been mutant strains engineered to selectively synthesize maximized amounts of various metabolic intermediates.

The Birth of Industrial Microorganisms and Metabolic Engineering



- Is it a new concept ?
- The use of yeasts dates back to ancient days. The oldest fermentation know-how, the conversion of sugar to alcohol by yeasts, was used to make beer in Sumeria and Babylonia before 7000 BC. By 4000 B.C.
- Back in the 1950s and 1960s, scientists were grappling with the concept of how microorganisms could be used for large-scale production. The idea of using microbes as factories wasn't new, but it was limited to basic processes like brewing beer or making cheese.
- **Real turning point** came in the 1970s ?

➔ when researchers discovered the potential of genetic manipulation (development of recombinant DNA technology).



- In 1978 - game-changer ➔ Humulin by Genentech.
- In 1982 Humulin was approved by the FDA, and it became the first biotechnology product to appear on the market.

Proc. Natl. Acad. Sci. USA
Vol. 76, No. 1, pp. 106–110, January 1979
Biochemistry

Expression in *Escherichia coli* of chemically synthesized genes for human insulin

(plasmid construction/*lac* operon/fused proteins/radioimmunoassay/peptide purification)

DAVID V. GOEDDEL*†, DENNIS G. KLEID*, FRANCISCO BOLIVAR*, HERBERT L. HEYNEKER*, DANIEL G. YANSURA*, ROBERTO CREA*‡, TADAAKI HIROSE‡, ADAM KRASZEWSKI‡, KEIICHI ITAKURA‡, AND ARTHUR D. RIGGS†‡

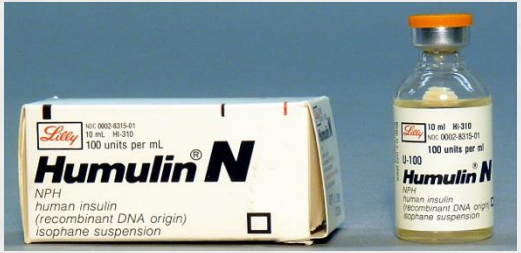
*Division of Molecular Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080; and ‡Division of Biology, City of Hope National Medical Center, Duarte, California 91010

Communicated by Ernest Beutler, October 3, 1978

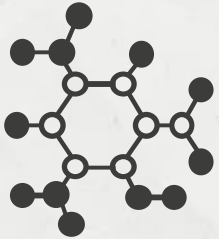
ABSTRACT Synthetic genes for human insulin A and B chains were cloned separately in plasmid pBR322. The cloned synthetic genes were then fused to an *Escherichia coli* β-galactosidase gene to provide efficient transcription and translation and a stable precursor protein. The insulin peptides were cleaved from β-galactosidase, detected by radioimmunoassay, and purified. Complete purification of the A chain and partial purification of the B chain were achieved. These products were mixed, reduced, and reoxidized. The presence of insulin was detected by radioimmunoassay.

Enzymes and DNA Preparations. T4 DNA ligase and T4 polynucleotide kinase were purified as described (6). Restriction endonuclease *Eco*RI was purified by the procedure of Greene *et al.* (7); *Hin*dIII was purified by a method developed by D. Goeddel (unpublished). Restriction endonuclease *Bam*HI was purchased from Bethesda Research (Rockville, MD); *E. coli* alkaline phosphatase was purchased from Worthington.

Plasmids, including pBR322 (8), were isolated by a published procedure (9) with some modifications. The chemical synthesis



Metabolic Engineering



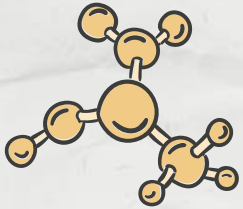
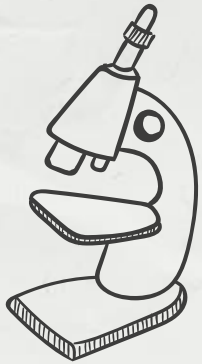
- **Definition:** *Metabolic engineering*, a new approach involving the targeted and purposeful manipulation of the metabolic pathways of an organism, is being widely researched to improve the quality and yields using molecular, genetic and combinatorial approaches.

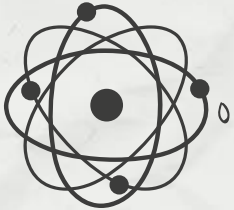
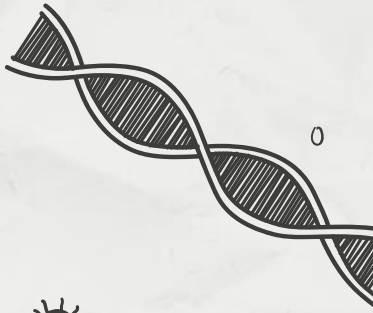
- **Key concept:** The purpose of the metabolic engineering is to generate a cell factory that produces cost-effective molecules at industrial scale.

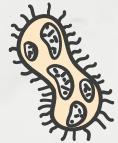
➔ Metabolic Cell factories (MCFs).

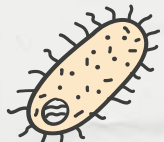
02

Why Use Microorganisms in Industry?



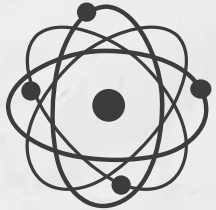
Why Use Microorganisms in Industry?

- The activities in industrial microbiology begin with the **isolation** of microorganisms from nature, their **screening** for product formation, **improvement** of product yields, **maintenance** of cultures and **optimization** of the growth condition, mass culture using **bioreactors**, and usually end with the **recovery** of products and their purification.

- **Properties of a Useful Industrial Microorganism:**

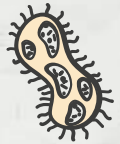
  - Grow rapidly in large-scale and in inexpensive media.
  - Produced spores can be easily inoculated.
  - Should not be pathogenic. (GRAS: Generally Recognized As Saf) by the FDA.
  - Produce the desired product in a relatively short period of time.
  - Amenable to genetic manipulation.

Industrial microorganisms Types:

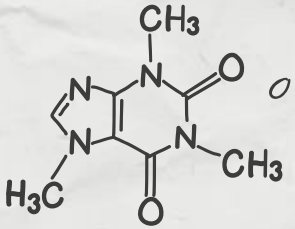


- **Industrial microorganisms generally fall into one of the following categories:**

  - Bacteria. *Escherichia coli, Corynebacterium glutamicum*
  - Fungi. most common: aspergillus species
  - Algae.
  - Yeasts. Common: Saccharomyces cerevisiae (baker's yeast)



- Viruses?

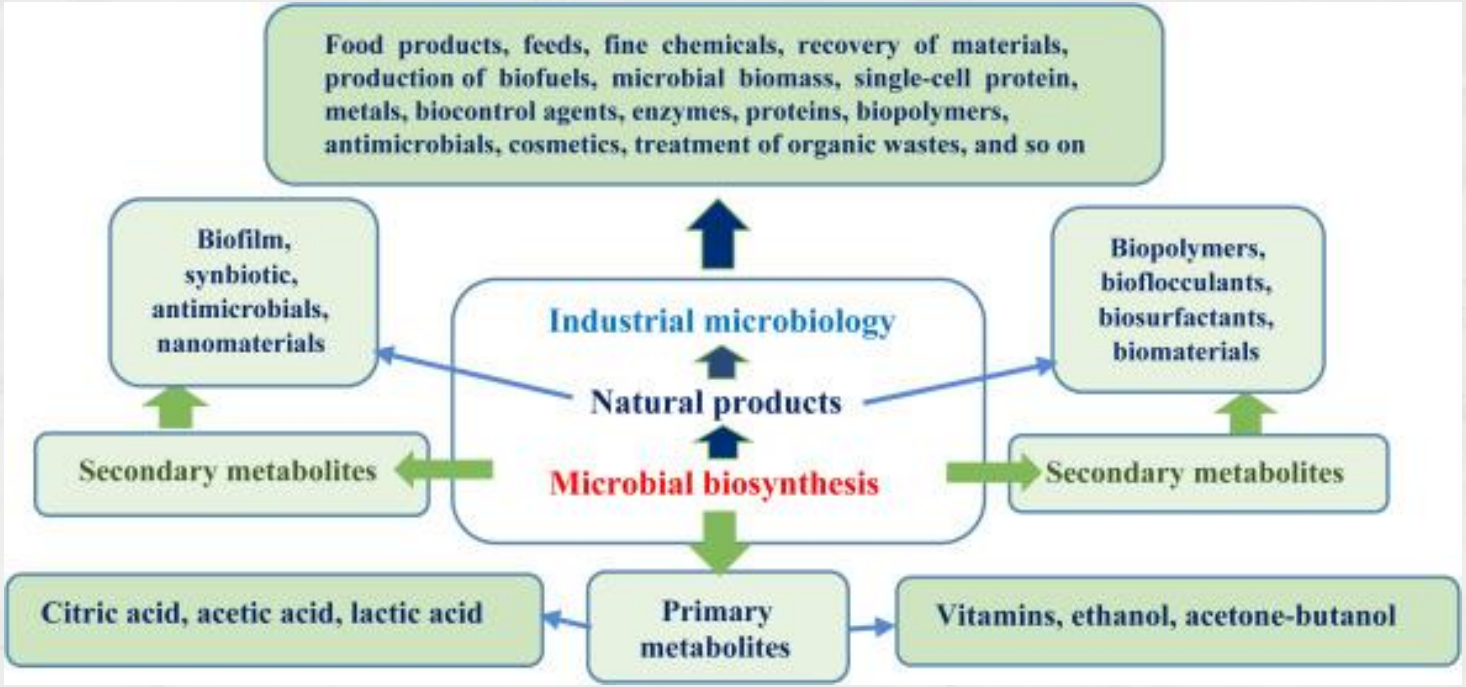


Microbial products :

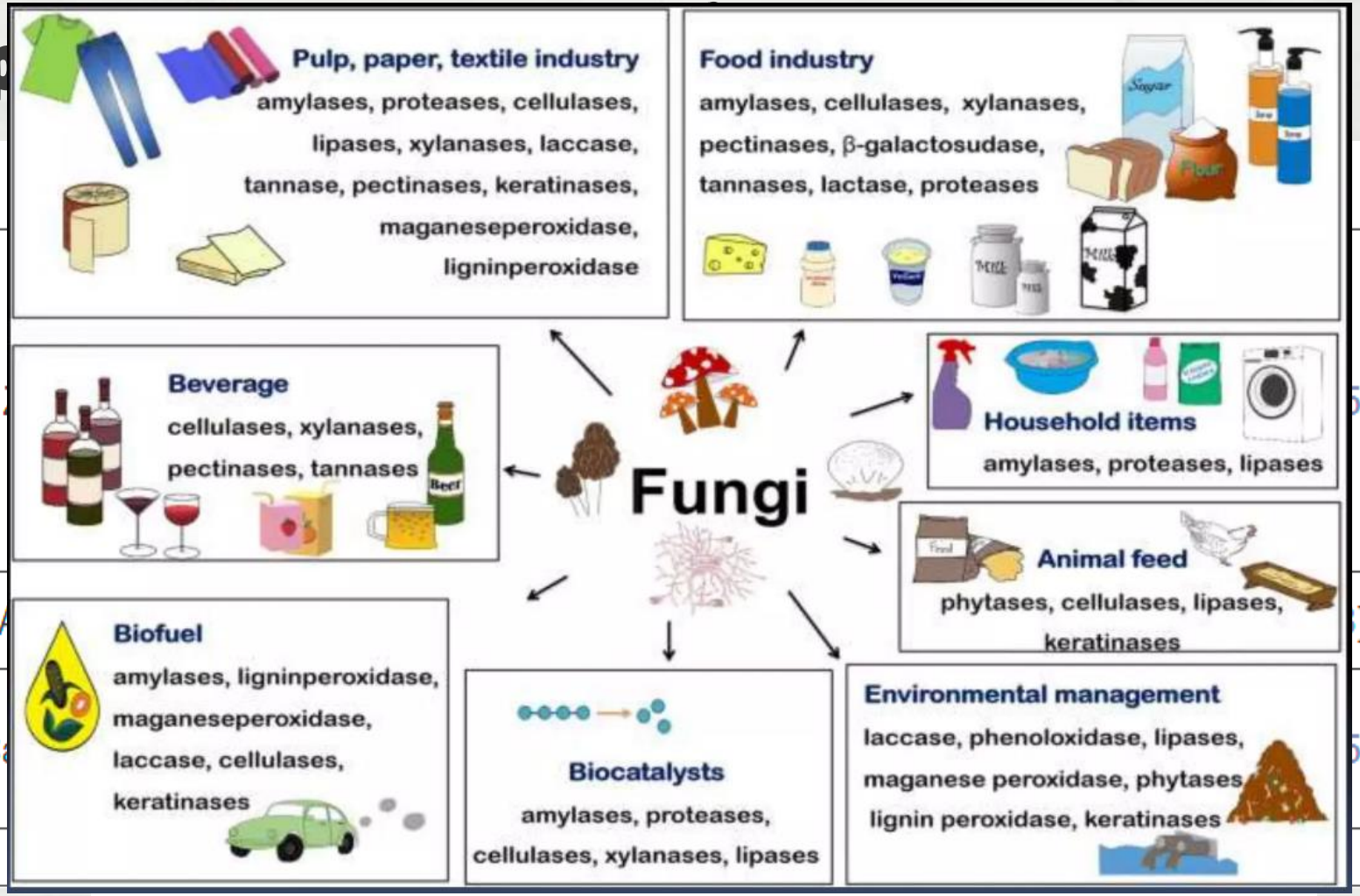
- **Microbial products can be broadly categorized into:**

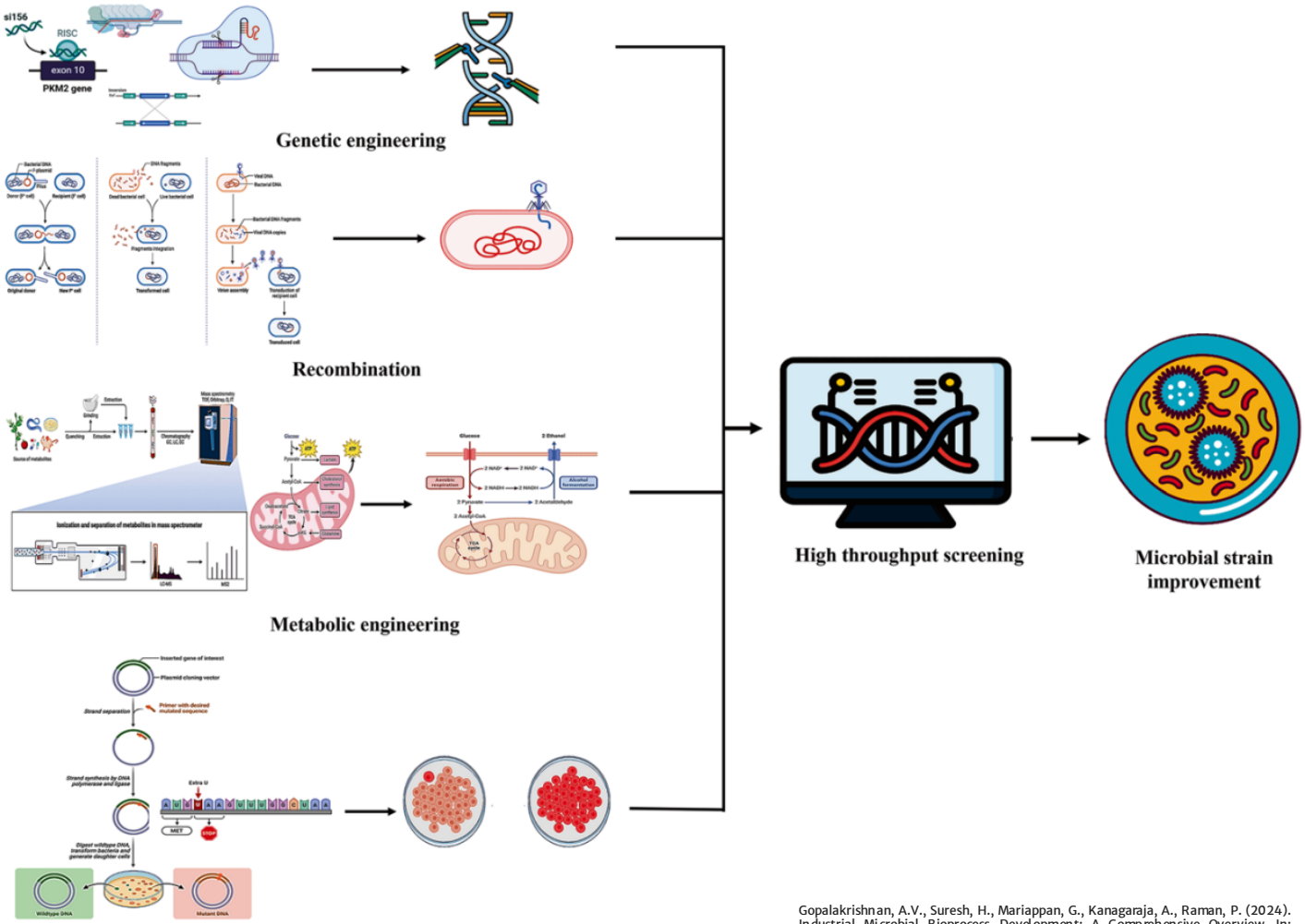
1. Metabolic production. can be divided into two broad classes: primary metabolites, and secondary metabolites.
2. Biotransformation.
3. Production of biofuels.
4. Treatment of organic and industrial wastes.
5. Recovery of metals.
6. Production of microbial biomass (microbial protein) for food and feed.
7. Production of biocontrol agents.
8. Fermentation of food products.

Microbial products :



Abdel-Aziz, S. M., Abo Elsoud, M. M., & Anise, A. A. H. (2017). Microbial biosynthesis: A repertory of vital natural products. In A. M. Grumezescu & A. M. Holban (Eds.), *Food biosynthesis* (Vol. 2, pp. 25–54). Academic Press. https://doi.org/10.1016/B978-0-12-811372-1.00003-8


Pulp, paper, textile industry
amylases, proteases, cellulases, lipases, xylanases, laccase, tannase, pectinases, keratinases, maganeseperoxidase, ligninperoxidase
Food industry
amylases, cellulases, xylanases, pectinases, β-galactosudase, tannases, lactase, proteases
Beverage
cellulases, xylanases, pectinases, tannases
Household items
amylases, proteases, lipases
Fungi
Biofuel
amylases, ligninperoxidase, maganeseperoxidase, laccase, cellulases, keratinases
Biocatalysts
amylases, proteases, cellulases, xylanases, lipases
Animal feed
phytases, cellulases, lipases, keratinases
Environmental management
laccase, phenoloxidase, lipases, maganese peroxidase, phytases, lignin peroxidase, keratinases



Gopalakrishnan, A.V., Suresh, H., Mariappan, G., Kanagaraja, A., Raman, P. (2024). Industrial Microbial Bioprocess Development: A Comprehensive Overview. In: Verma, P. (eds) Industrial Microbiology and Biotechnology. Springer, Singapore. https://doi.org/10.1007/978-981-97-6270-5_4

Top Technology Trends in The World of Microbial Products

Synthetic biol

Engineering principles wit
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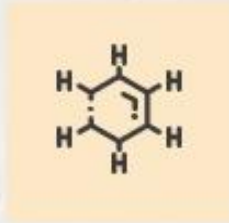
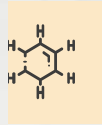
biological system



Metabolic engineering

Modification of metabolic pathways to enhance the production of specific products.



c engineering

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On

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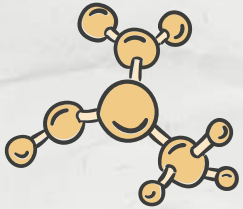
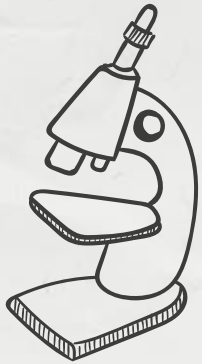
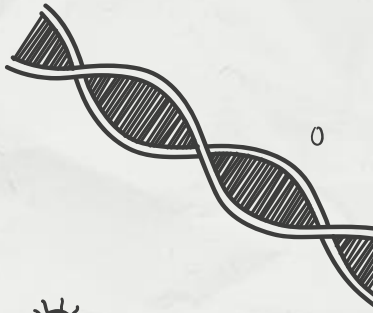
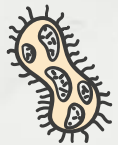
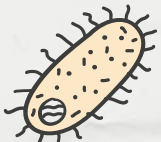
develop new microbial products.

microbial strains for specific
properties






03

Interplay between protein and metabolic engineering

Protein and metabolic engineering

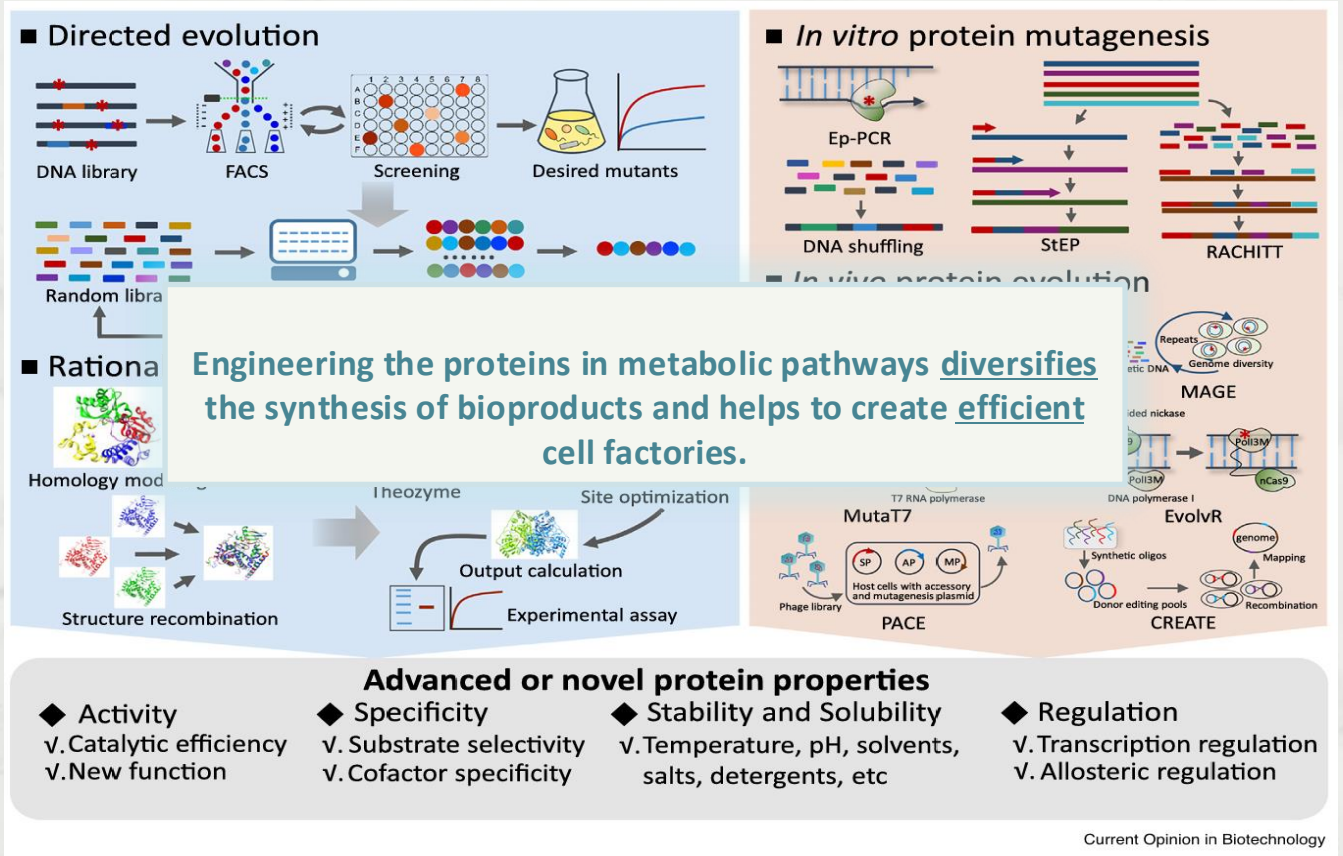
- Given that **enzymes** are generally protein molecules that function as biocatalysts to facilitate the biochemical reactions in metabolic pathways, many attempts of classical metabolic engineering focus on the improvement of specific metabolic fluxes by increasing gene expression levels and enzyme concentrations.

- However, the majority of naturally occurring enzymes show limitations. **SO?**

➔ protein engineering is effective toolbox.

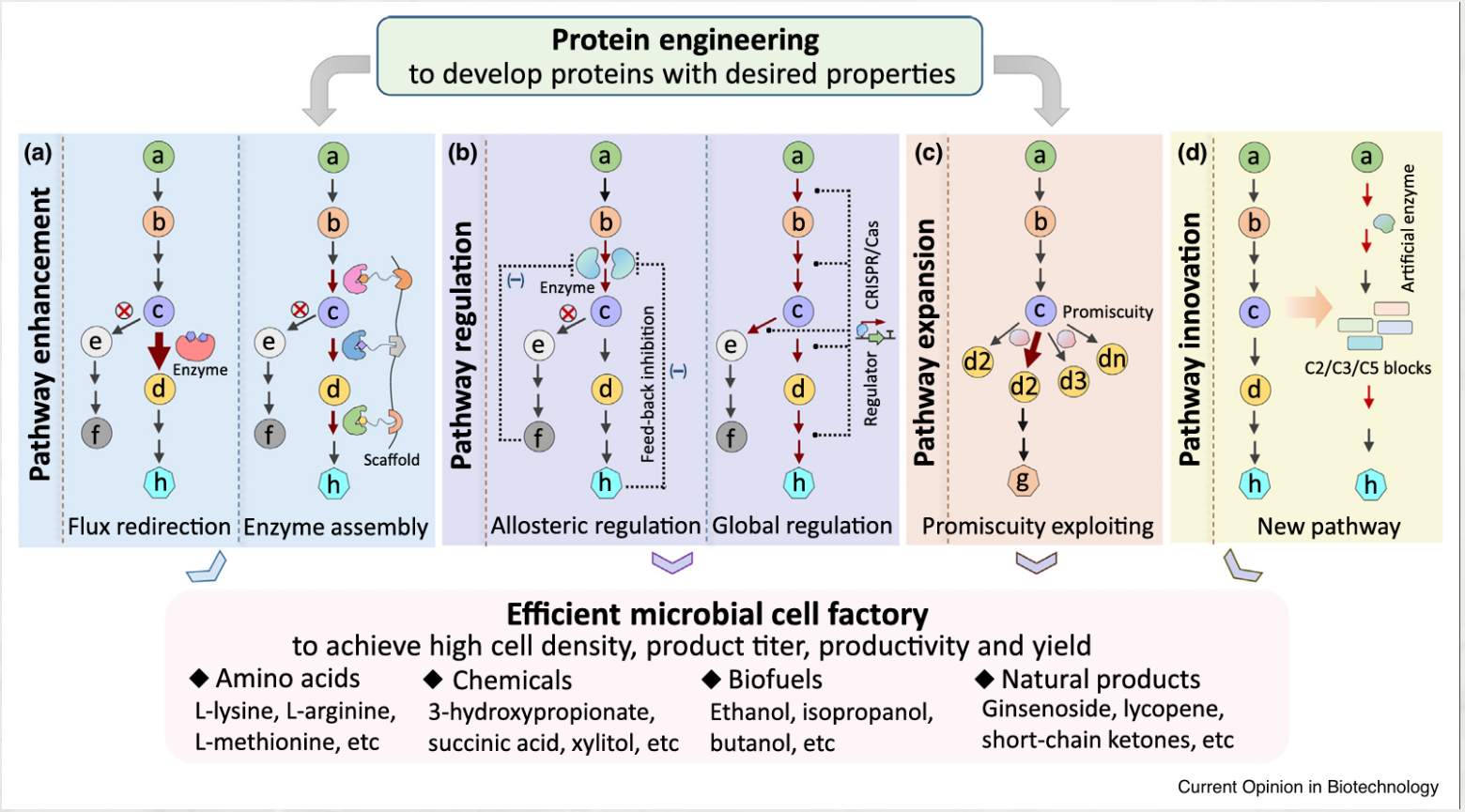
for the improvement of biological networks by **enhancing, regulating, expanding,** and **innovating** metabolic pathways.

Current toolkits and trends for engineering proteins with advanced or novel properties



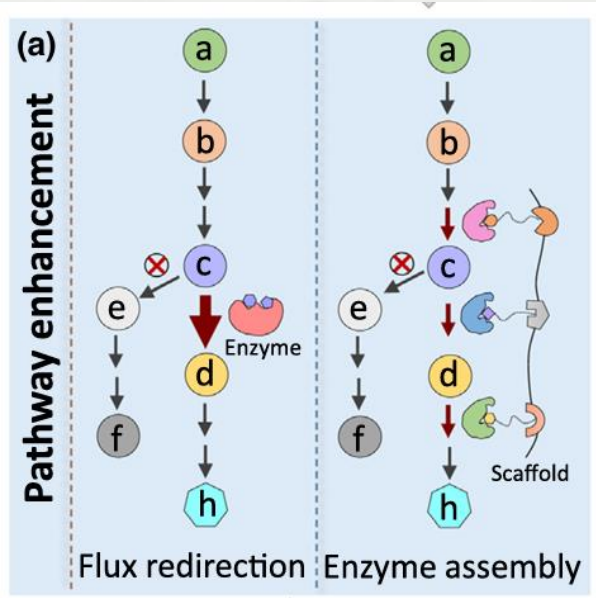
Xu, N., Liu, Y., Jiang, H., Liu, J., & Ma, Y. (2020). Combining protein and metabolic engineering to construct efficient microbial cell factories. *Current Opinion in Biotechnology, 66*, 27–35. https://doi.org/10.1016/j.copbio.2020.06.001

Role of Protein Engineering within Metabolic Engineering



Xu, N., Liu, Y., Jiang, H., Liu, J., & Ma, Y. (2020). Combining protein and metabolic engineering to construct efficient microbial cell factories. *Current Opinion in Biotechnology, 66*, 27–35. https://doi.org/10.1016/j.copbio.2020.06.001

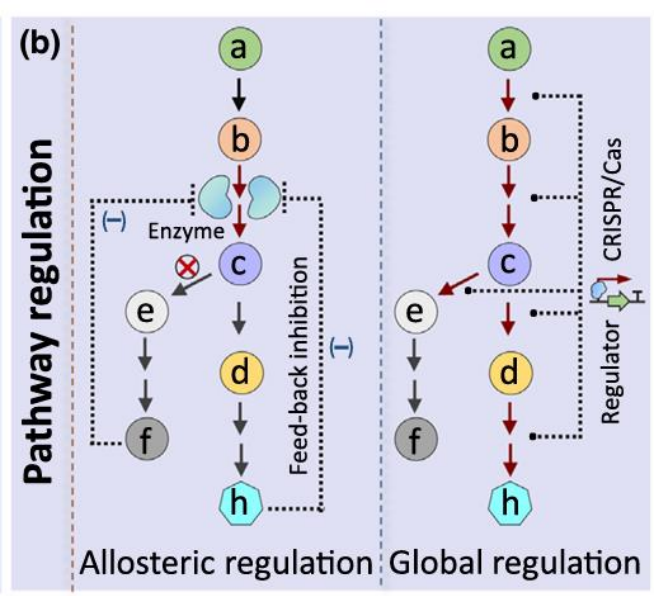
(1) Pathway enhancement:



1. By **modifying rate-limiting enzyme** properties such as catalytic activity and stability to redirect metabolic fluxes from the precursors toward target metabolites.

2. **Protein co-localization**: Protein scaffolds based on peptide motifs and adaptor domains bring driving forces for spatial organization of the enzymes that catalyze sequential reactions

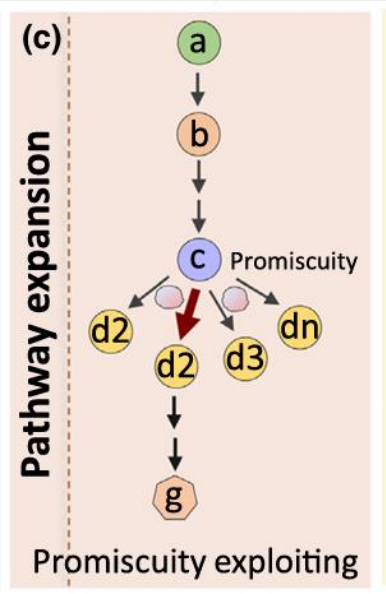
(2) Pathway regulation:



1. **Alleviating the native feed-back inhibition** of metabolic enzymes is considered to be an effective approach for improving end-product yields. (local control)

2. CRISPR-Cas9: using iCREATE "iterative CRISPR EnAbled Trackable genome Engineering.“ for modifying global regulators, transcription factors, and metabolic enzymes involved in targeted pathways.

(3) Pathway expansion:

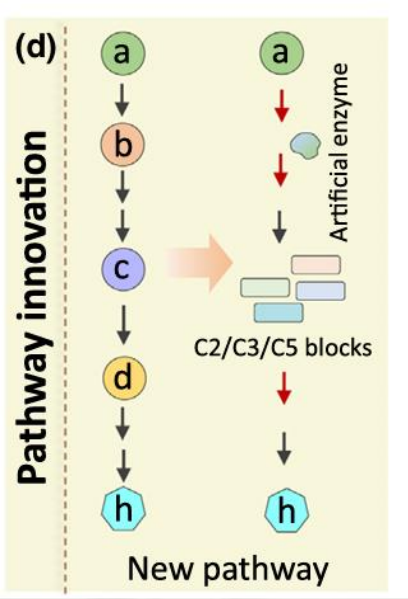


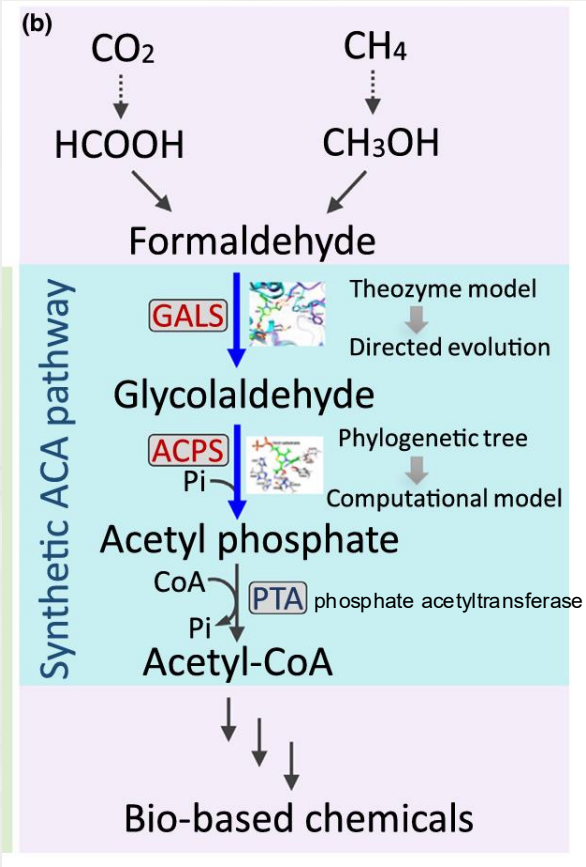
1. Modifying the enzyme's substrate specificity to increase the diversity and expand the pathway.

- **Example:** Terpenoids are a large group of specialized metabolites with numerous applications. Yet, being synthesized from five-carbon units (C10, C15, C20, etc.).

➔ *Ignea et al.* exploited substrate promiscuity of **monoterpene synthases by structure-based approach** to engineer a **single-residue switch** in the enzyme to catalyze the production of various C11 terpene scaffolds from 2-methyl-GPP (2meGPP) in engineered yeast cells.

(4) Pathway innovation:

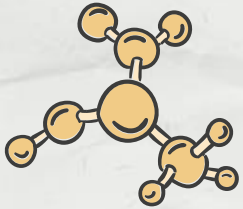
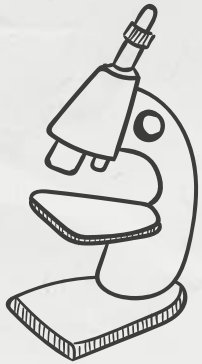


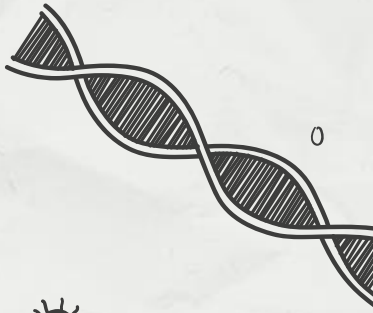


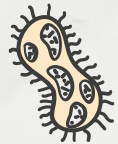
- *Lu et al.* designed an efficient **synthetic acetyl CoA (ACA) pathway** from C1 compounds by rationally engineering glycolaldehyde synthase (GALS) and acetyl phosphate synthase (ACPS).
- This pathway is made up of just three steps for the biosynthesis of acetyl-CoA in an ATP-independent manner, providing new routes for efficient production of acetyl-CoA-derived compounds.

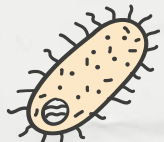
* glycolaldehyde synthase (GALS) ➔ referred to the catalytic mechanisms of ThDP-dependent enzymes and constructed a theozyme model.

* acetyl phosphate synthase (ACPS) ➔ selected eight candidates based on the phylogenetic tree of Phosphoketolases (PKs) from 111 bacteria families. (repurposing)

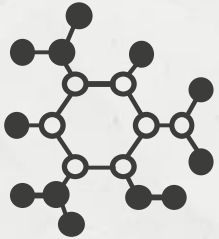


04

Metabolic Engineering and pathway design

Development of microbial cell factories

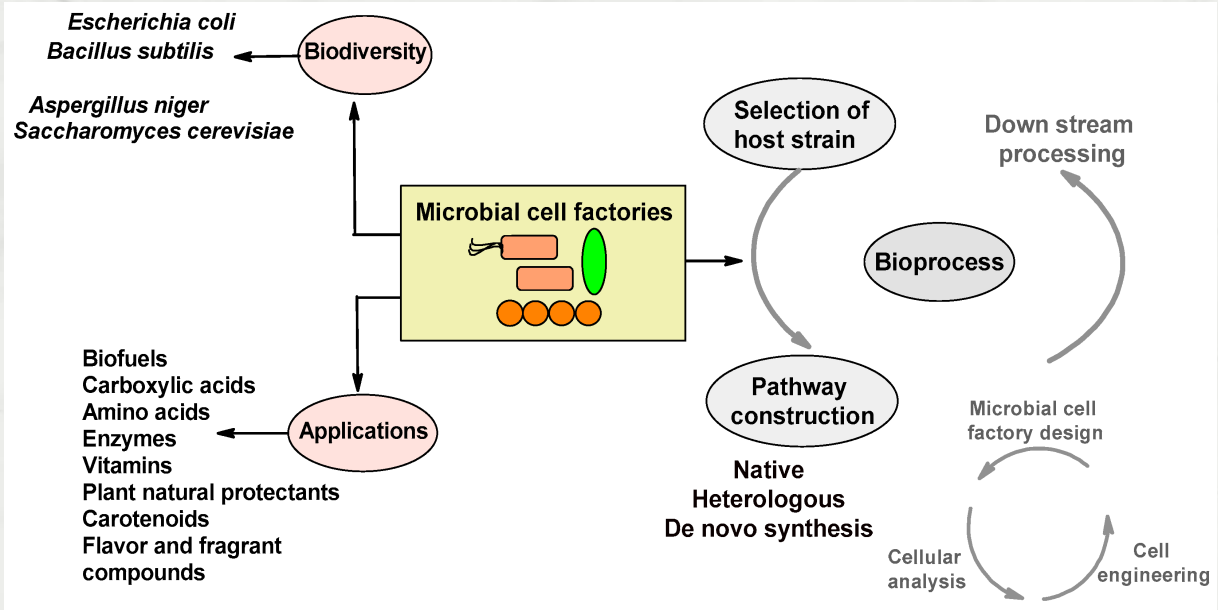


- The development of microbial cell factories by utilizing metabolic engineering involves the **rewiring of cell metabolism** in order to augment native metabolite production or enable cells to generate new products.

- Traditional strategies are based on eliminating metabolic shunt-related enzymes, modifying metabolic pathways, and balancing the reducing power and ATP to shift the metabolic flux for attaining desired end products.

Designing Microbial Cell Factories

Selection of the desired end product → Microbial Strain Selection → Pathway Construction



Chaudhary, R., Nawaz, A., Fouillaud, M., Dufossé, L., Haq, I. u., & Mukhtar, H. (2024). Microbial cell factories: Biodiversity, pathway construction, robustness, and industrial applicability. *Microbiology Research, 15*(1), 247–272. https://doi.org/10.3390/microbiolres15010018

Microbial Strain Selection

- **The criteria for selecting the starting strain comprise the following steps:**

1. easier manipulation of the respective organism;
2. whether the selected product is native, partially native, or non-native;
3. suitability and sustainability for large-scale production;
4. optimum growth and desired product formation on simple culture mediums;
5. metabolic capacity towards the end product;
6. bioprocess compatibility;
7. cost-effective recovery;
8. and purification procedures.

Pathway Construction

1.Native Pathway:

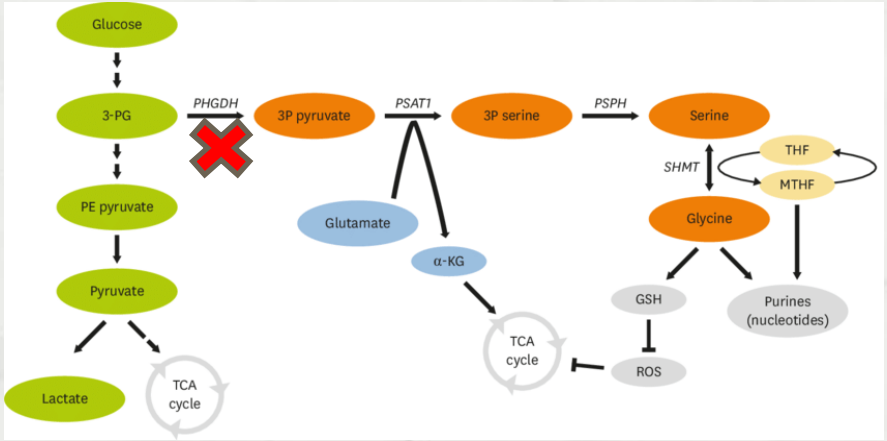
- Native metabolite **by native pathway.**
- Natural overproducers are favored host strains.

- **Examples:**

A. Metabolically engineered *S. cerevisiae* strains by "**removing enzyme**":

- *S. cerevisiae* is a natural producer of succinate.
- **Problem?** consumed during the TCA cycle.
- Solution? Metabolically engineered *S. cerevisiae* by removing succinate dehydrogenase and 3-phosphoglycerate dehydrogenase isoenzymes.
- RESULTS ➔ The mutant strain further upregulates the conversion of iso-citrate to succinate and glyoxylate, ultimately counteracting the deficiencies of serine and glycine.

Higher metabolic flux towards target



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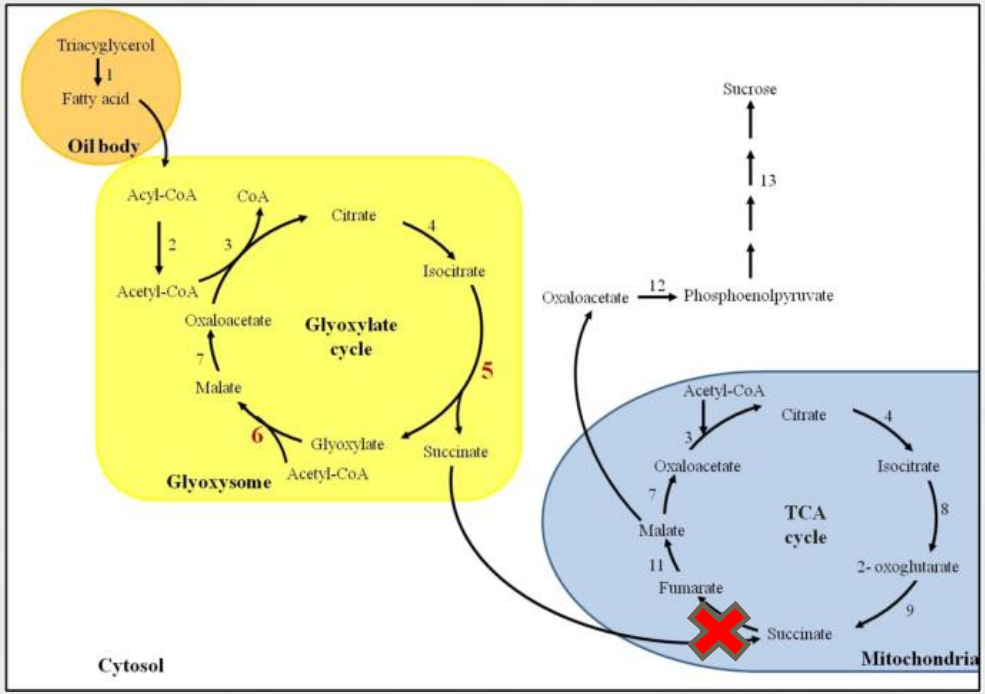


Wu, WL., Hsiao, YY., Lu, HC. *et al.* Expression regulation of *MALATE SYNTHASE* involved in glyoxylate cycle during protocorm development in *Phalaenopsis aphrodite* (Orchidaceae). *Sci Rep* **10**, 10123 (2020). https://doi.org/10.1038/s41598-020-66932-8

Pathway Construction

1.Native Pathway:

- Native metabolite **by native pathway.**
- Natural overproducers are favored host strains.

- **Examples:**

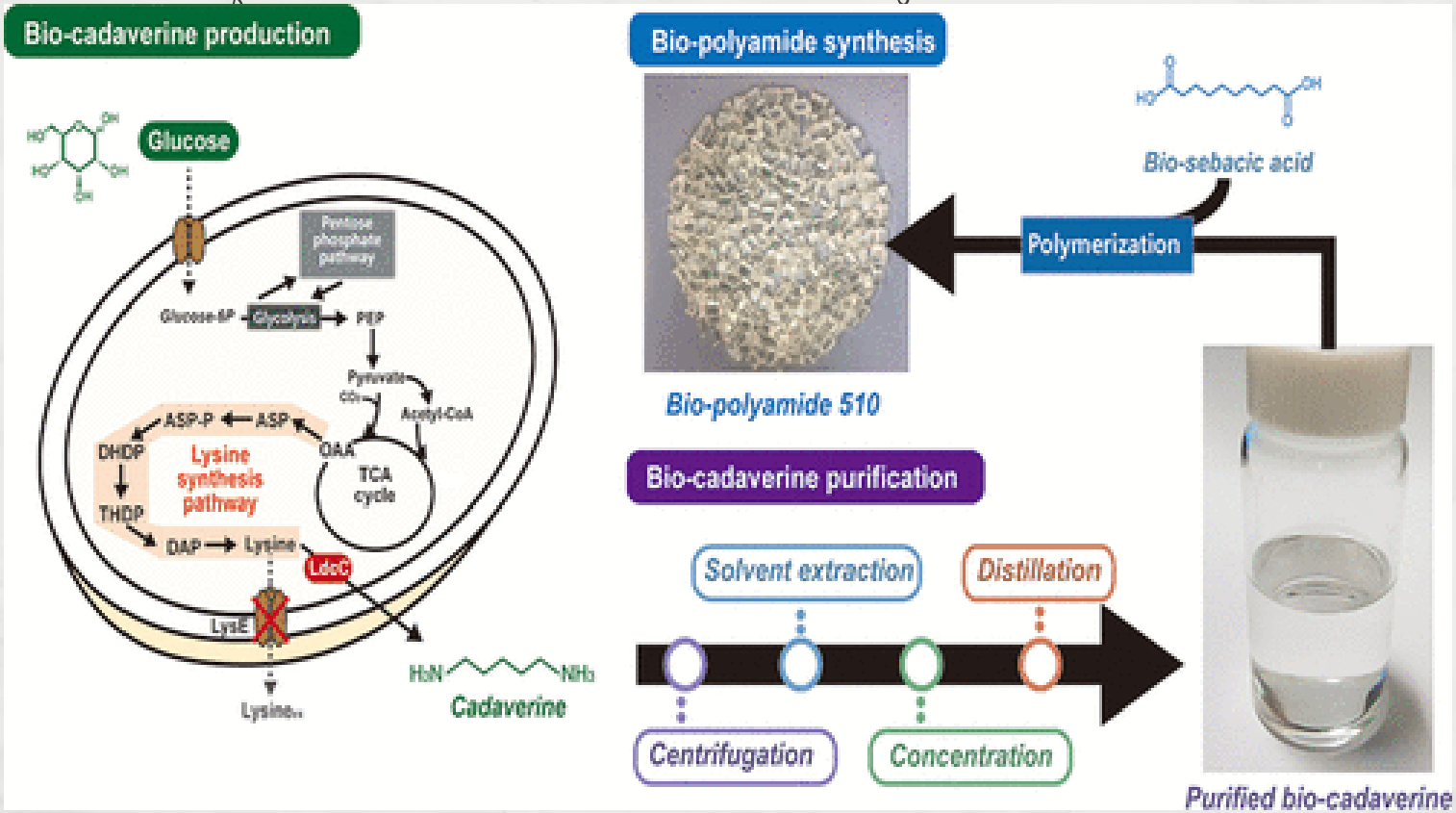
B. Metabolically engineered *C. glutamicum* strain by "**adding enzymes**" **:**

- Utilization of the L-lysine-producing *C. glutamicum* PKC strain as a chassis strain for **cadaverine** (polyamide monomer) production.
- **Cadaverine** is simply L-lysine that has lost one **CO_2 group** (decarboxylated).
- **Problem?** We want decarboxylated lysine.
- Solution?

➢ L-lysine decarboxylase (LDC) catalyzes a direct decarboxylation reaction to convert L-lysine into cadaverine.

➢ Metabolically engineered *C. glutamicum* PKC strain by integration of ldcC gene of E. coli into the genome of the C. glutamicum strain by disrupting L-lysine exporter (LysE).

- RESULTS ➔ producing greater yields of cadaverine under fed-batch glucose fermentation.



Kim, H.T.; Baritugo, K.A.; Oh, Y.H.; Hyun, S.M.; Khang, T.U.; Kang, K.H.; Jung, S.H.; Song, B.K.; Park, K.; Kim, K.; et al. Metabolic engineering of Corynebacterium glutamicum for the high-level production of cadaverine that can be used for the synthesis of biopolyamide 510. *ACS Sustain. Chem. Eng.* **2018**, *6*, 5296–5305.

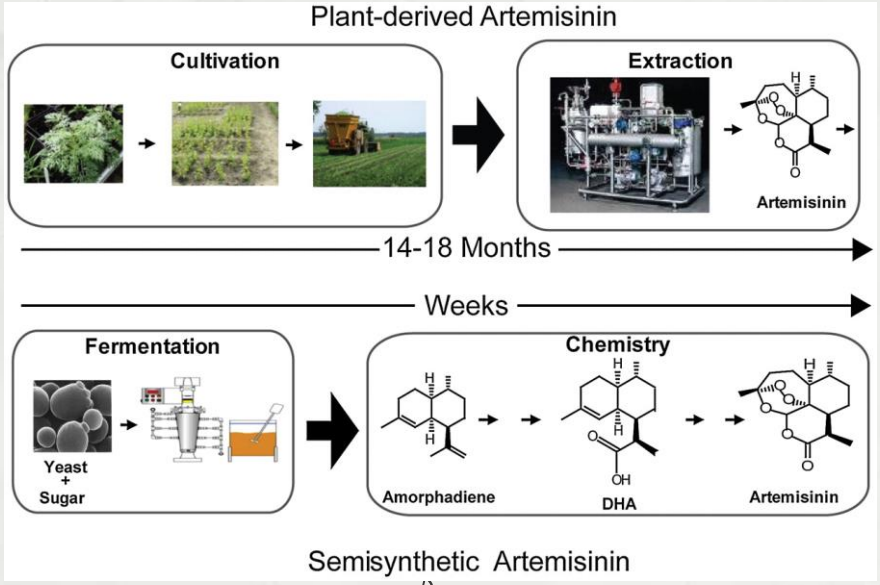
Pathway Construction

2. Heterologous Pathway:

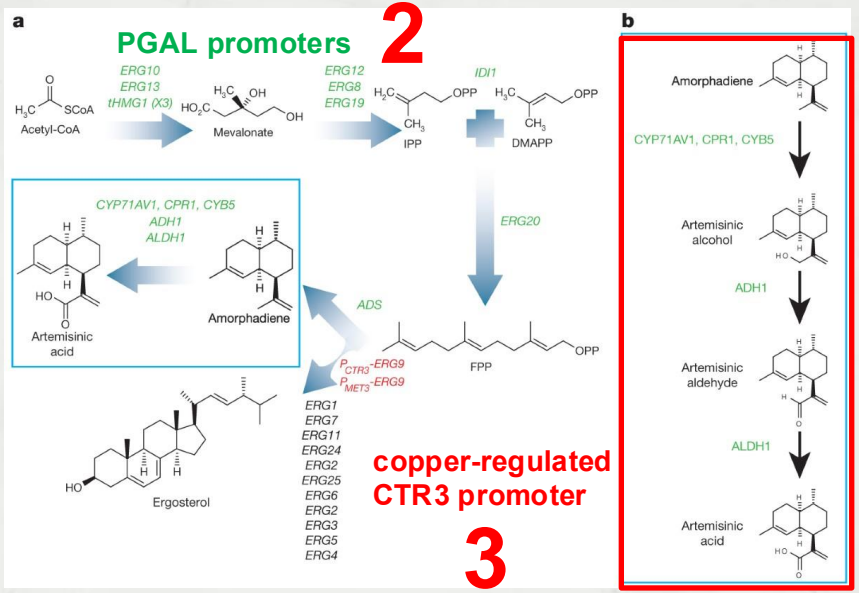
- New product **by installing heterologous pathways.**
- When?

- **Examples:**

A. Metabolically engineered *S. cerevisiae* strains for semi-synthetic production of the potent antimalarial artemisinin :



P.J. Westfall, D.J. Pitera, J.R. Lenihan, D. Eng, F.X.et al. ,Production of amorphadiene in yeast, and its conversion to dihydroartemisinic acid, precursor to the antimalarial agent artemisinin, Proc. Natl. Acad. Sci. U.S.A. ,118E-111E (3)109 .(2012)



Paddon, C., Westfall, P., Pitera, D. *et al*. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* **496**, 528–532 (2013).

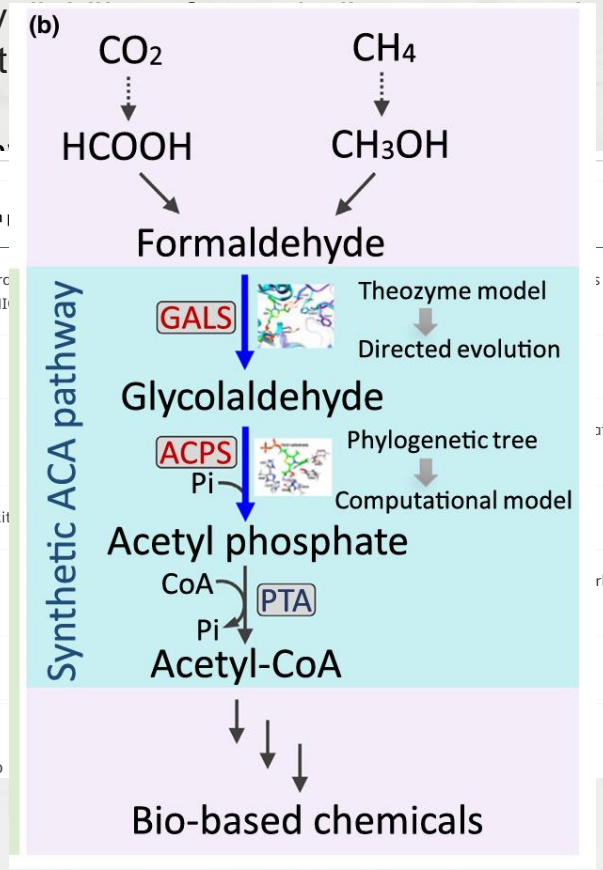
Pathway Construction

3. Artificial De Novo Pathway:

- **When?** unav… enzymes for several target…
- Artificial path… …uity **enzyme**…

De novo pathway prediction		References
Biochemical Network Integrc… Computational Explorer (BNI…	…s enzyme	[67,68,70]
BRENDA		[71]
DESHARKY	…atic reaction	[72]
From Metabolite to Metabolit…		[73]
L1SVM, L2SVM, BASELINE	…rk to predict	[74]
META		[75]

Table 2
Tools for designing de novo…



Metabolic Engineering Approaches

Metabolic

- The two most common rationally-based enzyme engineering approaches are:
(i) rational design and (ii) rational redesign.

- **Rational design** is not as commonly used as evolutionary approaches, and it is unique in its ability to completely design new (i.e., never before seen in nature) functional enzymes. This is done through computationally driven design using the Rosetta suite of programs.

➔ Ex: A serine hydrolase was recently developed possessing comparable activity to native enzymes through a focus on obtaining the correct serine-containing catalytic triad design.

- **In Rational redesign** the natural catalytic activity of an existing enzyme is altered through rational selection of mutations. The selection process is also computationally driven.

Genome

Random mu

Metabolic Engineering Approaches CONT'

- Tolerance engineering

Tolerance engineering focuses on developing microbes with improved robustness toward toxic or inhibitory end products.

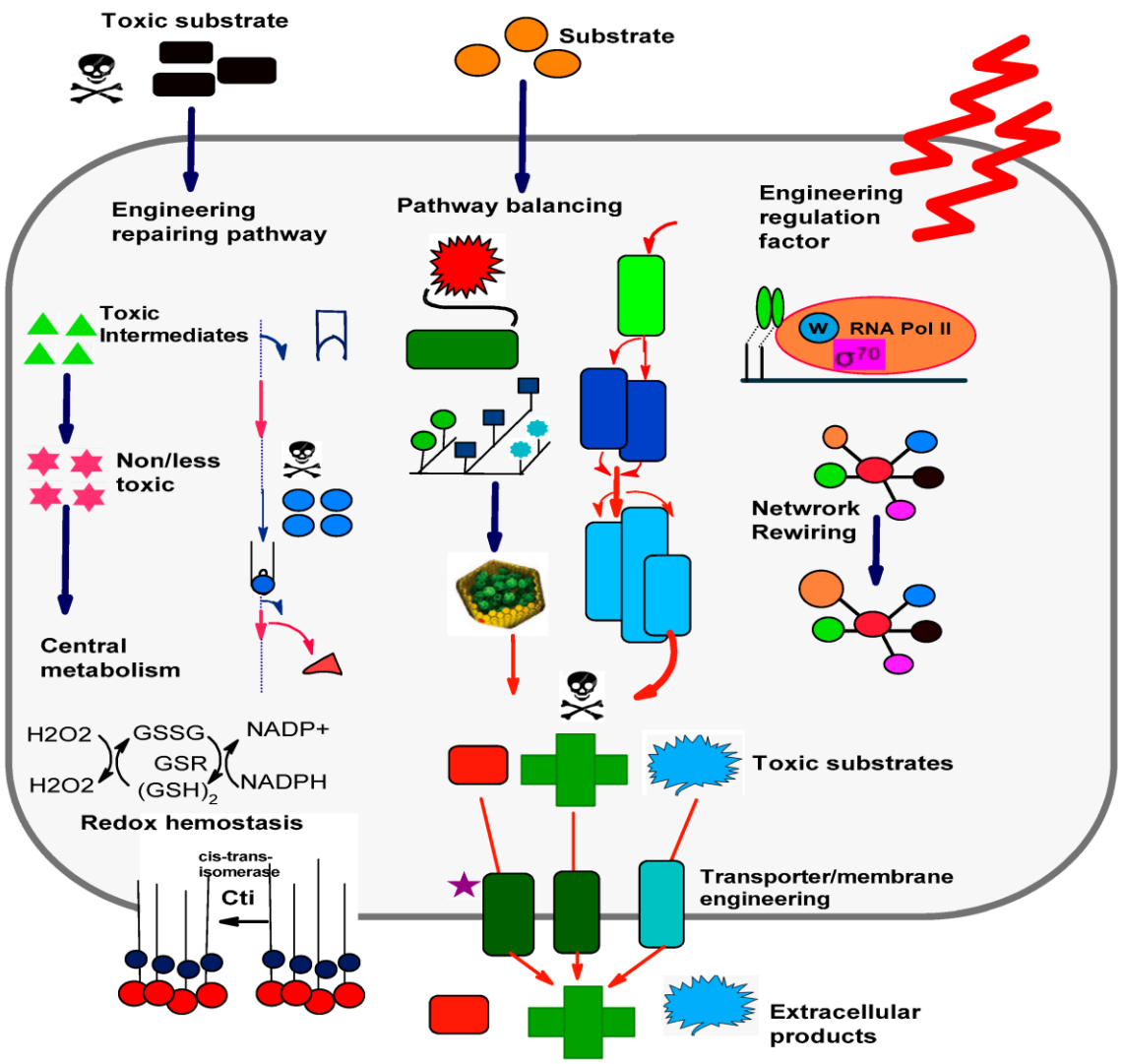
ngineering-

and transporter engineering

- Process engineering via two-phase (aqueous/organic) fermentation
- eVOLVER

- Transporter engineering

- Importer engineering
- Exporter engineering

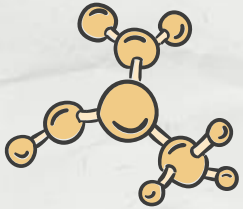


Engineering of robustness in microbial cell factories

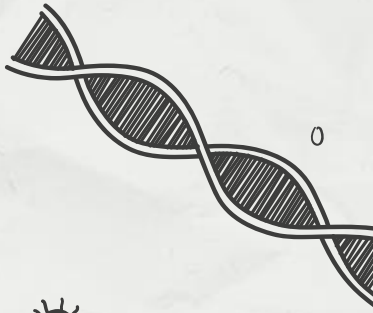
1. Introducing synthetic pathways and engineering **repairing pathways** sustain redox and cellular metabolic hemostasis and transform toxic substrate into less/nontoxic compounds, respectively.

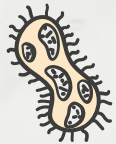
2. **Pathway balancing** evades the buildup of toxic intermediates in addition to refining the biosynthesis rate.

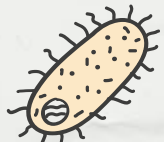
3. **Transporter engineering** relieves toxicity while **membrane engineering** generates tolerance in membrane against stress and toxic molecules.









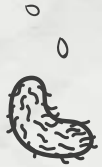
04

Challenges and Limitations

Challenges, Commercial Limitations, and Future Prospects:

- Metabolic cell factories face several limitations during industrial implementations, such as the production of toxic inhibitors, a low pH environment, and extreme temperature conditions, which leads to a relatively low industrial productivity compared to that acquired at the lab scale.
- Host selection & engineering limits.
- Cross-species pathway expression issues: enzymes from different organisms may not function efficiently in new hosts.

- Although metabolic engineering is a well-established strategy for transforming microorganisms into efficient cell factories, there is a high possibility that novel robust industrial phenotypes could emerge from the coupling of more database mining, omics technologies, and advanced genome engineering strategies.



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Thank you

Any questions?