Biotechnological Applications of Recombinant DNA

Dr. Selma Hamimed
Department of Molecular and Cellular Biology
Faculty of Natural Sciences and Life
University of Jijel
Email: selma.hamimed@univ-jijel.dz[1]

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Modification of metabolism by genetic engineering

1. Objectives

- Explore the mechanisms involved in modifying metabolic pathways through genetic engineering techniques.
- Examine case studies where genetic modifications have successfully enhanced or altered metabolic functions in organisms.

2. Introduction

Modification of metabolism using genetic engineering or Metabolic engineering is a fascinating field that combines biology, chemistry, and engineering principles to manipulate and optimize the metabolic pathways of living organisms. By understanding and modifying these pathways, scientists and engineers can enhance the production of valuable compounds, such as biofuels, pharmaceuticals, and industrial chemicals. At its core, metabolic engineering involves the design, construction, and optimization of genetic pathways within microorganisms or plant cells. These pathways are responsible for the conversion of raw materials into desired products. Through genetic manipulation, researchers can introduce or modify specific enzymes and metabolic reactions to enhance the production of target compounds.

One of the key tools used in metabolic engineering is recombinant DNA technology. This technique allows scientists to insert or delete specific genes in an organism's genome, altering its metabolic capabilities. By introducing genes from different organisms or even synthesizing entirely new genes, researchers can create novel pathways that enable the production of valuable compounds. The success of metabolic engineering relies on a deep understanding of the biochemical pathways involved. Scientists must carefully analyze the metabolic network, identify rate-limiting steps, and select appropriate targets for modification. They also need to consider the interplay between different metabolic pathways and the impact that changes may have on overall cellular function. Computer modeling and simulation play a crucial role in metabolic engineering. By using mathematical models, scientists can predict the behavior of engineered pathways and optimize their performance. These models take into account factors such as enzyme kinetics, substrate availability, and cellular metabolism. Through iterative cycles of design, simulation, and experimental validation, researchers can fine-tune their engineered pathways to achieve desired outcomes.*44*[44]*

(cf. What is metabolic engineering?)

3. How to conduct modification of metabolism?

Metabolic engineering refers to the deliberate and targeted modification of an organism’s metabolic pathways to better understand and exploit cellular processes for chemical transformation, energy transduction, and supramolecular assembly *45*[45]*. This interdisciplinary field integrates principles from chemical engineering, computational science, biochemistry, and molecular biology. At its core, metabolic engineering applies

engineering principles to the design and analysis of metabolic pathways to achieve specific goals, such as enhancing productivity in the production of antibiotics, biosynthetic precursors, or polymers, or extending metabolic capabilities for chemical synthesis or degradation.

Historically, metabolic engineering strategies relied heavily on trial-and-error experimentation. This scientific approach employs recombinant DNA technology and advances in cellular physiology to modify intermediary metabolism more precisely.

The growing interest in metabolic engineering is driven by its potential for commercial applications, particularly in developing microbial strains that can enhance the production of valuable metabolites. Recent efforts have centered on using biologically derived processes as sustainable alternatives to traditional chemical methods, aligning with goals of “green chemistry” and “sustainable development.” Notable examples include microbial production of indigo by Genencor and propylene glycol by DuPont, as well as advancements in the more established areas of antibiotic and amino acid production by various companies. Furthermore, the application of metabolic engineering in plant tissues and its role in understanding genetically determined human metabolic disorders broaden the field’s relevance beyond industrial fermentation, highlighting its future significance across multiple sectors.

3.1. Setting up a metabolic pathways

This is the first step in the process where the identification of desired goal to achieve through the improvement or modification of an organism’s metabolism.

It requires a **databases** and **scientific articles** to determine the metabolic pathways of our product of interest.

3.2. Analyzing a metabolic pathway

The completed metabolic pathway is modeled by different approaches using AI or mathematic to find the theoretical yield of the product or reaction fluxes.

Flux refers to the rate at which metabolites are produced, consumed, or transformed within a biological system.

3.3. Determining the optimal genetic manipulations

It is necessary to determine which reactions may be altered in order to maximize the yield of the desired product.

To determine what specific genetic manipulations to perform, it is necessary to use computational algorithms, such as OptGene or OptFlux.

3.4. Experimental measurements

In order to verify the effect of genetic manipulations on the metabolic network (to ensure they align with the model), it is necessary to experimentally measure the fluxes in the network.

To measure reaction fluxes, carbon flux measurements are made using carbon-13 isotopic labeling.

***Méthode* : Requirements**

- The biosynthetic pathway of the chemical to be produced;
- Genes encoding the related enzymes;
- Regulation of such enzymes, with ability to transfer and express or suppress the required genes in the host organism;

- Mutate the gene *in vivo* and *in vitro* to be able to alter propertied of the encoded enzymes;
- Assemble an array of genes for their expression inside the host cell.

***Fondamental* : Pathways**

Pathways are series of interconnected chemical reactions that occur within cells. These reactions are catalyzed by enzymes, which are biological molecules that speed up chemical reactions. Pathways are responsible for various cellular processes, such as energy generation, synthesis of biomolecules, and elimination of waste products.

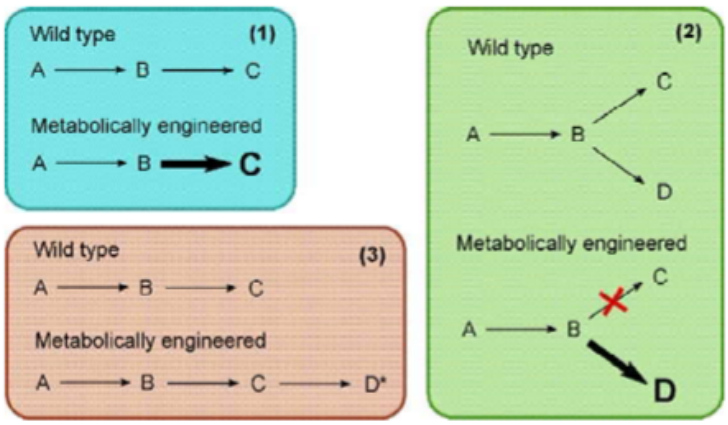
- Directly by manipulating the genes encoding the enzymes catalyzing the reactions in the pathways.
- Indirectly by altering the regulatory pathways affecting gene expression and enzyme activity.

3.5. Resulting approaches

1. **Over expressing the gene** encoding the rate-limiting enzyme of the biosynthetic pathway of the desired end-product: This can be achieved by introducing additional copies of the gene into the organism or by using genetic engineering techniques to enhance the gene expression.
2. **Inhibit the competing metabolic reactions** which involve the same substrate leading to metabolically channel the substrate towards the desired chemical
3. **The production of the desired biochemical can be carried out in the non-native organism.** Gene can be isolated from the organism which naturally produces the desired biochemical and can be expressed in another organism which might be easier to cultivate than the host organism.
4. **Engineered an enzyme that is not found in nature.** This mode relies on creating mutations in the related gene so that the amino acid composition of the enzyme is altered.

4. Engineering strategies for metabolic modifications

Engineering strategies for metabolic modifications often start with an understanding of the physiological characteristics of the host cell. Stephanopoulos and Vallino (*46*[46]*) introduced the concept of network rigidity, where metabolic networks consist of flexible and rigid nodes. The rigidity of these networks, or their resistance to metabolic changes, is governed by control mechanisms that maintain balanced growth.



Strategies for metabolic engineering for the production of a desired chemical: (1) overexpression of the rate-limiting enzyme, (2) inhibition of the competing pathway and (3) engineering a novel enzyme for the production of non-natural chemical.

4.1. Physiological factors

A successful engineering strategy requires a thorough understanding of the host cell's physiology to determine the most effective genetic modifications needed to achieve the desired outcome. Physiological factors such as the impact of genetic changes on growth and potential unintended effects on other systems must be carefully considered. These unintended effects, often attributed to metabolic burden, can hinder success. For example, the overexpression of phosphoenolpyruvate-forming enzymes has been shown to inhibit both the heat-shock response and nitrogen regulation[47]*.

In terms of DNA modification techniques, several approaches have been employed to modify the host cell for desired outcomes. These include: removing or inhibiting an enzyme to block a competitive pathway or eliminate a toxic byproduct; amplifying a gene or group of genes to enhance product synthesis [48]*; or expressing heterologous enzymes to extend substrate range, produce novel products, degrade toxic compounds , or enhance environmental resistance in plants[49]* .

In some cases, the deregulation of existing enzymes is necessary to bypass rigid control nodes in the metabolic pathway. A combination of genetic modifications is often required to achieve the overall goal. Additionally, manipulation of the central metabolic pathway may be necessary to generate the precursors, cofactors, and energy needed to support the modified pathways. An example of this approach can be seen in the production of aromatic compounds in *E. coli* grown on glucose [50]*.

4.2. Inverse metabolic engineering

nverse metabolic engineering takes a different approach from classical metabolic engineering. While the classical method requires extensive knowledge of enzyme kinetics, network systems, and intermediate metabolite pools before proposing genetic modifications for presumed benefits, inverse metabolic engineering starts by identifying a desired phenotype. Once the phenotype is identified, researchers determine the environmental or genetic conditions that produce it, and then genetically modify the host organism to acquire the same phenotype (. For instance, in the case of expressing the oxygen-binding protein VHb in *E. coli*, the high levels of heme cofactors observed in the obligate aerobe *Vitreoscilla* under oxygen-limiting conditions suggested that introducing hemoglobin synthesis into other organisms could improve their growth in low-oxygen environments[51]*.

4.3. Metabolic flux analysis (MFA)

Metabolic flux analysis (MFA) is a mathematical tool used for studying biochemical networks based on known biochemistry principles. It constructs a linearly independent metabolic matrix using the law of mass conservation and the pseudo-steady-state hypothesis (PSSH) for intracellular metabolites. This results in a set of linear equations, represented as a stoichiometric matrix A with dimensions m by n, with vectors r (net accumulation) and v (metabolic flux).

Typically, the system is underdetermined (m > n), though in some cases, certain inoperative pathways can be ignored, making the system determined or overdetermined, which allows for solutions using external or internal flux measurements . Non-invasive techniques like nuclear magnetic resonance (NMR) are also valuable in analyzing biochemical network structures and fluxes[52]* .

Metabolic Control Theory (MCT) was developed independently by Kacser and Burns ([53]*) and Heinrich et al. ([54]*) to address kinetic constraints in biochemical networks. Biochemical Systems Theory (BST), a related method, was introduced by Savageau ([55]*).

Based on kinetic data, control coefficients can be calculated to identify rate-limiting reactions, though in many networks, multiple steps may share control. MCT provides three key sensitivity measures:

1. **Flux Control Coefficient (FCC)**: Measures how enzyme E influences the steady-state flux through it.

$$CJ^E = \frac{dJ}{dE} \times \frac{J}{E}$$

2. **Concentration Control Coefficient (CCC)**: Evaluates how enzyme E affects steady-state concentration X.

$$C_X^E = \frac{dX}{dE}$$

3. **Elasticity**: Quantifies how the reaction rate responds to changes in concentration X.

$$\epsilon_v^X = \frac{d(v)}{d(X)}$$

Extensions of MCT allow for the application of control analysis even under large changes in enzyme activities. This enables the experimental calculation of deviation indices from MFA data, which been explored different facets of metabolic control analysis.

Additionally, a top-down approach was developed for determining control coefficients based solely on relative flux, eliminating the need to measure enzyme activities. However, the method has limitations, such as being invalid when more than one kinetic link exists between two points in a pathway (i.e., multiple enzymes catalyzing the same reaction). Additionally, a small overall FCC does not necessarily imply that individual FCCs are small, though the method remains useful in specific cases*56*[56]*.

(cf. Introduction to Metabolic Flux Analysis)

5. Applications

Metabolic engineering has wide-ranging applications in various industries. For example, in the biofuel sector, researchers aim to develop microorganisms capable of efficiently converting renewable resources, such as plant biomass, into biofuels like ethanol or biodiesel. In the pharmaceutical industry, metabolic engineering techniques can be employed to produce valuable drugs or drug precursors using microbial hosts. This approach offers a sustainable and cost-effective alternative to traditional chemical synthesis.

As the field of metabolic engineering continues to advance, researchers are exploring new frontiers, including the production of high-value chemicals, sustainable agriculture, and personalized medicine. By harnessing the power of biochemical pathways and leveraging genetic engineering tools, metabolic engineering holds great promise for addressing societal challenges and driving innovation in the biotechnology sector.

In conclusion, non-canonical amino acids offer a fascinating glimpse into the world of metabolic engineering. These unique molecules provide a pathway to Real-life biotech companies that use bacteria to create drugs or other compounds found it very helpful. By incorporating ncAAs into proteins, scientists could also use to make designer proteins, which might include new medicines or new ways to precisely deliver and control medicines.

***Exemple* : Non-canonical amino acids**

In the world of biology, amino acids are the essential building blocks of proteins. These tiny molecules play a crucial role in various biological processes, from supporting cellular functions to forming the structure of tissues and organs. While there are 20 standard amino acids commonly found in living organisms, scientists have also discovered a fascinating group of molecules known as non-canonical amino acids (ncAAs). In 1992, researchers were able to tweak cytosine to create what was essentially a 65^{th} codon, which could then code for a non-canonical amino acid.

Non-canonical amino acids are chemically similar to their canonical counterparts but possess unique properties and structures. These exceptional molecules offer researchers new avenues to explore in the field of metabolic engineering, where scientists manipulate cellular metabolic pathways to produce desired compounds or enhance organism capabilities.

In *E. coli* the non-canonical amino acids acids norvaline, norleucine, and β-methylnorleucine, which derive from an off-pathway of the branched-chain amino acid synthesis route are synthesized and incorporated into cellular and recombinant proteins

The study of non-canonical amino acids also sheds light on the natural processes of protein synthesis in living organisms. By investigating these unique molecules, scientists gain insights into the evolution and function of proteins. Understanding how ncAAs are incorporated into proteins provides valuable knowledge about the complexity of cellular machinery and the potential for expanding the diversity of life's building blocks.

(cf. Non-canonical amino acid)

6. Exercice : Prospects for crop improvement through the genetic manipulation

Through the chapter, provide an overview about the recent advancement in metabolic engineering in crops field and agriculture within 800 words text.

Glossaire

Polymerase chain reaction (PCR)

A method widely used to make millions to billions of copies of a specific DNA sample rapidly, allowing scientists to amplify a very small sample of DNA (or a part of it) sufficiently to enable detailed study.

Abréviations

DNA : Deoxyribonucleic acid ; is the molecule that carries genetic information for the development and functioning of an organism.

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