Metabolic Engineering

Biological Art of Producing Useful Chemicals

Ram Kulkarni

Metabolic engineering is a process for modulating the metabolism of the organisms so as to produce the required amounts of the desired metabolite through genetic manipulations. Considering its advantages over the other chemical synthesis routes, this area of biotechnology is likely to revolutionize the way in which commodity chemicals are produced.

Ram Kulkarni is an Assistant Professor at Symbiosis International University, Pune. His research interests are in metabolic engineering of lactic acid bacteria for increasing their nutraceutical properties.

Introduction

Living organisms have numerous biochemical reactions operating in them. These reactions allow the organisms to survive by processes such as generation of energy, production of fundamental building blocks required for structural organization and synthesis of biomolecules having specialized functions. Some of the chemicals generated during this process (called metabolism), are useful to mankind for various applications. These so-called value-added chemicals include various bioactive secondary metabolites such as an anti-malarial drug (artemesinin), chemicals required as the raw material for the synthesis of other molecules such as lactic acid, chemicals imparting flavor to food material such as terpenes, biofuels and associated chemicals such as ethanol and butanol, etc. The traditional way of utilization of such chemicals is to cultivate the host organisms producing these chemicals followed by harvesting the desired biochemical. However, in many cases, the quantities of the useful chemicals in the cells are very low, thus demanding cultivation of the organisms on a very large scale. To address this problem, organic chemists have come up with brilliant ways to chemically synthesize such value-added chemicals from the petroleum crude material. However, this strategy has its own disadvantages. Rapidly depleting petroleum resources and production of racemic mixtures[1] and harmful by-products are some of the issues which are forcing scientists to find

[1] Racemic mixtures contain equal concentrations of mirror image isomers (enantiomers) of that optically active chemical.

Keywords
Synthetic biology, commodity chemicals, microbial production, biosynthesis, enzymes.

Most of the metabolic engineering approaches are based on genetic engineering techniques.

out the alternative ways to generate these value-added biochemicals. Since living organisms already produce these chemicals, copying their biosynthesis mechanisms for their semi-natural production appears to be an excellent alternative to their isolation from natural resources and the chemical synthesis. Such an approach for the production of desired chemicals in living organisms is called metabolic engineering.

Strategies for Metabolic Engineering

Most of the metabolic engineering approaches are based on genetic engineering techniques. Some of the fundamental requirements for metabolic engineering are knowledge about (1) the biosynthetic pathway of the chemical to be produced, (2) genes encoding the related enzymes, (3) regulation of such enzymes, with ability to (4) transfer and express or suppress the required genes in the host organism, (5) mutate the gene *in vivo* and *in vitro* to be able to alter properties of the encoded enzyme, and (6) assemble an array of genes for their expression inside the host cell. Although bacteria and yeast are the pioneering hosts for metabolic engineering, other organisms such as fungi, animal as well as plant cells are also used nowadays for similar experiments.

There are different approaches of metabolic engineering for achieving the required production of the desired biochemicals. Some of them are described below along with examples.

(1) One of the most obvious approaches is overexpressing the gene encoding the rate-limiting enzyme of the biosynthetic pathway of the desired end-product. Using a similar strategy, the vitamin E content of Arabidopsis (a model plant system) has been increased by overexpression of a gene encoding the enzyme γ-tocopherolmethyltransferase [1]. See *Figure* 1.

(2) Another way of overproducing the product of a given pathway is to inhibit the competing metabolic reactions which involve the same substrate. In this way, the substrate is metabolically channeled specifically towards the desired chemical. An example of

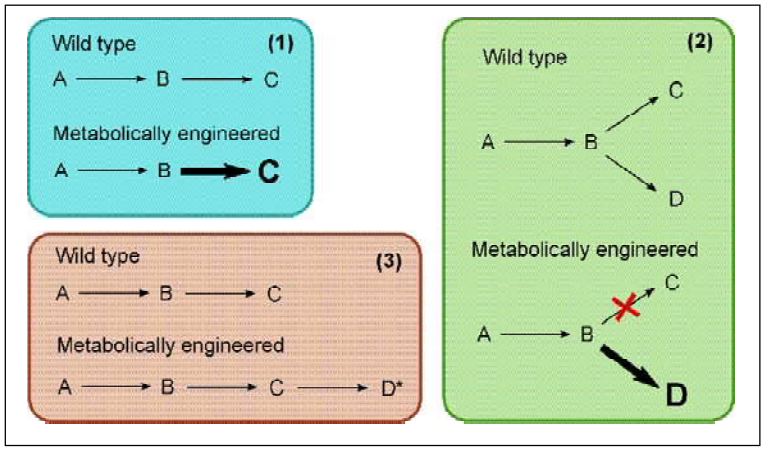

Figure 1. Strategies for metabolic engineering for the production of a desired chemical: (1) overexpression of the rate-limiting enzyme, (2) inhibition of the competing pathway and (3) engineering a novel enzyme for the production of non-natural chemical.

this strategy is increasing the production of 1,2-propanediol, which is mainly used for production of biodegradable polymers, by inhibiting the lactate dehydrogenase and glyoxylase genes which encode for the competing enzymes [2] (*Figure* 1).

(3) In some cases, the production of the desired biochemical can be carried out in the non-native organism, i.e., heterologous host (*Figure* 2). In other words, a 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. In such a case, an important factor is the availability of the substrate of the desired pathway. Thus, multiple genes encoding an array of the enzymes of a pathway can be expressed in the non-native host. Expressing the combination of genes encoding the most efficient enzymes from different organisms is another way to achieve the product which is otherwise not produced or produced at a very low level. One of

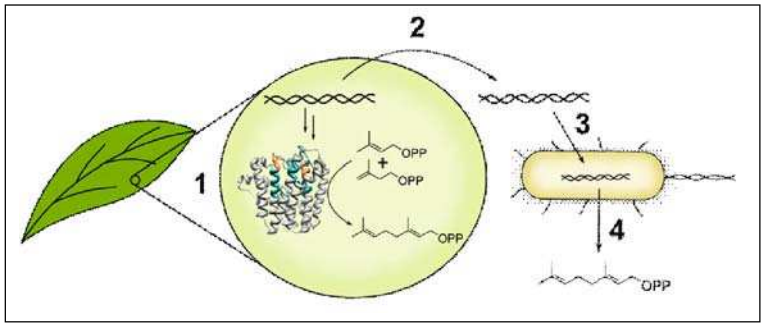

Figure 2. Any example of metabolic engineering by heterologous expression of a plant enzyme into bacteria involves (1) identification and (2) isolation of the gene encoding the enzyme catalyzing the desired reaction and (3) transfer and expression of the gene in the host which results in production of the desired chemical (4).

Metabolic engineering has become a well-recognized field of biotechnology with huge commercial potential.

the most successful examples of this strategy is the production of biofuel molecules – fatty acid ethyl esters – in *E. coli* by expressing the genes encoding the successive enzymes of the pathway which are obtained from various sources such as plants and bacteria [3].

(4) The most interesting approach for production of non-natural chemicals is to engineer an enzyme which is not found in Nature. This mode of metabolic engineering relies on creating mutations in the related gene so that the amino acid composition of the enzyme is altered. This might result in alteration in the substrate and product specificities of the enzymes, as they are dependent on the amino acid composition and sequence. There are two ways for achieving this. One involves generation of the mutations in a random manner followed by selection of the desired mutant and in the other rational mutations are created at predefined sites in the enzymes based on the available knowledge of its reaction mechanism. The latter way requires extensive computational modeling of the desired reaction in terms of the structure of the substrates and products, the active site and overall structure of the enzyme. Such a method has been used for the production of a non-natural amino acid L-homoalanine, (which is an important precursor of the many drugs), by creating rational mutations in glutamate dehydrogenase enzyme in *E. coli* [4] (*Figure* 1).

Metabolic engineering has become a well-recognized field of biotechnology with huge commercial potential. Some of the most successful examples of commercial metabolic engineering strategies include production of drugs such as artemesinin and peritaxel, overproduction of L-valine (a precursor for many value-added chemicals), production of amino acids including glutamic acid in Corynebacterium and production of biofuel related chemicals such as ethanol, alkanes and fatty acid esters (reviewed in [5]). In recent controversial research, scientists have discovered an enzyme which catalyses the production of morphine in plants [6]. Such genes have huge potential for the production of similar drug molecules in yeast by metabolic engineering[2]. Nonetheless, it is

[2] The Nobel Prize in Physiology or Medicine in 2015 was awarded for the discovery of artemisinin, a natural product, for the treatment of malaria. This molecule is now produced at large scale by metabolic engineering in yeast.

also feared that such genes can be misused for illegal production of the abusive drugs.

In a nutshell, metabolic engineering can be immensely beneficial to mankind as well as Nature by offering a biological tool for large-scale production of useful chemicals in an economical manner.

Metabolic engineering can be immensely beneficial for large-scale production of useful chemicals in an economical manner.

Suggested Reading

[1] D Shintani and D Della Penna, Elevating the vitamin E content of plants through metabolic engineering, *Science*, Vol.282, pp.2098–2100, 1998.

[2] N E Altaras and D C Cameron, Enhanced production of (R)-1,2-propanediol by metabolically engineered *Escherichia coli*, *Biotechnology progress*, Vol.16, No.6, pp.940–946, 2000.

[3] R M Lennen and B F Pfleger, Microbial production of fatty acid-derived fuels and chemicals, *Current opinion in biotechnology*, Vol.24, No.6, pp.1044–1053.

[4] K Zhang, H Li, K M Cho and J C Liao, Expanding metabolism for total biosynthesis of the nonnatural amino acid L-homoalanine, *Proceedings of the National Academy of Sciences*, Vol.107, No.14, pp.6234–6239.

[5] J W Lee, D Na, J M Park, J Lee, S Choi and S Y Lee, Systems metabolic engineering of microorganisms for natural and non-natural chemicals, *Nature Chemical Biology*, Vol.8, No.6, pp.536–546, 2012.

[6] S C Farrow, J M Hagel, G A W Beaudoin, D C Burns and P J Facchini, Stereochemical inversion of (S)-reticuline by a cytochrome P450 fusion in opium poppy, *Nature Chemical Biology*, In press.

Address for Correspondence
Ram Kulkarni
Symbiosis School of Biomedical Science,
Symbiosis International University,
Lavale, Pune 412 115
E-mail:
ramskulkarni@gmail.com