BIOTECHNOLOGY

METABOLIC ENGINEERING: ISSUES AND PROSPECTS

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When we visit a florist's shop we are fascinated by the variety of flowers with different colors and fragrances.' The rose, which has been one of the most beloved flowers, is now available in approximately 50,000 different colors. This enormous number of varieties was generated by traditional selection, mutation any hybridization methods. However, the blue rose, which has often been a theme of fairy tales, could not be made despite many efforts over the last 200 years. Recently, thanks to the development of recombinant DNA technology and advances in our understanding on the. metabolic pathways for pigment biosynthesis, a true blue rose was made! Metabolic engineering has brought a fairy-tale subject into reality.

What is Metabolic Engineering

Metabolism can be called as "chemical engine" that drives the living processes while Metabolic engineering can be defined as directed modification of cellular metabolism and properties through the introduction and modification of metabolic pathways by using recombinant DNA and other molecular biological techniques. In principle, metabolic engineering is not a new concept. Mutagenesis by physical or chemical means has been used to change the properties of a large variety of microorganisms for several decades. However despite widespread acceptance and many successes, mutagenesis remained essentially a random process where science was complemented with elements of art. .

Traditional mutagenesis and selection have successfully developed various industrial strains that can more efficiently produce antibiotics and amino acids. Animal and plant breeding although traditionally undertaken without any detailed knowledge of the reasons for the phenotypic changes, also can be considered to be traditional examples of metabolic engineering. Although creative ideas for selecting mutant strains possessing desired properties based on the known metabolic pathways were employed during the process, the overall approach was one of trial and error. The advent of recombiant DNA technique has enabled metabolic pathway modification by means of targeted genetic modifications instead of and in addition to the traditional black box approach.

The classical approach of metabolic engineering, as discussed above, requires detailed knowledge of the enzyme kinetics, the system network, and intermediate pools involved, and on such bases, a genetic manipulation is proposed for some presumed benefits. In contrast, the concept of inverse metabolic engineering is first to identify the desired phenotype, then to determine environmental or genetic conditions that confer this phenotype, and finally to alter the phenotype of the. selected host by genetic manipulation. As in the case of - expression of the oxygen binding protein, VHb, in *E. coli,* the observed phenotype of high heme cofactor levels in an obligate aerobe *Vitreoscilla* under oxygen limitation suggested that synthesis of the hemoglobin could improve growth of other organisms under similar limitations.

Examples of Metabolic Engineering

Since the mid 1980s, numerous examples of metabolic engineering have been reported. In 1993 Cameron and Tohg exhaustively reviewed the applications of metabolic engineering which they classified in the following five groups.

1. Enhanced production of metabolites and other biologicals that are already produced by the host organisms. Examples ethanol production by *Escherichia coli,* succinic acid production by *E. coli,* production of • bacterial cellulose by *Acetobacter xylinum etc.*

2. Production of modified or new metabolite and other biologicals that are new to host organisms.' Examples: various modified and novel polyketide antibiotics by *Saccharopolyspora erythraea* and *Streptomyus* spp., Octanoate production by *E. coli, Arabidopsis thaliana* and modified xanthan gum . by *Xanthomonas campestris* etc.

3. Broadenign the substrate utilization range for all growth and product formation. Examples: ethanol production from xylose by *Saccharonyces Cerevisine* and *Zymomonas mobiis,* L-tryptophan production from sucrose and thus possibly from molasses) by *E. coli,* and ethanol production from starch by *S. cerevisine* etc.

4. Designing improved or new metabolic pathways for degradation of various chemicals, especially xenobiotics. Examples include degradation of mixture of benzene, toluene and xylene by *Pseudomonas putida* and degradation of polychlorinated biphenyls by *Pseudomonas* spp. etc.

5. Modification of cell properties that facilitate bioprocessing (fermentation or product recovery). Examples include better growth of *E. coli* and other.

micro-organisms under microaerobic conditions, reduced acetate production in *E. coli*, and ammonia transport without ATP consumption in *Methylophilus methylotroplius.*

Importance of Metabolic Engineering - In the Future Context

Metabolic engineering is an area relevant to both fundamental and applied knowledge.

Factors Influencing its Wider Acceptability to Manufacturing Sector are:

1. Continuous increase in the worldwide production of carbohydrate raw materials. Whilst some products have now been developed and marketed, others particularly in, the field of materials are likely to find new business opportunities.

2. The decline in the manufacturing cost of biotechnology products compared to an increasing trend in the cost of products manufactured through traditional chemical processes.

3. The power of the techniques developed by modern molecular biology. Progress in the recent years have allowed . process biotechnologists to be optimistic regarding the. . development of methodologists that will permit the identification of the critical enzymes in metabolic networks..

Applications

There are three major applications

1. Improve the yield and productivity of native products synthesized by micro-organisms.

- 2. Extend the possible range of substrates in a specific process chain.

3. Biological synthesis of products that are new to the host cell as in case of polyketide and biopolymers , synthesis in micro-organisms and plants.

Besides manufacturing applications, metabolic engineering is having significant impact on the medical, field. The main focus here is one the design of drugs and new therapeutics by identifying specific targets for drug development and by contributing to the design of gene therapies. Current therapies presently target a specific single enzymatic step implicated in a particular disease. There is no certainity, however that the manipulation of a single reaction will translate to systematic response in human body. In this regard medical applications are not different from the ones mentioned earlier in the. industrial context. There is therefore a lot to be gained from development and engagement of metabolic engineering through better analysis of experimental results and applications to the rational selection of target for medical treatment.

Conclusions

Although it maybe difficult to accurately predict the near term directions of the field, the preceding analogy makes it easier to view the long term impact of metabolic engineering. This will be derived primarily from the development of a whole new industry around the fundamental core of and enabling technologies derived from applied molecular biology. One can envision a new system of biochemical engineering evolving for the purpose of developing the industrial applications of molecular biology. In this regard, ME aiming at the development of biocatalysts for process optimization, will play a very important role in biological systems similar to the role played by catalysts for many decades in chemical processes. Just as many chemical processes became a reality only after suitable catalysts were developed, the enormous potential of process biotechnology will be brought to realization when bicatalysts become available to a significant extent through metabolic engineering. When chemical ' synthesis pathways is multistep, microbial processing maybe preferred. The current research activities on sequencing the genomics of many different microbial and other species bring these possibilities much closer to reality: It is on this basis that the long term potential of metabolic engineering for industrial application should be assessed.

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