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A Brief Sketch of Strategies and Planning for Plant Metabolic Pathway Moderation

***Abstract*: In order to improve metabolic engineering potentialities, new transformation mechanisms have been developed to allow for gene specific silencing strategies or stacking of multiple genes within the same region of the chromosome. Metabolic engineering of plant production systems are now developed using these resources and much more complex products are synthesized in engineered microbial hosts. Plant secondary metabolism has vital role in functioning various functionalities in the plant's life cycle including their response to different interactions with environments. Due to various internal qualities of secondary metabolites, scientists have become interested to work for development in the metabolic engineering and route changing of plant secondary metabolism pathway. Various researchers have identified various strategies and methodologies for pathway alteration. Gene encoding biosynthetic enzymes and gene encoding regulatory proteins are important examples in this regard. To uncover the hidden mystery within the plant is one of the great passion for researchers. The present article sketches the foundation of strategies and planning for engineering metabolic pathway. This article will stimulate researchers to correlate their work, direction, and motivation with their work in this field to make their prosperous idea successful for future world.**

Keywords: Plant metabolism, secondary metabolites, PNP, gene-coding, enzymatic pathway

Plant secondary metabolism plays vital role in various functionalities in the plant's life cycle. Due to these capacities plants can response for different interactions with environments. These interactions are Plant-plant, plant- microorganism, Plant-Environments and plant–insect interactions. Development of plant defense system through production of antifeedants, phytoalexin, phytoanticipin have also an important role of secondary metabolites. Other important role of secondary metabolism also found in the case of plant reproduction, food quality, pigments etc. Scientists have been continuously working for development in the metabolic engineering and alteration of routes of plant secondary metabolism. Various strategies and methodologies have been adopted for pathway alteration, using of gene encoding biosynthetic enzymes, sometimes, gene encoding regulatory proteins.

Basis of Plant Metabolism: Plant metabolism can be classified broadly into two stages: primary and secondary metabolism. The primary macromolecules such as carbohydrates, lipids, proteins, nucleic acids, and hormones are synthesized in the primary metabolism pathway whereas the secondary metabolites derived from primary metabolism through the evolutionary and biosynthetic pathway[1]. For the plant growth and development, primary metabolism take part in directly and it is the basic stage in complex molecules formation, while secondary metabolism plays vital role for formation of unique and more advanced phytochemical products[2,3]. The seed and plant vegetative organs are the main source of primary metabolites. They play an important role in plant growth, reproduction, development and signal transduction, protein synthesis, respiration, photosynthesis, assimilation of nutrients and solute transport - these are in primary metabolism process types[4,5]. The primary metabolites are originated in all types of plant kingdom whereas; the occurrences of certain secondary metabolites are limited only found in some specific plant species with the influence of biotic and abiotic factors and it is not directly influence in plant development but it plays an impotent role in plant adaptation, and their survivability, defense mechanism against the various types of bacteria, fungi, nematoda, and pathogenic microbes. It helps the plant from being eaten by herbivorous or mammals[1,6]. Secondary metabolites are found by extraction methods and compounds can be identified by various chemical and spectroscopic technique.

Root of Biosynthetic Pathway of Natural Products: Natural products (NPs) are derived from organisms of all kingdoms in nature. Nearly 300,000 identified natural products have been summarized in libraries[7] and Super

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Natural II[8]. The generation of those major classes natural products and their hybrids have been possible due to four well-known biosynthetic pathways[9,10] which included:

(1) AA/MA (acetic acid and malonic acid) pathway which produces phenols, fatty acids, and polyketides;

(2) MVA/MEP (mevalonic acid or methylerythritol phosphate) pathway which generates steroids and terpenoids

(3) CA/SA (shikimic acid or cinnamic acid) pathway which produces flavonoids, phenylpropanoids, coumarins and lignans

(4) AAs (amino acids) pathway that is the key for construction of alkaloids.

Unfortunately, characterization and confirmation have been done only about 33,000 enzymatic reactions. Therefore, the overall biogenesis pathways and all intermediates involved in the process are not well established for a huge number of known natural products. Hence, it is most desirable to search on and reveal the biosynthetic pathway for various natural products.

Identification and Modification Through Engineering an Appropriate Host Organism: In order to fulfill various demands including industrial application, medicinal utility, agricultural purpose or scientific interest, a Plant Natural Products can be selected for target of metabolic engineering. Selection of an appropriate target host species for engineering the biosynthetic pathway is the primary step towards heterologus production. It has been proved that improvement of each host can be possible through adaptive evolution. In recent time, with the expanded journey of organism engineering afforded the various available methods, models, and tools for editing the host phenotypes. Improvements of the host organisms are made by a combination of a branch of operations like modification of the host, tuning, and optimizing unit operations. Utilization of pre developed strains which overproduce requisite metabolites can accelerate progress greatly.

Target Selection of Host Species for a Heterologous Plant Pathway: As there is a direct link of biochemical reactions happening within the host organism and heterologous pathways due to incorporation of foreign genes. Therefore, introduction of metabolic pathways into host organisms is an important strategy used for the greater productivity of valuable secondary metabolites. For that purpose selection of a host species is very important. Some properties such as ease of culture, cloning and suitability of host for compounds and enzymes are considered for selection of a host. Well known organism with good records in research specially in metabolic engineering and existence of developed technique for culturing, cloning, and make them for industrial scale-up which will make them most attractive choices. The plant cell for producing PNPs should be first choice as host where conservation of protein processing and specific sub-cellular compartments will be maintained[11]. On the other hand model plants like Nicotianabenthamiana are very useful during initial testing for transient expression of plant pathway enzymes, as enzyme functions, co-factors, and substrate pools are maintained in plant[12,13]. Microbial hosts are most preferable because it is comparable rapid process than genetic manipulation of plant.

Microorganisms like *Saccharomyces cerevisiae* and *Escherichia coli* have prosperity of well-established tools accessible for genetic manipulation, cultured easily and have a wide range of availability of advance platform strains. Other evolutionally well suited microorganisms are also employing for the said purposes. For the production of antibiotics, Streptomyces originated from Stretomyces species is also often used[14]. Use of *Corynebacterium glutamicum* is attributed for the production of amino acids[15]. For using lipid as substrate, *Yarrowialipolytica* is employed frequently[16]. Although it is true microbial hosts like *E. coli* or *S. cerevisiae* are generally used for production of PNP's. Hence, seeking of heterogeneous host whether to use *E. coli* or *S. cerevisiae* for the production of compounds is a great question in this regards. *S. cerevisiae* may get recommendation due to its high rate of homology and ease of genomic integration gives extra advantage.

Yeast harbors several organelles that are also present in plant cells. Several enzymes involved in the biosynthesis of para-nitrophenol (PNP), including cytochrome P450s, are characterized as transmembrane proteins that necessitate the presence of a suitable membrane, specifically the endoplasmic reticulum(ER), for their accurate anchorage and conformational folding. An obstacle during semi-synthetic Artemisini project was observed which had the potential to impede progress. This event served to highlight the challenges inherent in such endeavors. The project involved the modifications of *S. Saccharomyces cerevisiae* were genetically modified to generate elevated levels of artemisinic acid, a pivotal precursor in the production of the essential antimalerial compound, artemisinin. This project explores the implications of studying both *E. coliprotein* structure and function. *Escherichia Coli* and *salmonella enteric* are also

two bacterial species commonly studied in microbiology. *Saccharomyces cerevisiae* was evaluated as candidate host, and noteworthy titers of the intermediate. In the metabolic pathway involving *E. coli*[17], the ensuing stage is executed by P450AMO, a member of the plant cytochrome P450 family. *E. coli* failed to achieve high enzyme activity, requiring a switch to production. Using *S. cerevisiae* as a host for pathways with transmembrane proteins avoids extra modification work[18]. *S. cerevisiae* has micro compartments that mimic PNP biosynthesis in plants[19,20]. *E. coli* doubles faster than *S. cerevisiae*, by 3-4 times. *S. cerevisiae* is ideal for high enzyme expression and possesses unique metabolites. Using a native pathway, *E. coli* was engineered to produce taxadiene 2,400 times more efficiently than *S. cerevisiae* strains for taxol production. *S. cerevisiae* engineered for taxadiene[21].

When selecting a host organism for PNP biosynthesis, an alternative approach is to adopt a multi-organism co-culturing strategy, whereby constituents of a metabolic pathway are distributed among different organisms, either within the same species or across diverse species[22-25]. The advantages of this approach are manifold encompassing the amelioration of the load assigned to the host via the heterologous pathway, the enhanced potential to leverage the species that are optimally equipped to effectuate the expression of targeted enzymes within the pathway. As well as the modular nature of the process, this enables the combination of disparate pathways through the co-cultivation of distinct strains. In a specific instance, the chemical compounds known as benzyl-isoquinoline (BIAs) were synthesized within the context of an E. coli biochemical system. The bacteria *E. Coli* and Salmonala are commonly studied in microbiology due to their significant impact on human health and environment[24].

An additional illustration entails the attainment of elevated titers of an anthocyanin PNP via the dispersion of the metabolic load within four *Escherichia coli* cells. The *Escherichia coli* strains that underwent co-cultivation were evaluated[23]. The limitations associated with cultures are characterized by pathway specificity and encompass deficiencies pertaining to the transportation and diffusion of intermediate metabolites between cells within the co-culture. In addition, the co-culture also requires the imposition of a balanced growth of multiple hosts within a single culture, which may exhibit apparent differences in their preferred growth conditions and rates.

Decision taking for host strain choice of PNP precursors: Following choice of a host species, engineering the host to boom titers of main native metabolites that are biosynthetic precursors to the desired product can greatly accelerate downstream manufacturing of PNP molecules. The middle metabolic networks of version organisms are well-characterized and can be used to manual over expression and knockout changes for overproduction of critical metabolite precursors and to cope with not unusual challenges (e.g., comments inhibition or different metabolic law).

One of the blessings of biosynthesis over chemical synthesis is way of simple biosynthetic distribution. Once, a pressure has been engineered to produce a compound, researchers trying to extend on that paintings inside the destiny want now not repeat tedious syntheses of beginning fabric. Strains of *E. Coli* and *S. Cerevisiae* that overproduce alkaloids, fatty acids, terpenes, and other valuable compound training have been engineered. Producing more central metabolites or a heterologous secondary metabolite can both be beneficial. Central metabolites, such as geranyl pyrophosphate or amino acids, can lead to the production of a variety of PNP compounds. Secondary metabolites can make it easier to engineer biosynthesis of a specific PNP product.

A standard platform strain might be functional not only because it creates an indispensable initial material, but also for other factors like sustainability, inexpensive and easy handling by the researchers. The creation of an effective simultaneous saccharification and co-fermentation (SSCF) strain for bioethanol synthesis in *E. coli* that uses lignocellulosic biomass [26], a cheap waste product from agriculture and forestry, in place of pricy refined sugars, serves as a demonstration of this.Another illustration is the development of an enzyme that incorporates formate into the central metabolic process[27]. This enzyme may enable the manufacture of pharmaceuticals and common compounds from formate, which is anticipated to be readily accessible from the electrochemical reduction of CO_2.Last but not least, scientists created an *E. coli* strain that can photosynthesise CO_2 to make its own biomass[28]. The ability to engineer CO_2 fixation in well-researched, genetically tractable industrial microorganisms like E. coli and S. cerevisiae may be advantageous, despite the high interest in using naturally occurring photosynthesizing microorganisms (such as cyanobacteria) for the production of PNPs[29].

The other PNP-producing strains could potentially be integrated into these platforms, even though the aforementioned strains have not yet been used to make PNPs. The production of complex PNPs are allowed from agricultural waste, using renewable energy, or even directly from atmospheric CO_2. The engineering of E. coli to use the one-carbon fuel methanol and ultimately convert it into

the flavonoid naringenin[30] serves as a demonstration of this idea. Such approaches might help more environmentally friendly bioprocesses produce an expanding range of goods, including PNPs, on an industrial scale.

Host Metabolism Modification for Smooth PNP Biosynthesis: After choosing a host or pre-existing platform strain, alterations to the host, such as gene deletions, replacing indigenous enzymes with more active homologues, or overexpressing endogenous metabolic genes, may be made to increase the availability of biosynthetic precursors.

A recent masterstroke[31] integrated all of these methods to rewire the core metabolism of yeast to overproduce acetyl-CoA for the production of isoprenoid and fatty acids, which serves as the precursor for numerous PNPs, including the antimalarial artemisinin. A more advantageous reaction stoichiometry, which was described as having a reduced ATP need, reduced loss of carbon to side reactions, and better pathway redox balance, was found using yeast reaction stoichiometriesa model for acetyl-CoA, sugar, and redox cofactors.

By expressing four acetyl-CoA biosynthesis-related enzymes from other organisms to increase acetyl-CoA biosynthesis, the best acetyl-CoA stoichiometry was achieved. This allowed yeast to produce 25% more of the isoprenoid farnesene with an equal amount of sugar while using less oxygen, which is crucial for industrial fermentation settings with oxygen restrictions For instance, scientists developed yeast that produced 1.9 g/L of p-coumaric acid by combining six genetic changes to yeast's natural metabolism. These included eliminating competitive side routes, overexpressing enzymes near bottlenecks, and designing feedback-resistant enzymes[32]. Researchers watched biosynthetic intermediates in their designed route during work on the de novo synthesis of strictosidine, an alkaloid produced from plants, in order to spot competing side pathways[33].

Key Strategies for Planning and Engineering a Metabolic Pathway: An appropriate host can be selected, and a route can be planned to reach the desired PNP. In order to lay out a candidate pathway, a series of chemical intermediate first selected, leading to a host metabolism to target molecule, and then enzymes are chosen to perform each step necessary. In order to facilitate pathway engineering into a heterologous host, certain PNPs have detailed information of the native biosynthetic pathway that may be utilized to delineate all intermediates and enzymes in a pathway. Such in-depth knowledge, however, is frequently lacking or insufficient and can require years or even decades of devoted investigation in plant. The next sections go over the unique difficulties such as candidate pathway design, enzyme choice, and pathway testing provide under such circumstances.

Conclusion

The valuation of natural secondary metabolites insisted scientists to uncover the hidden mystery within the plants. The production of specific secondary metabolites in the plant species is of very limited amount. Hence, increasing the amount of production in various ways is the prime and important desire to the scientists. The present article analyzed, focused and summarized the basis of key strategies for engineering metabolic pathways. The present article will encourage the researchers in this field to create more and more successful and productive ideas to develop this field. ❐

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