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Review Article

Secondary Metabolites Synthesis in Microorganisms

*P.Pradeepa

Department of Genetic Toxicology, GLR Laboratories Private Limited, Chennai.

Abstract: Bacterial pigments are classified as natural products, which are the important classes of highly active natural compounds and also plays a wide range of biological roles such as antioxidant, antibacterial and anticancer etc., Bacterial pigments are categorized as secondary metabolites, these molecules are economically important as a potential applicant in biomedicine. They are widely used in biopharmaceutical industries, due to their needs in various health illnesses in humans. The bacterial secondary metabolites productions are regulated through physical, chemical and biological factors, the scientific based modern tools are playing major role on signaling processing, growth composition regulation and impaired metabolism in bacteria. To know the general mechanisms involved in enhancing the production of bacterial pigments, this review outlines the application of various modern techniques applies to identify the production of secondary metabolites in microorganisms.

Keywords: Pigments, Strain improvement, Feedback inhibition, Genetic engineering

1. Introduction

Secondary metabolites are organic compounds that form at the end of near the stationary phase of microorganisms and are not directly associated with growth, development and reproduction of microorganisms [1]. The source of secondary metabolites in microorganisms functioning as antibiotics, pigments, toxins, effectors, symbiosis, pheromones, enzyme inhibitors, immunomodulating agents, receptors antagonists and agonists, pesticides, antitumor agents, growth promoters, bio-indicators, pesticides, herbicides, feed additives and preservatives, etc., [2, 3]. Recent years have been particularly approaches the scientific based techniques, tools to enhance the secondary metabolites synthesis in bacteria. Generally, the bacteria biosynthesize range of distinct molecules with remarkable biological activities, as bioregulation, quorum-sensing molecules and antibacterial agent [4]. Through a negative control mechanism, the

*Author for Correspondence. E-mail: pradeepa_21@yahoo.com

microorganisms may generate the signals to activate regulatory events by binding or inactivating a regulatory repressor/ receptors proteins, resulting in chemical differentiation. The signaling process is concluded in microorganisms by a low molecular weight encouragement, transfer RNA synthesis, variation in sigma factors and gene products formed during post exponential development. Since the secondary metabolites may have unusual structure, alteration either in nutrient/ growth rate/ inducer signals in microorganisms presumably activates a master gene by the following pathways. This master gene either acts at the level of translocation by encoding a rare tRNA, or by encoding positive transcription factors, hierarchy genes control one branch of cascade, which in turn controls the formation of particular groups, follow up of smaller groups and ends in controlling of individual biosynthetic pathways [5].

The secondary metabolites showed a most important application in biomedicine. The overproduction of microbial products can be based on the microbial response through elicitors, genetic engineering, metabolic engineering, ribosomal engineering and also methods for the amplification of secondary metabolites by biosynthetic genes, inactivation of competing pathways, disruption of regulatory genes, manipulation of secondary mechanisms, expression of convenient heterologous proteins, combinatorial biosynthesis [2].

2. Natural pigments in microorganisms:

Color of food material is vital to show its freshness and safety, which also indicate good aesthetic and sensorial values of food. Pigments have been identified with essential properties to many industries. Recently the colouring of food with pigments were produced from the natural sources are gaining worldwide interest and importance due to the undesirable and unsafe market of synthetic pigments, natural dyes are considered the safe replacement for food use. The demand for natural sources is increasing linearly, thus it is imperative to explore diverse natural sources of food grade colorants and their potentials. The main sources of natural pigments are plants and microorganisms. The use of plant pigments has many drawbacks such as non-availability throughout the year, pigment stability and solubility. Microorganisms are known to elaborate a variety of pigments and hence they are the promising source of food colorants [6]. Pigment producing bacteria are ubiquitous and present in various ecological niches, such as soil, desert sand, freshwater and marine samples [7-11]. Microorganisms are readily available alternative source of naturally derived pigments and have enormous advantages over plant pigments including easy and rapid growth in low cost medium, easy processing and the growth that is independent of wealth conditions [12]. In recent times, the microbial pigment production such as colors, are considered natural when derived from biological sources, it has now been one of the emerging fields of research to display its potential for a variety of industrial applications.

3. Classification of pigments:

Pigments are either organic/inorganic or natural/synthetic. Structural affinities and natural occurrence of biological pigments has been used as the basis of classification of biological pigments. Pigments are majorly classified as carotenoids, melanin, prodigiosin, violacin,

riboflavin, pyocyanin [3]. Those molecules are arising from the generic classes of polyketides, nonribosomal peptides, isoprenoids and shikimate derivatives in the biosynthetic system have been reported most frequently [13-15].

4. Production of microbial pigments:

A bacterium express various pigments at different stages of growth, when it is more resistant to oxygen and nitrogen derived oxidants, the production of melanin against reactive oxygen species and ion binding to protect from damage caused by hydrogen peroxide, confers resistance to oxygen stresses against hydrogen peroxide and superoxide. The following biological mechanisms are involved in the production of pigments in bacteria.

- Morphological Mutation: Strain improvements involve regulatory genes, especially as regulatory mutants obtained in basic genetic studies are sometimes found to be altered in colonical morphology. These include mutants affected in mycelium formation, which produce colonies with a modified appearance or a new color [16]. Especially in hierarchy genes control one branch of cascade and control the formation of particular groups play a major role in mycelium formation in filamentous organisms. The level of sporulation genes lower in the cascade and control particular stage of sporulation [5].
- Feedback inhibition: Auxotrophic mutation has been a major factor of primary products such as amino acids, nucleotides synthesis. When auxotrophs are grown in nutrionally complete and even complex media, the secondary metabolites production markedly affected in the negative direction. Primary metabolites are produced by a single branches pathway, sometimes auxotrophic mutants modify the over production of secondary metabolites. Reverse in the auxotroph leads to new prototrophs possessing higher enzyme activity than the parent prototroph. Changes in the enzyme activities are subjected to more/less feedback inhibition in the secondary metabolite production [16].
- Selection of regulatory mutants: Which over produce the end products of the primary pathways and function as anti-metabolites, may control the enzymes that are insensitive to feedback inhibition or enzyme forming system resistant to feedback repression.
- Essential growth composition regulation: Nutritional usages may alter by mutants eg., when the selection of 2 deoxyglucose in enzyme pathways can controlled by carbon source regulation, methyl ammonuim regulated by nitrogen source, arsenate for phosphate regulation.
- Impaired metabolism: Rate of product excretion in certain microbial strains is based on their internal cellular uptake/ catabolism. Genetic variations in microbial cellular permeability, which may eliminate active uptake, enhance excretion of metabolites in microorganisms. Certain mutants play feedback regulation to impaired ability to uptake the product in intracellular levels. [16].

5. Regulation of secondary metabolites production:

Microorganism can generate new genetic characters by two ways, means, mutation and genetic recombination [16]. Availability of cultivation technology (genetic engineering), have made it now possible to modify the bacteria to produce the pigment of interest. The formation of secondary metabolites is regulated by nutrients, growth rate, feedback control, enzyme inactivation, enzyme induction. The fundamental biotechnological approaches are applied when producing microbial pigments, firstly a search for new sources, and secondly enhancing the yield of already recognized sources either through optimization or strain improvement [17].

Optimization refers to the following suitable conditions that favor the growth of microbes and improve the secondary metabolite production such as the influence of incubation time, influence of moisture content, influence of fermentation processes, influence of carbon sources, influence of nitrogen source, influence of pH and influence of UV [18]. A number of physical activities of microbes such as symbiosis, competence, conjugation, sporulation, biofilm formation, virulence, motility may change the secondary metabolite production.

Through quorum sensing technology, the communication may generate between cells by the release of chemical signals when cell density reaches a threshold concentration. Biosynthesis/addition of an inducer or growth rate decreases may generate signals which may affect the cascade of regulatory events resulting in chemical differentiation. The signal is often a low molecular weight inducer which acts by the negative control (either binding/inactivating a regulatory protein) which prevents the secondary metabolites production [5].

Strain improvement may process through the chemical source (e.g., nitrosoguanidine, 4-nitroquinolone-1-oxide, methyl methane sulfonate, ethylmethane sulfonate, hydroxylamine) and physical source (UV radiation, pH, nitrogen, oxygen, antibiotics, inorganic ions) [19, 20]. It also can produce a variation/ modification in the color of pigment and chemical structure.

Genetics has had a long history of contributing to the production of microbial products. The following classes of genetic controls are involved in metabolites producing microorganisms. The keys are structural genes coding for product synthesis, regulatory genes determining the onset and expression of structural genes, resistance genes determining the resistance of the producer to its own antibiotic, permeability genes regulating entry, exclusion and excretion of the products and regulatory genes controlling pathways providing precursors and cofactors [21].

The main strategies are being used in molecular genetics tools for identification of biosynthetic pathways, adequate vectors and effective transformation protocols by the following pathways.

- Improvement in strain advantages includes increasing yields of the desired metabolites, removal of unwanted co-metabolites, improving utilization of inexpensive carbon and nitrogen sources, alteration in cellular morphology for better mycelium production, improved oxygen transfer in the fermenter.
- Basic understanding of product metabolism and pathway regulation for the selection of a particular characteristic of the desired genotype, differ from the one of the final interest, but easy to detect.

- Recombination by protoplast fusion between related species of microbes with genetic development results in high productivity of the newly identified secondary metabolites from two strains.
- Targeted gene duplication (amplication), identify a neutral site on the chromosome where genes can be inserted without altering the fermentation properties of the strain. The neutral site is cloned and incorporated into the vector with the antibiotic gene. After transformation, the gene is inserted into the chromosomal neutral site by homologous recombination.
- Block a pathway that competes for common intermediate key precursors such as cofactors, reducing power and energy supply are being used to perform this strategy.
- Incorporation of new enzyme in the bacterial strains, which will lead to the formation of a new related product.
- Including the directorial biosynthesis, mutagenesis, combinatorial biosynthesis and ribosomal engineering are useful essential tools in the production of secondary metabolites in microorganisms.

6. Conclusion

Natural products from the microbes are still important sources for drug research with biological importance. There are many research activities has been initiated to find the various sources for the production of secondary metabolites in microorganisms. Utilization of industrial waste, optimization of media components, culture parameters and strain improvement are essential tool to improve the performance of the bacterial system, which helps to increase the yield of its products [22].

Microbes are easy adapting to any changes in their environment, at the manipulation in their genetic materials is easy more potential choice for extracting secondary metabolites. Most natural pigments are so complex that they property will never be made commonly by chemical synthesis. Synthetic pigments contain toxic and carcinogenic amines that are not environmentally benign, particularly pigments of microbial origin are very rare, but offer great commercial availability due to the ease of mass production by fermentation. The power of microbial culture in the competitive world of commercial synthesis can be approached by the fact that even single molecules are made by fermentation rather by chemical synthesis. Since industrial production of natural pigments by microbial fermentation is economical, extraction is simple and higher yielding.

This observation may be attributable to the use of high-throughput techniques; metabolomics is a new emerging field of research with the comprehensive characterization of small molecules metabolisms in biological systems. The promise for the future, such as, association analysis, massive parallel signature screening, directed evaluation, molecular breeding with DNA and genome shuffling, combinatorial biosynthesis are the recent areas of improvement progress in strain techniques and identification of new genetic targets. Strain improvements in

microorganisms by metabolic engineering could approach high yielding properties of microbes in secondary metabolites production.

This review has concludes that the contribution of modern science and techniques could significantly enhance the production of secondary metabolites (biologically potent pigments) in microbial sources.

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