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TAXOL PRODUCTION USING MICROBES: A REVIEW

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ABSTRACT

Taxol is a powerful, effective and often-used anticancer drug, also called paclitaxel. Since the first paclitaxel-producing fungi were described more than 15 years ago, microorganisms have been researched as potential substitutes for an ethical, straightforward, and affordable method of taxol production. Nevertheless, despite extensive studies on paclitaxel-producing bacteria, no commercial fermentation method has yet been applied. This study aims to review the microbes involved in taxol production to date and the present status of ongoing efforts to produce and analyze the process of microbial fermentation for paclitaxel production.

***KEYWORDS*:** Taxol, Taxomyces, Paclitaxel, Anti-cancer, Microbial biotechnology, Biosynthetic pathway.

INTRODUCTION

Individuals, families, healthcare systems, and economies as a whole are all impacted by cancer, which has a tremendous social and economic impact. Therefore anticancer treatment is of utmost importance since it has significant societal and economic repercussions. The anticancer medication taxol, also referred to as paclitaxel, has completely changed how different cancers are treated. Originally, it was made from the bark of the Pacific yew tree (*Taxus brevifolia*). But due to its scarcity and environmental issues, other ways of making taxol have been devised. Taxol, a complex molecule with a special chemical structure, has remarkable anticancer capabilities because it prevents cancer cells from dividing normally.

Isolating taxanes, a class of chemical compounds that includes taxol, from plant material is the initial step in the manufacturing process. Then taxanes are extracted utilizing a variety of solvents and purifying processes to provide a crude extract that is concentrated into taxol. This procedure frequently uses synthetic or semi-synthetic chemical changes. To fulfill the rising demand for this life-saving anticancer medication and to increase access to it for patients around the world, effective and sustainable techniques for taxol production must be developed.

Purpose of microbial biotechnology in paclitaxel production:

In recent years, microbial biotechnology techniques have also shown prominence as a means for producing taxol. To produce taxol through a biosynthetic pathway, genetically modified microorganisms, such as bacteria or fungi, are used in microbial biotechnology. By overcoming the drawbacks of conventional techniques, this strategy may enable the manufacturing of taxol on a wide scale that is sustainable and affordable.

In comparison to genetically modified bacteria, taxol is often produced by fungi at lower yields, and can be difficult to produce enough taxol for commercial purposes. Fungi normally develop more slowly. The less efficient taxol manufacturing methods may result in higher production costs due to the longer production time needed for fungal cultures. Therefore microbial biotechnology is important in the production of taxol most effectively by altering their genetic sequences involved in biosynthetic pathways.

Microbes used in taxol production

A variety of techniques have been tried to categorize and quantify the paclitaxel-producing capacity of microorganisms. Some microbes naturally produce taxol and genetically engineered host systems to express taxol-producing genes. Fungal species include *Taxomyces andreanae*, *Pestalotiopsis microspora*, *Cladosporium* sp., *Alternaria alternative*, etc. Taxomyces, an endophytic fungus present in the bark of Pacific yew trees, served as the initial source of taxol. From 100 to 400 mg/l, relatively in high concentrations. *Pestalotiopsis* is said to produce taxol at a concentration of 1 to 5 mg/l. Bacterial strains include *Streptomyces* species and *Burkholderia* species. It has been determined that specific strains of the bacteria *Streptomyces* naturally create taxol in a few micrograms per liter, but it is typically modest. Further optimization is necessary for improved yields because the reported taxol concentrations in *Burkholderia* strains are low. The genetic alteration of a variety of microorganisms, including *E. coli, S. cerevisiae, P. putida, Streptomyces spp., Aspergillus*

spp., and others, has been utilized to produce taxol. These strains are engineered to manufacture taxadiene, a crucial step in the biosynthesis of the chemical compound taxol. The selection of the host organism is determined by several variables, including the accessibility of genetic tools, the compatibility of the host's metabolism with the taxol biosynthesis pathway, and the capacity for increased output.

Taxol production process from industrially engineered bacteria

The process is described in the following flowchart Fig1. The genes required for taxol production were found in taxol-producing plants and other sources. The selected genes are introduced into the host bacteria using genetic engineering techniques. Inducible promoters, genetic regulatory elements, or metabolic engineering approaches are used and grown in bioreactors by nutrient-rich media to enhance gene expression and enzyme activity. After fermentation, the bacteria are collected and taxol is extracted from the bacterial biomass using suitable solvents and extraction methods. The recovered taxol is next processed to remove impurities and unwanted byproducts using techniques like chromatography or filtering.

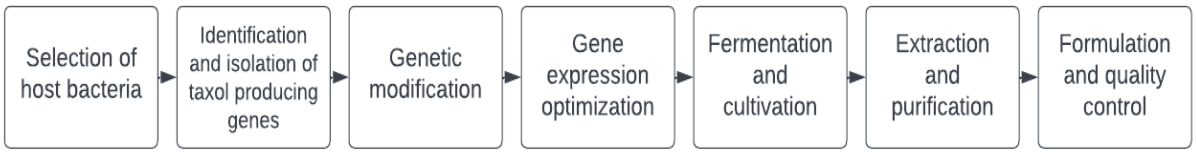

Fig. 1: Flowchart for engineering microbes.

Taxol biosynthetic pathway in bacteria

In bacteria, taxol is produced through several enzymatic processes that transform precursor molecules into taxol. The prerequisite isoprenoid substrates required to facilitate chemical biosynthesis must be provided by the new hosts as the foundation. The MVA or MEP pathways are the two options accessible for this use. The MEP pathway is frequently connected to bacterial metabolism, whereas the MVA pathway is typically seen in eukaryotic organisms.

The host naturally uses the MEP pathway to produce cellular isoprenoid compounds, including tRNA prenylation and stages in quinone and cell wall synthesis. To provide the common isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) substrates for heterologous biosynthesis, this intrinsic support route was used. Dxs, idi, ispD,

and ispF were identified as pathway genes linked with bottleneck steps in precursor support and have subsequently been the focus of numerous engineering efforts to increase intracellular precursor availability. IPP and DMAPP are mixed to create geranylgeranyl diphosphate (GGDP), which is the building block for taxol. The precursor molecule GGDP is transformed into taxadiene as the first step in the biosynthesis of taxol. The enzyme taxadiene synthase (TS), which is encoded by the taxadiene synthase (TXS) gene, catalyzes this reaction. To create taxadien-5-ol, taxadiene goes through a number of oxidations. The oxidation of taxadiene at particular locations is one of multiple enzymatic events that take place during this stage and are catalyzed by enzymes including cytochrome P450 monooxygenases and oxidoreductases. Several enzymatic processes change taxadien-5-ol. These adjustments include esterifying the carboxyl group at the C13 position and acylating the hydroxyl groups. The enzymes acyltransferases and acetyl-CoA transferases are involved in these changes. Taxadien-5-yl acetate is transformed into taxol in the last stages of taxol biosynthesis. The C10 position of taxadien-5-yl acetate is acetylated in this step, and subsequent rearrangement processes result in the formation of the taxol core structure.

Genetic elements present in taxol production pathway

Paclitaxel is created by adding an acetate group at position C10 and a side chain at position C13. However, it is still essentially unknown what molecules in fungus allow for the synthesis of paclitaxel. For the fungus *M. rouxianus*, which was isolated from *T. chinensis*, THE first partial fungal TS-coding sequence (ts) was discovered. The sequence (632 bp) matched *T. brevifolia* with 98% identity. Only two full fungal paclitaxel biosynthetic genes—belonging to *C. cladosporioides* and *A. candidus*, both isolated from T. x media are listed in GenBank. In the first instance, the 1546 bp sequence shared 97% identity with *T. wallichiana* var. Mairei and 99% identity with T. x media (EF028093). The *T. wallichiana* var. mairei and T. x media homologous genes shared 97% and 99% similarity, respectively, in the A. candidus MD3 dbat sequence (1545 bp). The TS- (EC 4.2.3.17) and BAPT (EC 2.3.1.) coding sequences from *T. andreanae* were recently partially amplified using PCR. For ts and bapt, respectively, 58 Similarities of 97 and 96% were reported about Taxus sp., however the matching sequences appear to not have been released.

Mechanism of resistance by taxol

Taxol binds to the microtubule's tubulin subunit, causing it to polymerize and stabilize the microtubule structure. The dynamic assembly and disassembly of microtubules required for

efficient cell division is prevented by this stabilization. Then it impairs the normal progression of the cell cycle, particularly during the mitotic phase, by stabilizing microtubules. By blocking the separation of duplicated chromosomes, it induces mitotic arrest and cell cycle arrest by activating the mitotic checkpoint. Taxol-induced prolonged mitotic arrest causes the activation of apoptotic pathways, which ultimately results in cell death. Cancer cells are destroyed by apoptosis, which occurs in cells with halted mitosis.

Advances and Perspectives

The manufacturing of taxol has made significant strides due to microbial biotechnology by incorporating numerous engineering techniques. In order to improve taxol production, this entails designing and constructing synthetic gene circuits and metabolic pathways inside microbial hosts. Heterologous expression is a different technique that includes moving the yew tree's taxol biosynthesis pathway to a more manageable host organism, like *E.coli* or *S. cerevisiae*. The goal of metabolic engineering is to alter the microbes' metabolic pathways to increase the production of taxol by adjusting the precursor supply, balancing metabolic fluxes, and maximizing the activity of important enzymes. Better process control and increased taxol production can be achieved by investigating various fermentation techniques, such as optimizing culture media composition, pH, temperature, oxygen availability, and the use of bioreactors with cutting-edge monitoring and control systems. To effectively recover taxol from the microbial cultures, new developments in scalable purification procedures, including chromatography and extraction techniques, have been created.

Even though there has been a lot of development, more study and improvement are still required to produce taxol utilizing microbial biotechnology in an economically viable way.

CONCLUSION

Previously, Taxol was extracted from yew trees typically ranging in a concentration from 0.01% to 0.5%. The taxol yield from fungal sources is generally higher compared to plants, ranging from 0.05% to 2% of the dry weight of fungal biomass. Whereas, in genetically modified bacterial species, it is generally reported to be in the range of 1% to 2.5%. However, research has shifted to a semi-synthetic and culture-based approach. Two objectives have been the main focus of microbial production host engineering. First, the entire Taxol route must be successfully implemented to enable semisynthesis. The second goal is to properly plan production to simplify later metabolic and process engineering. To make compounds like Taxol feasible for microbial production with widespread accessibility, the availability of

emerging engineering options associated with the genetic, metabolic, and process scales will each need to be utilized and optimized. Furthermore, the ability to rationally tailor the biosynthetic pathways for molecular diversity would open up a new frontier.

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