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Taxol Precursor Production in Physcomitrella patens

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ABSTRACT

Taxol is a cancer fighting drug that was initially isolated from the Pacific Yew. However, the isolation process is not very efficient and the tree is being excessively harvested and faces extinction. To synthetically make Taxol is an inefficient and costly process. If the precursor taxadiene-5α-acetoxy-10 beta can be produced with ease, then the synthetic modification of that precursor would be an efficient solution to this cancer fighting drug. Several genes from the Pacific Yew were isolated and amplified so that they could be inserted into the moss Physcomitrella patens. Several of these genes were transformed into E. coli and then amplified in vitro to produce the precursor taxadiene-5α-acetoxy-10 beta. Physical and biochemical analysis of the precursor taxadiene-5α-acetoxy-10 beta-synthetic genes was performed. When the final transformation was run, a transient expression of the genes resulted in small amounts of product being obtained. After gene chromography mass spectrometry analysis, the chromatogram peaks showed a few more promising peaks representing other Taxol precursors. With a permanent transfer to the moss, a much larger sample could be analyzed and more Taxol precursor could be produced.

INTRODUCTION

Taxol, also known as Taxus brevifolia, is better known as the Pacific Yew. The compound was discovered during the U.S. Department of Agriculture's random collection program (Lewis 2003). In 1977 it was selected for a more rigorous clinical trial and was found to have cytotoxic properties, and acts by stabilizing microtubules and preventing depolymerization (Lewis 2003). Pacific yew is a first class cured ninety percent of the participants with late stage ovarian cancer (Success). Taxol's effective cancer fighting traits have made it a high priority for production and use in the medical community. In fact, Taxol sales reached 1.6 billion dollars in 2000 and became the best selling anticancer drug (Success). However, there are issues that arise because of Taxol's popularity. Taxol's presence in the bark of the Pacific Yew is very minor, on the range of 0.01 percent. Therefore, it takes the death of four Pacific Yews to produce one human dose of Taxol. For these reasons, the Taxol production is being extensively harvested and faces extinction (Davidson). The synthetic production of paclitaxel requires thirty steps and has a yield of less than 0.05 percent (Azu 1997). Because of its eleven chiral centers, as can be seen in Figure 1, synthetic production of Taxol will not be cost effective or efficient until the production is reduced to twenty five or fewer steps (Azu 1997). By inserting genes into Physcomitrella patens, a pacific precursor will be made in the plant that will allow production of the compound to be more time and cost effective.

MATERIALS AND METHODS

Several precursors must be synthesized before the final Taxol product can be produced (Figure 2). P. patens has already been altered to produce taxadiene and taxadiene-5α which are the first and second precursors respectively (Figure 3). Three plasmids, which contained either T5AT, T10H, or T13H were amplified so that they could be inserted into the moss Physcomitrella patens. Transform Into Moss

RESULTS

Version six was transformed with just T133 and had the largest peak at fifty minutes. Version four was transformed with T133 and T10H. It had a significantly smaller peak at fifty minutes, representing T132 and a larger peak at the 48.75 retention time minute, which represents T10H. As can be seen in Figure 2, T10H can only be seen after T132 or T45 has acted. Therefore, when T1H and T133 are combined, the T133 product is used by T10H to make the final product. Finally, version one was transformed with T10H, T133, and T45. The proposed T113 and T10H peaks were small but present. They could be smaller because their products are being outcompeted by that of T45 whose product is not seen on the 304 MW chromatogram. Based on these results, it is believed that T133 and T10H were both successfully transformed to the moss and expressed. The results from the 330 MW chromatogram showed some promising peaks; however, the peaks were not very large. Based on this, it is believed that T45 was not successfully transformed to the moss. However, with the results from the screens, electrophoresis gel, and sequencing, it is believed that the plasmids isolated were the correct versions and in working order. Therefore, a viable T45 could have been present in the moss, but in so small a quantity that it did not produce visible results. All of the results were obtained from a transient transformation of moss and a small amount of product being analyzed. With this said, more products need to be analyzed before stronger conclusion can be made. To create more products, a permanent transformation must be completed. Upon completion, the pathway's viability will be determined and the precursor product can be made.

REFERENCES


Figure 1. Paclitacl (Taxol) Structure

Figure 2. Taxol Precursor Pathway and Target Genes

Table 1. Moss Versions With Vectors Added and the Protoplasts That Were Already Present

<table>
<thead>
<tr>
<th>Version</th>
<th>Vector Added</th>
<th>Protoplast Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T5AT</td>
<td>TS6, T45</td>
</tr>
<tr>
<td>2</td>
<td>T3H, T5AT</td>
<td>TS6, T45</td>
</tr>
<tr>
<td>3</td>
<td>T10H</td>
<td>TS6, T45</td>
</tr>
<tr>
<td>4</td>
<td>T3H, T13H</td>
<td>TS6, T45</td>
</tr>
<tr>
<td>5</td>
<td>T5AT</td>
<td>T13H, TS6</td>
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<tr>
<td>6</td>
<td>T13H</td>
<td>T13H, TS6</td>
</tr>
<tr>
<td>7</td>
<td>T10H</td>
<td>T45, T13H</td>
</tr>
<tr>
<td>8</td>
<td>n/a</td>
<td>T45, TS6</td>
</tr>
<tr>
<td>9</td>
<td>n/a</td>
<td>W3H Type</td>
</tr>
</tbody>
</table>

Figure 3. Moss Prior to Transformation With T10H, T3H, and T5AT

Figure 4. Experiment Flow Chart

Figure 5. Electrophoresis Gel After PCR Amplification

Figure 6. Electrophoresis Gel After Screening and Collection

Figure 7. Sequenced Plasmid Variants

Figure 8. GCMS Results For 304 Molecular Weight

Figure 9. GCMS Results For 330 Molecular Weight