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Popular Science Summary
KBTM01 Master's Degree Project in Biotechnology, 30 credits

Cloning and expressing the genes encoding Glycerol dehydratase (GDHt) and 1,3-Propanediol dehydrogenase (1,3-PDDH) in *E. coli*

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The production of biodiesel, a type of renewable fuel, has been increasing rapidly, leading to a significant increase in the production of glycerol by-product. Scientists are now looking for ways to use this excess glycerol in a sustainable manner. One exciting solution is to convert glycerol into a valuable chemical called 1,3-propanediol (1,3-PDO). 1,3-PDO is a valuable compound with various applications in industries like manufacturing, textiles, and cosmetics. It can be used to make polyester fibers, which are used in clothing and fabrics, as well as in the production of personal care products like lotions and creams. This conversion process of glycerol into 1,3-PDO can be done by using microorganisms, like bacteria, which have special abilities to transform glycerol into 1,3-PDO.

To make this conversion happen, certain enzymes are involved. Enzymes are special proteins which help carry out specific chemical reactions inside the cells. In the case of glycerol transformation, two key enzymes are responsible for the process: glycerol dehydratase (GDHt) and 1,3-propanediol dehydrogenase (1,3-PDDH). Here's how the transformation of glycerol into 1,3-PDO occurs: First, GDHt takes glycerol and converts it into a different chemical called 3-hydroxypropionaldehyde (3-HPA). Next, 3-HPA goes through another reaction facilitated by 1,3-PDDH, resulting in the formation of 1,3-PDO.

In this research project, these two enzymes were used to transform glycerol into 1,3-PDO. The focus was on studying GDHt and 1,3-PDDH enzymes and how they work together. A common bacterium called *Escherichia coli* (*E. coli*) was used, and the genes responsible for making GDHt and 1,3-PDDH were inserted into these bacteria, to produce the enzymes. When GDHt and 1,3-PDDH were produced together in a single bacterial cell, there was an indication that glycerol was being transformed into 1,3-PDO. However, the actual production of 1,3-PDO couldn’t be detected, and there were other factors affecting the measurements. It was also discovered that GDHt's activity could be increased with the presence of glycerol dehydratase reactivase (GDHR), another important protein. However, measuring GDHt's activity accurately was challenging because there were other components in the bacteria that interfered with the measurement.

To improve these experiments, it is important to optimize the reaction conditions. This means finding the right temperature, pH level, and controlling the presence of oxygen. It is also suggested using purified enzymes, which are enzymes that have been carefully separated from other substances. Additionally, different methods should be explored for measuring the transformation of glycerol into 1,3-PDO. They aim should be to better understand the transformation reaction and find ways to make it more efficient. This research will contribute to developing sustainable ways to utilize glycerol by-product and produce valuable chemicals like 1,3-PDO, which can have various important applications in different industries.