Heading

"Production of the protein thymidine kinase 1 from tomato in bacteria and insect cells"

Introduction

I produced the protein thymidine kinase 1 in bacteria and in insect cells. This protein, which originally comes from tomato, could have important medical applications, such as therapies to treat brain cancer.

Main text

Some types of cancer, brain tumors for example, are very difficult to treat. Many patients die only a few months after being diagnosed and there are very few treatments available. For that reason, the medical community is looking for alternative therapies. One of those therapies is called suicide gene therapy and it consists of combining an enzyme (a protein that carries out a chemical reaction) with certain types of drugs called nucleoside analogs in order to kill cancer cells. The aim of my degree project was to use bacteria and insect cells that have been modified by genetic engineering in order to make them produce an enzyme called ToTK1 (tomato thymidine kinase 1), which is likely candidate to be used in suicide gene therapy.

The first step in my project was to use genetic engineering in order to make bacteria and insect cells produce ToTK1. This is done by introducing a piece of DNA that contains the gene that codes for ToTK1. Next, cells were grown in solutions that contain all the necessary nutrients and under the right temperature and stirring speed. To make bacteria produce ToTK1, a chemical known as IPTG was added to the culture. On the other hand, insect cells were infected with a special type of virus (called baculovirus) that contains the ToTK1 gene necessary to produce the protein. At the end of the cultivation, the cells were harvested and broken open to release all the protein they contain inside. I used a method called affinity chromatography in order to separate ToTK1 from all the other proteins. The next step was to determine if the protein was active, in other words, if it was able to carry out its chemical reaction. To my surprise I found out that ToTK1 produced by bacteria was *not* functional, which was a pity because we were able to get very high amounts of protein. I also found out that this protein was not very soluble, and after some time it precipitated (similar to what happens to milk when it curdles). Fortunately, insect cells were able to produce soluble and active ToTK1. This is most likely due to the fact that insect cells have a more sophisticated machinery to produce proteins that come from other eukaryotic organisms (such as plants, yeasts, humans, etc.).

Now that I have successfully shown that it is possible to produce ToTK1 using insect cells, other scientists can use my methods at a larger scale to produce higher amounts of the protein and to purify it to make samples of very high quality. I hope that these samples will then be used to figure out the three-dimensional structure of ToTK1. Right now we don't know what the protein looks like! Once the structure has been determined, it will be possible to better understand how the enzyme works and how to improve it by, for example, creating mutant versions of the protein. In the end, I hope that all of these efforts will result in more effective and successful therapies for the treatment of brain cancer, and possibly other tumors as well.