Microfluidic biofermenter mimics plant tissue

It is estimated that as many as one million secondary plant metabolites can be used as medical agents. Plants produce these compounds to aid their growth and development as well as to discourage herbivores from eating them. However, it is still very difficult, or even impossible, to produce secondary plant metabolites industrially. Researchers from the Karlsruhe Institute of Technology (KIT) are now developing a microfluidic bioreactor to make the industrial production of plant metabolites more practical.

One of Professor Nick's team members with a microfluidic biofermenter.
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A historic building on the edge of the Karlsruhe Institute of Technology South Campus where a few lab coats hang on hooks in a long corridor – not the kind of place where you would expect pioneering work to be done. But if you take a look, you can see Peter Nick, professor at the KIT Botanical Institute, and one of his team members carefully unpacking a transparent chip the size of a microscope slide.

Upon closer inspection, you can see a bean-shaped chamber below a transparent layer where up to 200 plant cells can be grown on a nutrient-permeable membrane. The extraordinary thing about this chip is that the bean-shaped chamber can be connected by way of small tubes to other chambers where other types of cells grow. The tubes continuously supply the cells with nutrients as well as removing metabolites produced by the plant cells. This chip is a two-chamber microfluidic bioreactor that mimics plant tissue on a small scale. The researchers have in the meantime filed a patent to protect their invention.

Botanists playing metabolic Lego

"We play metabolic Lego," said Nick roughly describing what he and his team are doing. "We combine different metabolic modules, in our case cell types or genetically-modified cell lines, to generate different plant compounds." Nick's team of researchers have taken the two-chamber microfluidic bioreactor and adapted it for tobacco cells. Like natural tobacco plants, the tobacco cells in the bioreactor produce nornicotine, which appears to be an important ingredient in the treatment of Alzheimer's. One of the bioreactor's chambers contains transgenic tobacco cells, an enzyme that normally triggers nicotine synthesis in plant leaves, and the second carries transgenic cells with an enzyme derived from tobacco plant roots that converts the nicotine from the first chamber into nornicotine. "The combination of leaf-specific and root-specific synthesis steps in one chamber only produces small amounts of nornicotine because nornicotine downregulates the first step, i.e. nicotine synthesis," says Nick explaining the benefit of spatially separating the two synthesis steps into two different chambers.

Capricious plant cells

"It took us five years to grow the first plant cells in the reactor. You can imagine how happy I was when we achieved it," admitted Professor Nick, laughing. Working with colleagues led by Professor Andreas Guber and Dr. Ralf Ahrens from the KIT's Institute of Microstructure Technology, Nick's team carried out meticulous and detailed work on the bioreactor design. Although bioreactors are commonly used for animal and bacterial cells, cultivating plant cells in bioreactors is much more difficult. This is because many plant cells grow relatively slowly and tend to become woody, causing them to commit programmed cell death. Moreover, plant cells have a cell wall which prevents them from adhering to the bottom of the culture vessel, making them difficult to cultivate.

Worldwide, there are only a handful of companies that specialise in plant cell cultivation. But even though it is possible to grow plant cells, many biotechnologists nevertheless fail to produce the desired secondary metabolites. In contrast to plant proteins that can be produced in bacterial and animal cells into which the genetic blueprint of the proteins has been transferred, producing secondary metabolites usually requires a relatively large number of steps. "What usually happens is that people just throw a piece of plant tissue into a fermenter, without taking into account that different cell types are needed to produce the required secondary metabolite," lamented Professor Nick.

In the case of a plant called periwinkle (Catharanthus roseus), around ten different cell types that specialise in various parts of the vincristine biosynthesis pathway work together to assemble the chemotherapy drug. Vincristine is used to treat a number of cancers. Chemical synthesis of this drug is very time-consuming and expensive, which is why it is usually directly isolated from the plant. A number of medically valuable plants are threatened with extinction. "There

Prof. Dr. Peter Nick

are only around 20 plum yew (Cephalotaxus hainanensis) trees left on the island of Hainan, off the coast of China. The bark of the plum yew is believed to be antileukaemic and is therefore a widely used herbal remedy. Plum yew bark is worth eight times its weight in gold," said Nick.

The king of fermenters

The biologist is aware that his team can only produce modest quantities of secondary plant metabolites in the small bioreactor and the bioreactors are therefore not suitable for mass production. At present, the researchers can produce around one fifth of a gram in a shot glass filled with nutrient solution. "Although the quantities are still quite low, we can nevertheless use our chip to identify cell types that produce larger quantities of the desired product and optimise the culture conditions," said Nick.

Ideally, the findings from this work with microfluidic bioreactors need to be transferred to industrial scale production. "It can take months to grow plant cells in fermenters of around 75,000 litres," says Nick highlighting the importance of using the microfluidic fermenter to select the most efficient cell types. In a project funded by the BMBF as part of the BioEconomy 2030 programme, the KIT researchers and a company called Phyton Biotech GmbH aim to establish microfluidic bioreactors for various applications. The Ahrensburg-based company uses Pacific yew cells to produce a secondary plant metabolite called paclitaxel, which is used for treating cancer.

In December 2015, Nick and Prof. Dr. Mathias Gutmann from the KIT's Institute of Philosophy received the Baden-Württemberg Teaching Award for broaching ethical issues with biology students. Nick has no ethical concerns about his research, quite the contrary in fact. "Biofermenters represent ethical progress. We can use them to protect rare plants and also increase product quality thanks to standardised production processes," said Nick, highlighting the advantages of using biofermenters.