

Plants producing pharmaceutical active ingredients

Health-bringing tobacco



One in four new medicines today is what is termed a biopharmaceutical – a drug whose active ingredient was produced by a bioreactor, for example bacteria, brewers' yeast, or insect or hamster cells. The market share of these active ingredients is likewise increasing sharply. Icon Genetics, a subsidiary of Bayer Innovation GmbH, uses tobacco plants to manufacture pharmaceuticals and believes that it has excellent prospects in this market.



Gene immersion bath: Dr. Sylvestre Marillonnet places tobacco plants upside down in a vacuum tank filled with a bacterial solution. The bacteria transport genes into the plants which are then capable of producing medicinal active substances (left). Alternatively, the active substance genes can be implanted into individual tobacco cells which then grow into whole plants (above right).

At first glance, it looks like the greenhouse of an uninspired gardener. Row upon row of flowerpots, each with an approximately 40-centimeter-tall plant growing out of it. Each plant has developed strong, healthy leaves – but there is not a flower in sight.

However, these green plants growing on the top floor of a research building just outside Halle in Saxony-Anhalt, Germany, are not intended to be aesthetically appealing and brighten up living rooms. None of these plants will ever bloom, as they will all be destroyed before they get to that stage. Long before they have started to blossom, their leaves will contain substances that could potentially save lives.

"We use these tobacco plants," explains Dr. Viktor Klimyuk, "to produce large quantities of pharmaceutical active ingredients – considerably faster, more easily, more safely and more cheaply than was previously possible." The 48-year-old biochemist is Development Director at Icon Genetics, a biotechnology company which was acquired by Bayer at the beginning of 2006. He believes that, "with our expertise we will achieve a leading position in the promising molecular pharming market." 'Pharming' is the term used by experts to describe the production of high-quality, recombinant proteins in plant biofactories.

Klimyuk's confidence is founded not least on the company's many years of research and the resultant large number of patents. "We can already produce at least 50 therapeutically active

proteins with our tobacco plants today," confirms his colleague Dr. Anatoli Giritch, "and with some we have even already reached the maximum theoretical yield."

Plants can produce complex active ingredients

The Halle researchers are not only concerned with quantities of pharmaceutical products, however – what is also important is how easily and how quickly a few hundred grams of a certain active ingredient can be produced. This is because in future "personalized medicine" could mean an increased demand for small quantities – a problem which cannot readily be solved economically using today's production processes.

Despite impressive successes, the experts at Icon Genetics are constantly researching other proteins, such as antibodies. The aim is to produce more and more complex active substances, as this is one of the major advantages of pharming over previous manufacturing processes which rely on bacteria, fungi or animal cell cultures. Only relatively simple proteins can be produced by fungi and bacteria, while neither microorganisms nor yeasts can form and correctly fold complex proteins. These processes are unable to deal with complicated protein structures, producing only useless lumps of protein.

Animal cell cultures are different, however. They can also manufacture more complex proteins and have therefore been used for many years for the



Tell-tale light: under ultraviolet light (right), the tobacco plants reveal that Bayer's scientists have successfully implanted the DNA for a luminescent protein into their leaves using the immersion bath procedure (see page 41). The green fluorescent protein (GFP) serves as a test for later active proteins. Using the immersion bath procedure, large quantities of proteins can be harvested very rapidly. After a while the tobacco plants lose the new gene.

production of pharmaceuticals. However, extreme sterility and very time-consuming, costly tests are needed to ensure that no pathogens such as viruses find their way into the cultures and replicate there. This is the only way in which to guarantee the safety of the products for humans.

In contrast, plants are free from animal and human pathogenic viral contamination, yet so closely related to humans and animals that they are able to correctly process and configure complex proteins of human or animal origin. "We naturally have to decide," explains biologist Dr. Sylvestre Marillonnet of Icon Genetics, "which particular proteins we want to produce in the future. One thing is already certain, however: we will achieve much more than was possible with previous methods."

A virus transforms tobacco into a medicinal plant

The idea of using plants as protein bio-factories is not that new, however. The

first pharmacological substance was created in a plant as long ago as 1986. A tobacco plant was modified in such a way that it formed the human growth hormone somatotropin. Three years later, the first antibodies – much more complex proteins than somatotropin – were produced using recombinant technology, again in a tobacco plant. Since then, scientists have referred to such antibodies as "plantibodies".

In all these experiments, the plant's DNA was changed in exactly the same way as had previously been done to modify their properties, for example to confer them with herbicide resistance, insecticide resistance, drought tolerance or improved nitrogen uptake. To give a specific example, modified tobacco plants were used which carried a gene from the bacterium *Bacillus thuringiensis*, which made them resistant against insect pests.

"For qualitative modifications like that, it is sufficient if the plant produces relatively small quantities of the new or modified proteins. With medical active

substances it's different, however. Here we're interested in harvesting large protein quantities," explains Professor Yuri Gleba, the founder of Icon Genetics. "This allows us to produce the active ingredients on small growing areas such as greenhouses, for example." The scientists therefore combine the gene for the medical active substances they need with the genetic material of plant-specific viruses. Such viruses multiply rapidly in plant cells. The viruses themselves are used solely as vectors and cannot infect the plants.

In the initial stage, the scientists modify the genotype of a plant virus, the tobacco mosaic virus, from the natural RNA (ribonucleic acid) form to the chemically more stable DNA (deoxyribonucleic acid) form. The desired genes are then incorporated into the DNA and the viral genome modified in this way is introduced into the tobacco cells using bacteria of the *Agrobacterium tumefaciens* species. The plants subsequently produce the desired substance.



Plant researchers: Carola Engler, Dr. Yuri Simonenko and Dr. Romy Kandzia (left to right) breed transgenic tobacco plants that retain the new genes permanently but must first grow to maturity before sufficient quantities of the new proteins can be harvested. Right: Professor Yuri Gleba, founder of Icon Genetics, in the greenhouse with fully grown tobacco plants.

To do this, the scientists use not a syringe, but an immersion bath. The plants are dipped upside down as far as the bottom of the stalk into a bacterial solution and exposed to a vacuum. The process takes one or two minutes and then the pressure is slowly brought back to normal. Says Klimyuk, "We have achieved far better results with this

method of vacuum infiltration than with spraying the plants, for example."

Immersion bath for gene transfer to the plants

The immersion solution contains *Agrobacterium tumefaciens*, which is subsequently found in all fully developed

leaves on the tobacco plant. The bacterium itself only has one job – to introduce the modified tobacco mosaic virus into the plant – "and this is precisely what interests us," explains Klimyuk. This virus is not complete – the sheath protein is missing, for example. Instead, it has been equipped with genes which enable the plant to produce the desired proteins or active substances after the infiltration. Plant geneticist Giritch explains: "With this method, the plants don't incorporate the foreign genes into their genotype. They are not transgenic and lose the foreign hereditary information again after a little while."

Antibodies from tobacco plants

The treated plants continue to grow under normal greenhouse conditions for a further ten days or so. They recover very quickly from the immersion bath and infection. During this period, they form considerable quantities of the desired products in their cells. It

Hamsters, chickens and bacteria

Isolating genes from the DNA of one species and incorporating them into the genotype of the desired organism is a tried and tested practice in modern medicine. For example, in the production of the Bayer product Kogenate®, which is used for the treatment of hemophilia, human Factor VIII gene is implanted into hamster kidney cells, thereby achieving a much higher level of safety and purity than can be obtained by deriving it from whole blood. The industry and patients also place their trust in recombinant technology for the production of insulin, since it has become apparent that insulin produced from bacteria is better tolerated than insulin derived from pigs. One of the first products with a recombinant plant protein is avidin. This protein derived from hens' eggs is used in medical laboratories for diagnostic purposes. This is possible because extensive clinical studies in humans were not necessary for this application.



Interview with Dr. Detlef Wollweber

Extremely good starting position

Dr. Detlef Wollweber is the managing director of Bayer Innovation GmbH. *research spoke to him about the acquisition of the biotech company Icon Genetics.*

What exactly are the particular strengths of Icon Genetics?

Icon Genetics has outstanding expertise in the creation of special medicinal substances in plants. The takeover of Icon Genetics was concerned in particular with patent-protected technologies for the production of these biological pharmaceutical products. This technology platform allows us to produce a large number of different complex proteins, ranging from simple protein units such as are required for the manufacture of certain vaccines, to complex antibodies such as are used in the treatment of infectious diseases or malignant tumors. Irrespective of the complexity of the molecule, initial tiny quantities of protein can be produced within a week. Larger quantities, such as will be necessary in a defined quality for clinical studies, can be produced more quickly without investing in fermenters and, because of the high yields, probably also considerably more cost-effectively than established production methods.

What were Bayer's aims with the takeover of Icon Genetics?

We want to use the company's expertise to strengthen our activities in the creation of certain medicinal substances in plants. This whole subject is not new to Bayer. Over the last few years, Bayer CropScience has carried out excellent preparatory research and development work in the area of bioscience. Bayer is also in an extremely good starting position because we have pharma know-how and plant know-how all in one group of companies.

What are the next steps in the plant-made pharmaceuticals project?

We anticipate interesting possibilities for using the new technology in the area of vaccine development, with expected benefits to all users and consumers. For example, we are expecting to be able to produce the vaccine more quickly and in much better quality so that high-risk individuals and if necessary the whole population in affected areas can be given more extensive protection against certain pathogens. This is particularly important so that the risk of epidemics can be nipped in the bud.

Why was the entire project assigned to Bayer Innovation and not to Pharma or BioScience?

Bayer Innovation is a small limited company whose role is to identify and expand innovative areas of business. Plant-made pharmaceuticals are not in the immediate area of interest of Bayer CropScience and at present are still a little too remote from Pharma. Our role is that of a project base which works in areas above and beyond those of the subgroups, but naturally with their support. This also means that we are not in competition with established areas of research and development. Our expertise covers project management and the entire business area.

has since become apparent that the possibilities of this technique are far greater than scientists realized only a few years ago. For example, hormones can be produced, as well as vaccines or enzymes for technical applications. Even antibodies, which play an important part in the immune defense against pathogens by identifying and fighting foreign structures, can now be produced by tobacco plants.

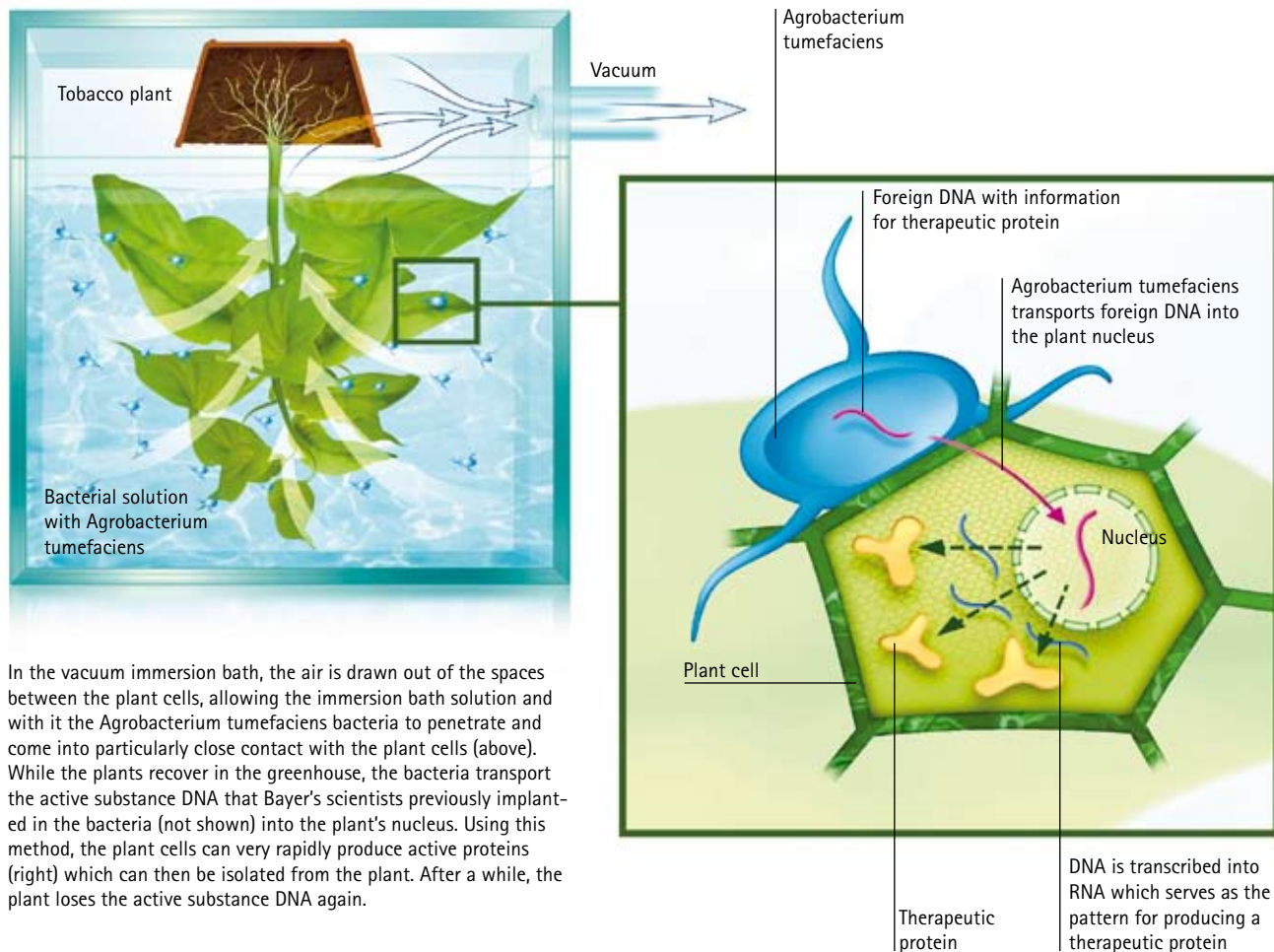
As soon as these products are harvested from the plants, all biological remains are destroyed as a precautionary measure to prevent genetically treated plants from finding their way into the environment in some way. "Tobacco also has the particular advantage that it is neither eaten nor fed to animals," explains Anatoli Giritch. And finally, there is also the fact that the viruses which are used present no danger to the environment anyway. "They are unable to survive in their vector form and present no danger to anyone."

Nevertheless, his boss Gleba is closely following the debate surrounding genetically modified plants: "The public's concern must be taken very seriously," he says. "That's why it's all the more important that everyone knows what we are doing here and the benefits it will bring to man."

The benefits: speed and high yield

However, there is a long way to go before patients can make use of drugs produced using this technology. Dr. Detlef Wollweber, Managing Director of Bayer Innovation GmbH, expects it to take at least ten years before medicinal products made from plants are granted marketing authorization. This is due to the comprehensive clinical tests which medicinal products have to undergo. "It is quite possible, however," says Wollweber, "that on the way to regulatory approval we may perhaps be able to harvest a few low-hanging

Transforming tobacco into a medicinal plant in the immersion bath



In the vacuum immersion bath, the air is drawn out of the spaces between the plant cells, allowing the immersion bath solution and with it the *Agrobacterium tumefaciens* bacteria to penetrate and come into particularly close contact with the plant cells (above). While the plants recover in the greenhouse, the bacteria transport the active substance DNA that Bayer's scientists previously implanted in the bacteria (not shown) into the plant's nucleus. Using this method, the plant cells can very rapidly produce active proteins (right) which can then be isolated from the plant. After a while, the plant loses the active substance DNA again.

fruits – perhaps products which are important in the technical area, such as proteins, which play an important part in the production of recombinant antibodies, or trypsin, which at present is derived from animals and which is used in large quantities in the production of medicinal products such as insulin. The focus of our activities at present, however, is to make use of the unique potential of Icon Genetics' technology in terms of speed and yield. This may be a decisive advantage in the production of vaccines. This is why we are concentrating on this class of substance at the moment."



www.icongenetics.com

The Icon Genetics website shows techniques and projects for the production of plant-made pharmaceuticals.