Using white biotechnology to develop new crop protection products

The **bacterial workshop**

Nature offers many models for new technologies: Bayer's research scientists are now using biotechnology-enhanced bacteria cells to produce substances used in crop protection products. Without these efficient micro-organisms, the active ingredient indaziflam for the new herbicide Alion™ might have failed at the development stage. But scientists reached into their box of biotech tricks to boost the efficiency of their miniature helpers.

Some of the chemical factories of the future are tiny: bacteria cells just a few thousandths of a millimeter in size can control hundreds of chemical processes in a very small area. They produce highly complex molecular structures in an extremely efficient manner. The particular talents of these tiny organisms lie in their molecular tools: enzymes, or biocatalysts as they are also known. They manage cellular metabolism and work as accurately as machines: starting reactions, juggling groups of atoms and putting them together to form larger chemical compounds. It is the vast repertoire of enzymes that makes these micro-organisms into desirable chemical factories on a miniature scale.

We normally tend to associate bacteria with diseases such as gastrointestinal disorders or infected wounds. But bacteria come in useful and even vital forms as well. For example, intestinal bacteria help us digest nutrients and make vitamins. Lactic acid bacteria not only give yoghurt its characteristic sharp taste; without them, it would quickly spoil.

Micro-organisms help in herbicide production

The special properties of micro-organisms can also be put to commercial use thanks to white biotechnology (see box text: "The colorful world of biotechnology"). Says Dr. Mark Ford from Process Research at Bayer CropScience in Frankfurt, "Biotechnology processes are now just as useful a tool for manufacturing substances on an industrial scale as purely chemical processes." The micro-organisms helped him and his team in the production of a new crop protection product. Bayer's experts were finding it impossible to determine the physical arrangement of a particular group of atoms in the molecular structure of the active ingredient indaziflam, and this was holding up large-scale production. They teamed up with external partners to look for a biotechnology solution and developed genetically modified bacteria cells (a harmless version of Escherichia coli) which resolved the

Optimization team: analyst Dr. Uwe Döller and process researcher Dr. Mark Ford (photo, left, left to right) have been working hard on the production procedure for the new active ingredient at Bayer CropScience. After examining countless bacteria cultures (photo, right), two suitable genetically modified variants were discovered and optimized. It is thanks to these micro-organisms that a new crop protection substance can now be manufactured.

Photos: Sabine Bungert (4)/Bayer AG, Gunnar Knechtel/laif (1)

problem of manufacturing the new herbicide. Scientists spent months fine-tuning the micro-organisms and improving their enzymes' properties. About half a million genetically modified bacteria were tested. The most ingenious aspect that the scientists came up with was using two different, genetically modified strains of E. coli at once to make the substance in the bioreactor. As Dr. Ford explains, "Their genetic material is only very slightly different, but each bacterium specializes in a particular chemical reaction, and we need both."

Competition in the bioreactor: the fight for nutrients

Ford and his colleagues, together with their external partners, adjusted the tiny cell factories so that they focused on dividing particular chemical compounds. It is only thanks to this biotechnological process that the crop protection product indaziflam, to be sold under the brand names Alion™ and Specticle™ all over the world, can be produced. It can be used to control a wide range of weeds affecting crops such as fruit and grapevines, but also in plantations with citrus fruits, olives and sugar cane. Special formulations of the active ingredient are also being developed for applications in forestry, industrial facilities, golf courses and sports fields. Marketing under the brand name Specticle™ for weed control on golf courses and sports fields has already begun in the United States.

But before reaching this stage the Bayer experts had to overcome a few hurdles in their bacteria fine-tuning workshop. Says Ford, "It isn't easy to control two different biological reactions in a reactor accurately." This is why Bayer's scientists first had to conduct a close examination of how bacterial enzymes work, because the two different proteins compete with each other. Therefore the scientists had to adjust the ratio of starting material and intermediate products very precisely, as this has a considerable impact on the work of the tiny biochemistry factories. But they found the right mix: millions of customized bacteria cells are now floating around as creamy yellow flakes in giant stainless steel containers, finely adjusted to achieve the maximum output for industrial production.

The new weedkiller Alion™ is now being manufactured in large quantities, thanks to nature's box of magic tricks: "We were not able to produce the compound efficiently by means of conventional chemical synthesis. We needed enzymes," explains Dr. Norbert Lui, Head of Research Technologies at Bayer CropScience in Monheim. Fortunately the experts were able to start down the biochemical manufacturing route at an early stage, which saved money. Comments Lui, "Refining a process like this takes up a lot of time at the start of the process."

Scientists working on development must also make sure that the process they select is the best in terms of safety, use of resources and costs. Says Lui, "We compare many different

A sharp-eyed biochemist: laboratory manager Dr. Ulrike Hänsel checks the tubes supplying the bacteria in the fermenter with the ideal amounts of oxygen and the other nutrients they need.

The colorful world of biotechnology

A color code is used as a handy method of classifying the different applications in biotechnology. **White biotechnology** *uses the tools of nature like bacteria, yeast cells or enzymes in industrial production. Products made using white biotechnology have been enriching human life for thousands of years, particularly in the area of food: micro-organisms are essential to products such as wine, bread, beer, cheese and yoghurt. Scientists now also use tools derived from nature to produce basic or specialist chemicals.* **Red biotechnology**, *by analogy with human blood, is currently the largest field and refers to the development of new therapeutic and diagnostic processes. Drugs such as antibodies and hormones are well-known examples for which modern genetic research lays the scientific foundations.* **Green biotechnology** *takes its name from the green pigment in leaves, chlorophyll. This form of biotechnology involves breeding new plant varieties. Molecular methods are used to produce improved crop plants and higher-yielding varieties, for example.* **Blue biotechnology** *focuses on marine organisms. Scientists are particularly fascinated by thermostable enzymes found in deep-sea bacteria that live close to hot underwater volcanoes.*

A bright outlook: farmers with apple orchards like this one in Argentina (photo, left) can benefit greatly from the new herbicide in future. The substance indaziflam not only targets weeds very accurately, but it is also long-lasting. To optimize production of the crop protection agent, Dr. Mark Ford (photo, right) and his colleagues in Process Research and Development tested its synthesis in a series of trials in the laboratory and finally applied biotechnology methods to scale up production.

processes – sometimes we test up to 20 different routes for their general technical feasibility and their suitability for upscaling."

But everything starts in the laboratory, with the detective work of the crop protection researchers as they try to guide individual molecular elements into the correct position and devise chemical reaction paths which will lead to substances. Once they have found a suitable compound, it is time to consult the route planners produced by process research specialists like Ford and Lui. This is because, although many reaction paths are identified in the research lab, only a few are suitable for industrial production. The reagents or starting materials which the laboratory only needs in minute quantities are often too expensive for large-scale production.

The latest analytical methods mean that their colleagues working in crop protection, as well as the scientists working on pharmaceuticals, already know very well what a compound needs to look like in order to work effectively. They can predict how groups of atoms need to relate to each other or be arranged. A weed dies only if a substance fits in exactly the right place, like a lock and key. Then the substance can act, for instance by blocking a particular receptor in the plant cell, and the harmful plant dies. As Ford explains, "The extent of the herbicidal action of a compound can depend on the physical arrangement of a single chemical group, or on whether an atom is facing backwards or forwards." For example, mirror-image molecules (what specialists refer to as chiral compounds) have the same composition and connection of atoms, but can produce quite different effects, because it may be that only one of the two structures can dock properly onto a receptor.

A smart combination: standard chemistry and biotechnology

But the costs of a process are important, in addition to purely technical aspects, when a company is manufacturing an innovative crop protection product like Alion™: there is a specific price per hectare for each market segment. Says Ford, "The application volume, that is the amount of herbicide that a farmer has to use in his orchard, for example, and the cost of goods need to be taken into account when we make our plans." The benefits of Alion™ are that it is targeted specifically on weeds, and is very long-lasting, so that farmers do not need to apply it as frequently as other products. Bayer CropScience's many experts are therefore very upbeat about the prospects for the new product.

Both the scientists we spoke to put their success down to the ideal combination of conventional chemistry with methods used in biotechnology. Here again, the work of the Bayer CropScience scientists differs hardly at all from the approaches followed in pharmaceutical research. Experience is not all that counts: creativity is essential too. As Lui says, "You have to look at nature with open eyes." And nature is bountiful: scientists estimate that naturally occurring enzymes can catalyze over seven thousand different chemical reactions. But only around 130 of these are used in industry. So the tasks that lie ahead for the biotechnological production of crop protection products, and therefore also the chemical route planners devised by Lui and Ford, are vast.

www.research.bayer.com/indaziflam
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