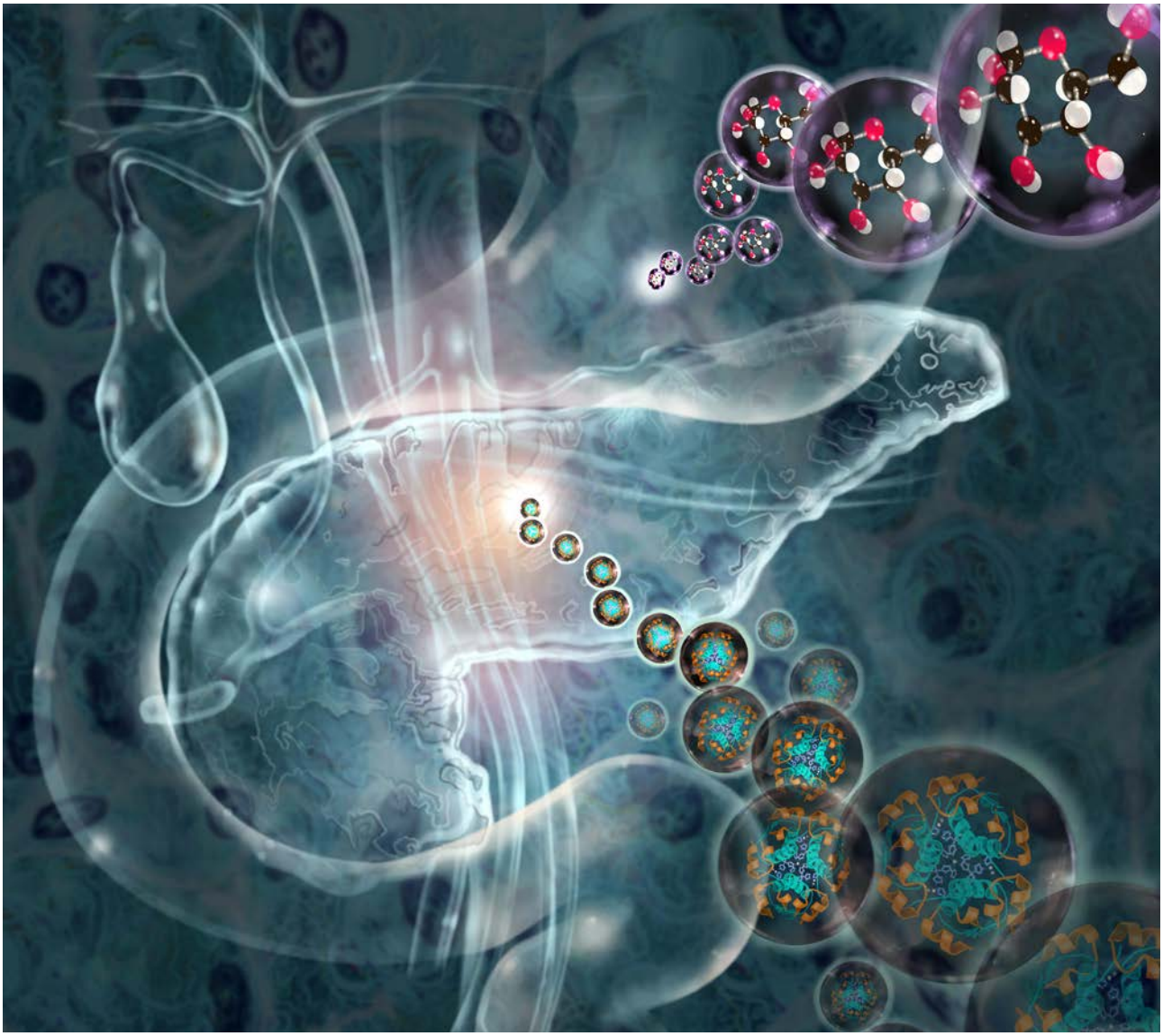
Sweet blood in the Middle Kingdom

Diabetes is on the increase all over the world, but China has been hit hardest. The pharmaceutical compound acarbose helps Asian diabetes patients manage their blood glucose levels. Bayer's Active Ingredient Production is now optimizing the bacteria that produce the substance using cutting-edge molecular biology technologies.



Sugar jam: body cells usually absorb glucose (top globes in the graphic) after the pancreas has released the signal hormone insulin (globes below). In patients with type 2 diabetes, this mechanism only functions inadequately and the harmful glucose accumulates.

Photos: Michael Rennertz/Bayer AG (1), Bayer AG (2), All Mauritius Images/Jim Dowdalls/Science Source (1), dpa Picture-Alliance/Zhang Tao Zz (1)

The Far East is gradually moving closer to the western world, and not least in terms of lifestyle and daily food. In China, burgers and chicken wings are increasingly common dishes on the menu. "The growing economy and modernization in this country has led to changed lifestyles as well," says Xiaoqing Li, medical affairs manager at Bayer in China. "Over the past decades, the level of obesity in the population has increased sharply, while physical exercise has declined."

Obesity is a growing problem in China

The economic developments are related to higher incidences of some diseases in China, above all diabetes mellitus. Almost one-tenth of the Chinese population is now affected by this condition; in 1994, the figure was just 2.5 percent. "Diabetes mellitus (type 2 diabetes) is on the rise in China, and affecting not only elderly people but now also children," says Li. What's more, only one in three affected people are aware that they have the disorder.

Diabetes is actually a condition that, depending on which stage it is at, responds well to treatment. As a result, the demand for antidiabetic medications is increasing in China. One Bayer antidiabetic product with the active ingredient acarbose is manufactured in Wuppertal, the site where Bayer was founded. Approximately 80 percent of the produced substance is exported to China. "Acarbose is produced biotechnologically by the Actinoplanes bacterium," explains Till Zemke, plant manager for acarbose active ingredient production at Bayer in Wuppertal. In view of the huge demand, Bayer researchers have been working steadily for more than 20 years to improve the manufacturing conditions for acarbose. In recent years, cutting-edge technologies have made it possible for researchers to scrutinize the strains of Actinoplanes bacteria that are used in the production of acarbose in great detail.

In the body, acarbose delays the production of monosaccharides, notably glucose, by inhibiting specific enzymes on the brush-border membrane of the small intestine, which are responsible for the digestion of complex polysaccharides and sucrose. In this way, acarbose can significantly reduce rising glucose levels after a meal.

In healthy people, when the small monosaccharides pass into the bloodstream, the hormone insulin allows other body cells to absorb glucose from the bloodstream and use it as a source of energy. In patients with type 2 diabetes, their body cells develop a form of resistance to insulin, which leaves the cells unable to absorb glucose from the blood normally. The sweet blood containing high levels of glucose can cause major damage to the body: elevated blood glucose levels have a negative effect on the blood vessels in the long term. Particularly affected are the fine capillaries in the eyes and kidneys. The consequences are damage to the retina, kidney failure, ulcers or stroke.

In type 2 diabetes patients, acarbose inhibits specific digestive enzymes of the small intestine and delays glucose release from complex carbohydrates and thus reduces rising glucose levels after a meal significantly.

New tools to optimize bacteria

With the increasing demand for acarbose in Asia, Bayer scientists are now planning to make targeted modifications to the genome of the bacterium in order to make acarbose production more efficient while at the same time further optimizing the quality of the manufactured acarbose.

"Technical developments have made possible completely new approaches, allowing us to refine even established processes," says Dr. Winfried Rosen, plant manager for acarbose active ingredient production in Wuppertal. The scientists created a kind of roadmap of the Actinoplanes bacterium genome, with all known genes and their properties. It shows where each gene lies and how the DNA of each strain, including those used in the past, differs from the others.

"For this task, we got help in the shape of the specialists from the Center for Biotechnology – CeBiTec for short – at the University of Bielefeld," says Zemke. "The experts there are engaged in cutting-edge research into bioinformatics and genome sequencing." Professor Alfred Pühler and his "Genome research of industrial microorganisms" workgroup at CeBiTec sequenced the entire genome of the various strains of Actinoplanes. "We were also able to analyze which genes are particularly active and which proteins and metabolic products are produced by the bacteria," explains Pühler. The researchers were particularly interested in the metabolic pathways that are involved in the production of acarbose. For example, they compared how the gene activity varied with different sources of nutrients or during different growth phases.

Their work generated a wealth of knowledge. "We can now use these findings to make targeted changes to the current production strain as needed," says Pühler.

Overweight Asians: Chinese boys exercise in order to lose excess weight.

92.4 million

adults in China suffer from diabetes.

Source: Xu et al, 2013

Till Zemke (photo left) in Bayer's acarbose production facility in Wuppertal. Bacteria produce the natural substance in huge fermenters (photo right), watched by Bayer employee Thomas Kiesl.

His team and their colleagues at Bayer are planning to modify a regulator gene, for example. "It influences the DNA region that is responsible for acarbose synthesis," says Pühler.

A regulator gene promises increased AI production

If the researchers manage to modify this regulator in such a way that it increases activity in this acarbose DNA region, the bacterium could produce more active substance. Another objective could be to suppress the production of secondary components which otherwise have to be removed by means of complex purification stages.

Dr. Winfried Rosen, plant manager for acarbose active ingredient production at Bayer

"Technical developments allow us to refine even established processes."

In theory, these kinds of improvements can be relatively simply planned using gene maps. But in reality, the processes and interrelationships in the bacterial cells are significantly more complicated. "It's not ever likely to be just a switch that we have to flip. Much more probable is that we have to combine lots of switches to achieve our objective," explains Zemke.

Bayer researchers have spent 20 years optimizing the strain

Targeted modification of specific genes is not possible without the genome map. In the past, Bayer researchers would deploy chemicals or ultraviolet radiation to create random mutations in the genetic material of Actinoplanes, and then run tests to find out if any of these changes increased the acarbose yield. "These analyses were tremendously time-consuming. We had to pick out from among thousands of bacteria the few ones that grew well and produced lots of acarbose," remembers Zemke.

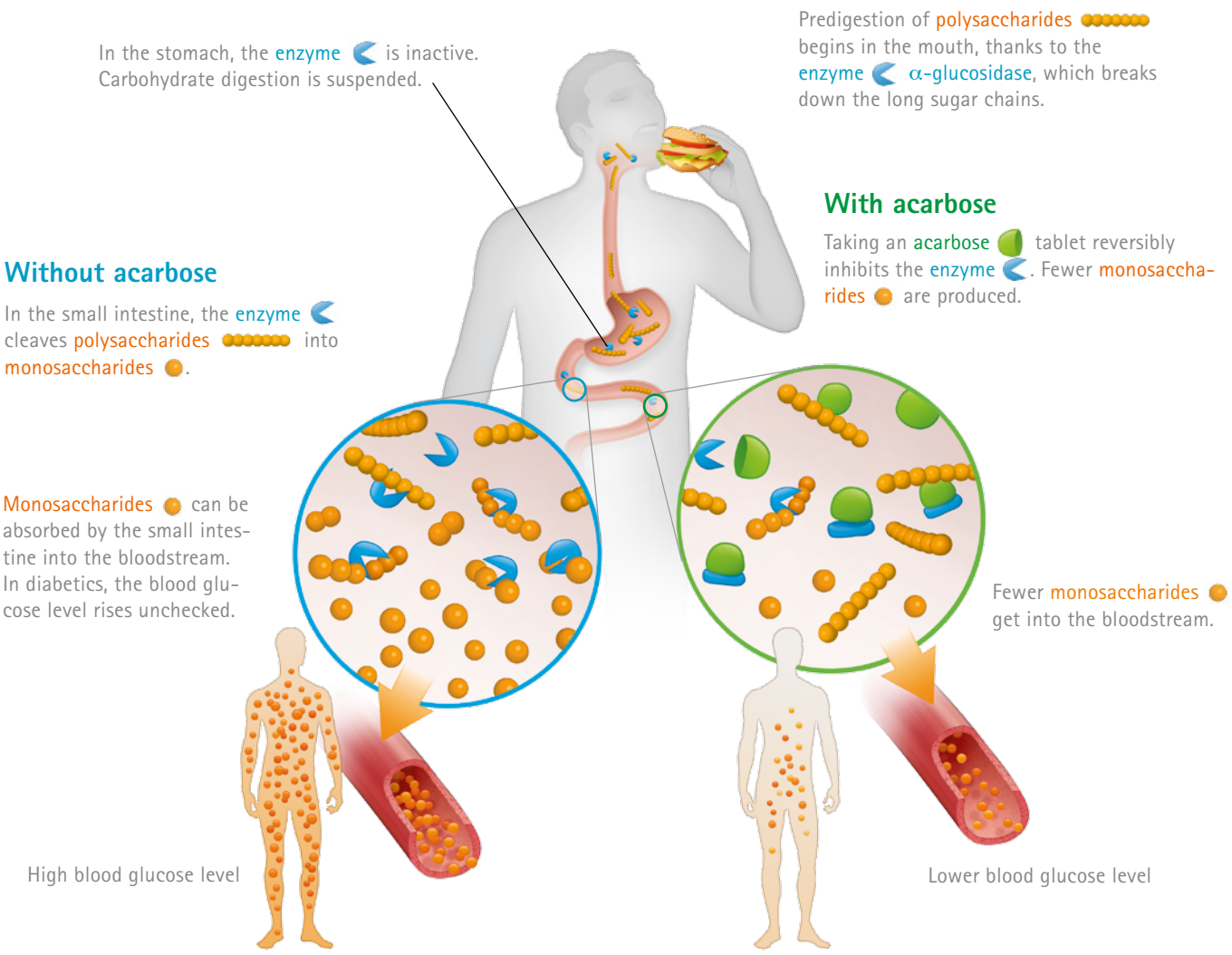
Bayer's experts have already repeatedly enhanced the performance of the little active substance producers over the past 20 years using this painstaking technique. By comparison, while the original strain of Actinoplanes bacteria produced less than 0.5 grams of acarbose per liter of culture medium, the strain used

For more efficient microbes

Big helpers can be tiny: Bayer researchers have been using the Actinoplanes bacterial strain to produce the natural substance acarbose since 1967. Over the past decades, they have continuously optimized the strain through random mutations and judicious selection of strains with beneficial gene variations. Thanks to state-of-the-art molecular biology – omics technologies – the scientists are now able to make targeted improvements to the microbes. For example, the researchers use genomics to read all of the genes in a bacterial strain and then compare the production microbes with their standard siblings. This method allows them to locate genes that are associated with greater biosynthesis activity. Using transcriptomics and proteomics, they can analyze which genes are read – i.e. used – by the bacterium. The scientists can take a snapshot of the cell condition by carrying out metabolomics testing to determine all of its metabolites. By modifying the identified genes, for example, they can create high-performance bacteria carrying the gene for elevated productivity and thus increase the efficiency of acarbose production.

Acarbose lowers the blood glucose level

The body cells of diabetes patients only inadequately absorb glucose from their blood. The glucose accumulates. The active ingredient acarbose reduces the absorption of glucose from food in the digestive tract and thus helps prevent high glucose harm.



at present produces approximately 80 times as much. "Using new technologies and thanks to our cooperation partners at CeBiTec, we now also understand the changes that we randomly created in the past," says Zemke, underlining the potential of genomics and bioinformatics. "We can trace the managed evolution from the original strain right through to the one currently used in production and draw new conclusions from it again." In this way, the researchers were able to characterize and file for patenting some 2,000 such mutations in the genome of Actinoplanes which led to its enhanced performance.

And CeBiTec and Bayer are already preparing the next technological step, which will involve genome editing. Today, new methods in molecular biology can be used to selectively modify DNA in the genome, almost like molecular scissors which enable scientists to cut out and replace genes. "We have adapted genome editing to Actinoplanes," reports Pühler. "We are now able to genetically modify each individual gene in the organism selectively." The scientists at Bayer and CeBiTec are working together in this project to make Actinoplanes an even more effective active ingredient producer, so that diabetes patients in Asia and all over the world can continue to rely on their therapy. ■