Achieving the Seemingly Impossible

Biotechnological Production of Rare Functional Sugars on an Industrial Scale

Rare functional sugars provide huge market opportunities. The German company Jennewein Biotechnologie, headquarterd in Rheinbreitbach near Bonn, specializes in in the industrial manufacturing of these products for a wide range of applications, including nutritional, pharmaceutical and cosmetic products and has a strong scientific foundation. As well as developing new and more efficient production processes for scarce functional sugars, Jennewein collaborates with selected partners in the preclinical development of its products, including the investigation of their health-promoting properties. The development of innovative processes for sugar molecules calls for broad expertise in biotechnology, process engineering and carbohydrate chemistry. Ralf Kempf asked Katja Parschat, deputy-head R&D, and Benedikt Engels, head of Production, about current projects and collaborations.

Benedikt Engels,
Jennewein Biotechnologie

Katja Parschat,
Jennewein Biotechnologie

CHEManager: Ms Parschat, Mr Engels, Jennewein Biotechnologie specializes in the production of human milk oligosaccharides (HMOs) on an industrial scale. Why was it so difficult to develop a profitable manufacturing process for these functional sugars? What hurdles had to be overcome?

Katja Parschat: HMOs were known for more than 100 years and for a long time people tried to synthesize them. Chemical and biocatalytic synthesis proved to be uneconomical and also not scalable into volumes relevant for food applications. Fermentative approaches suffered initially from low productivity and no cost efficient and scalable technology existed to purify HMOs from fermentative processes. We at Jennewein have addressed both aspects, the metabolic engineering part, as well as the development of suitable purification processes for HMOs and other oligosaccharides and monosaccharides. In 2005, when the company was founded, no industrial processes existed for defined oligosaccharides using a heterologous host.

After trying to synthesize human milk oligosaccharides by chemical synthesis and biocatalysis, meaning performing chemical reactions with isolated, enriched enzymes, the third and most successful attempt was to use metabolically engineered bacteria as cell factories. However, although harmless bacteria like Escherichia coli do provide some of the enzymes needed to synthesize complex sugar molecules, the genetic pathways involved had to be optimized and expanded by genes from other organisms to finally establish bacterial cells that are able to produce, and — what is most important — to secrete the complex sugars into the fermentation medium. By growing these optimized bacteria in a defined medium, the HMOs can be obtained simply from the culture supernatant. In addition, Jennewein developed a unique process to purify the HMOs from the cell-free fermenter solution and optimized the method for drying the sugar to obtain a product that complies with the high-quality standards for ingredients of infant formulae.

Starting at the lab bench, bacterial strains have been developed and optimized to produce individual human milk oligosaccharides with economic and feasible results, whilst processes for the recovery of the sugars from the fermentation solution were invented and refined.

Benedikt Engels: Developing an efficient process which lacked, for example, any use of organic solvents resulting in a high quality and safe product for use in infant formula was a milestone. All these processes had to be scaled up to an industrial size, to run fermenters in a scale of several hundreds of cubic meters and to handle these volumes in the recovery process. These development steps took about a decade with a substantial financial investment.

In October 2018 Jennewein bought the facilities of the former Artus Mineralquelle in Bad Hönningen to build an integrated production plant for HMOs and other sugars. What is the status of the project?

B. Engels: The purchase of the Artus Mineralquelle was a fantastic opportunity to acquire a suitable industrial estate for building an integrated production plant for HMO production. The facility is close-by and due to own water rights and existing infrastructure, perfectly suited to build large fermentation capacity. Currently we are in the planning and design phase of these production facilities. We hope that we will be able to start construction of the plant at the beginning of next year.

Why is it necessary to expand your production capacity?

B. Engels: After being qualified by several infant formula producers and receiving the approval for 2'-fucosyllactose in multiple countries, demands are increasing. In addition, Jennewein is currently bringing further HMO products like Lacto-N-neotraose on the market, and we are also launching the 5HMO Mix, containing the five most abundant neutral and acidic HMOs. These products will all be used in a higher concentration, thus getting closer to the natural concentration found in human milk. Therefore, we are strongly growing in production capacity to cover the increasing demands of 2'-fucosyllactose and at the same time bringing the next innovation cycle of HMOs to the market.

What are the company's current projects?

K. Parschat: After starting with single HMOs we are now producing different mixtures of HMOs. Using its 5HMO Mix, Jennewein is currently sponsoring a multi-center clinical study to confirm the benefits of using infant formula containing HMOs, in a concentration that is similar to the natural concentration in human milk, compared to infant formula not containing HMOs. The study is being conducted in Germany, Italy and in Spain and comprises of three study arms and the enrollment of over 300 in-

fants. The participating babies are assigned either to one of the double-blinded randomized formula groups, receiving either HMO Mix containing formula or the reference formula, or to the third group representing the gold standard of breast-feeding. Besides the evaluation of growth and behavioral parameters, the development of the gut microbiome of the subjects will be analyzed and by determining the HMO profile of mothers' milk from moms in the breast-feeding group, a correlation of HMOs in breast milk and the resulting gut microbiome in the offspring will be drawn. The clinical study started at the end of 2018 and will be finished in 2020. We have applied to the European commission for the approval of the five HMO Mix as a Novel Food and will shortly apply to the US FDA to obtain the "Generally Regarded as Safe" (GRAS) status to open up the opportunity to commercialize this new product.

In the last two decades science discovered diverse effects of HMOs on the human body and on the interaction between the human being and its microbiome. These effects range from supplying energy to support growth of beneficial gut bacteria to direct interaction of the HMOs with certain human cells on a molecular level and supporting brain development in neonates. Thereby different effects can be assigned to specific HMOs or groups of HMOs, like neutral sugars, HMOs containing fucose residues or acidic HMOs containing a sialic acid moiety. By combining HMOs with different properties, diverse effects can be addressed. Therefore, we stress the development of even more complex HMOs to enlarge the possibilities of combinations and to even offer personalized solutions.

In addition to the products mentioned earlier, we are currently extending our portfolio of monosaccharides. Besides L-Fucose and sialic acid, we are also offering Mannose as a monosaccharide in crystalline form.

You are also doing research on the production of designer organisms using metabolic engineering. What improvements do you expect for your production processes?

K. Parschat: Jennewein has always based its metabolic engineering on genomic integration or genomic modification. When designing organisms from scratch, we use modular systems to easily construct large synthetic parts of a bacterial genome. Having the needs for different metabolic pathways comprising several genes for the synthesis of different HMOs, using exchangeable modules makes the development of new strains faster compared to approaches using single genes for single production strains.

B. Engels: Production strains are highly efficient cell factories, customized not only for production of individual functional carbohydrates. Besides giving the strains the ability to produce HMOs by genetic engineering, we address production efficacy by also adjusting primary metabolism pathways as well as import and export mechanisms for building blocks and individual HMOs.

In June 2018, Jennewein signed a long-term lease for a site in Bonn and announced plans for the construction of a new R&D center. How is the development of this center progressing?

K. Parschat: The development is progressing very well, offices for our IP team and scientific communication team are already fully occupied and the R&D department will most likely move to Bonn, when the labs are fully operational, which we expect to be the case by the end of the year.

What will be the focus of the work in this new research center?

K. Parschat: At the new R&D center in Bonn most of the molecular biology used to design new and to improve bacterial strains for the manufacturing of new products will be established. The R&D department is equipped with high quality analytic devices that are implemented in the support of production strain development, but also in the analyses of metabolites from HMO degrading gut bacteria. Jennewein now also forces, besides the production of rare sugars, the investigation of beneficial effects of these products on the human body, like the prebiotic effect of HMOs on the neonatal and adult gut microbiome and the metabolites produced by these gut bacteria while metabolizing HMOs. These findings will hopefully strengthen the conviction that supplementation of diet, and especially infant formula, with HMOs is preferred over the addition of artificial fibers, when looking deeper into the metabolic outcomes of diverse supplements.

We believe that consumers will become more aware of the physiological effects of a healthy microbiome and that HMOs will definitely play an essential role in maintaining and restoring a healthy microbiome not only in infant nutrition.

Jennewein has been cooperating with the Yili Group, China's leading dairy company, since February of this year. What is this partnership about?

K. Parschat: The cooperation with the Yili Group will merge the expertise of Yili in the Chinese breast milk composition and our expertise in producing HMOs and their application. The aim is to obtain a deeper understanding of HMO related effects on the microbiome of newborns and to develop tailor made infant formula for the Chinese market.

The 2'-fucosyllactose produced by Jennewein is already approved and used as a food additive for baby food in the USA and Europe. Are there other countries where the product is used?

K. Parschat: 2'-Fucosyllactose is already approved in the Philippines, Israel, Canada, Malaysia, Thailand and Singapore. Furthermore, products containing 2'fucosyllactose are available or launched in Russia, Mexico, Hong Kong, Saudi Arabia, Colombia, Ecuador, Chile, Kuwait, Oman, Qatar, Vietnam, Cambodia and Myanmar.

What other products or projects does the company have in its pipeline?

B. Engels: Extending our monosaccharide portfolio, which now comprises of L-fucose, sialic acid and mannose. In addition, we are working on additional HMO blends, for example a mix of four sugars (LNnT, LNT, 3-FL and 2'-FL) for microbiome applications.

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