Chito Bioengineering

Metabolic and enzyme engineering for the biotechnological production of partially acetylated chitosans

Partners involved in the Chito Bioengineering project

Project coordinator

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Project leaders

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Chito-oligomers constitute an interesting class of specialty carbohydrates, used (among other applications) in plant protection, animal nutrition, bio-stimulants and wound-healing products. Today's commercially available chitosans are produced via a chemical process in which chitin is first isolated from shrimp or crab shell wastes and then further converted into chitosans. These chitosans are a mixture of molecules with a different chain length, a different degree of acetylation (i.e., the number of acetyl groups found on the chain) and a different pattern of acetylation (i.e., where these acetyl groups are positioned on the chain). Chitosans from shrimp waste can contain up to 30 different molecules. Until now, it was unknown which of the molecules in these mixtures are responsible for the bio-activity in the different applications described and whether or not the molecules work synergistically.

To resolve these issues, the partners in the Chito Bioengineering project chose a radically different approach. Instead of breaking down longer chain molecules (like chitin) into short chain molecules, they attempted to build up chito-oligomer structures starting from inexpensive and renewable resources, such as sugars and glycerol. The key technology developed in the project was the design of a bacterial catalyst that allows the conversion of simple sugars into longer chain chito-oligomers. By a combination of genetic and metabolic engineering, the University of Ghent, the Westfälische Wilhelms University of Munster and the Institut Quimic de Sarria of Barcelona have succeeded in designing a battery of bacterial strains that are able to produce well-defined and pure chito-oligomers in high concentrations. An illustration of the old and new approaches is shown in Figure 1.

The availability of larger amounts of these pure compounds would allow testing for bio-activity to be carried out on the pure compounds themselves, instead of on the mixtures, as has been done previously. To make this possible, a fermentation process was designed to further enhance yields and production rates. In addition, a purification process was developed in which chito-oligomers could be easily and inexpensively extracted from the fermentation broth. Once this process was fully optimized, it was demonstrated at larger scale in the pilot facilities of the Bio Base Europe Pilot Plant in Ghent, where up to 1 kilogram of chito-oligomers was produced. The fermenter, in which the bacteria were cultivated to produce the chito-oligomers, is shown in Figure 2.

Hendrik Waegeman, business development manager at the Bio Base Europe Pilot Plant, acknowledges: "The production of significant amounts of these new, innovative molecules does a great deal to enable further application research. To date, only miligrams of these molecules were available on the market at unreasonably high prices. Thanks to the effort of all partners in the Chito Bioengineering project, real application research using pure and single compounds has been started."

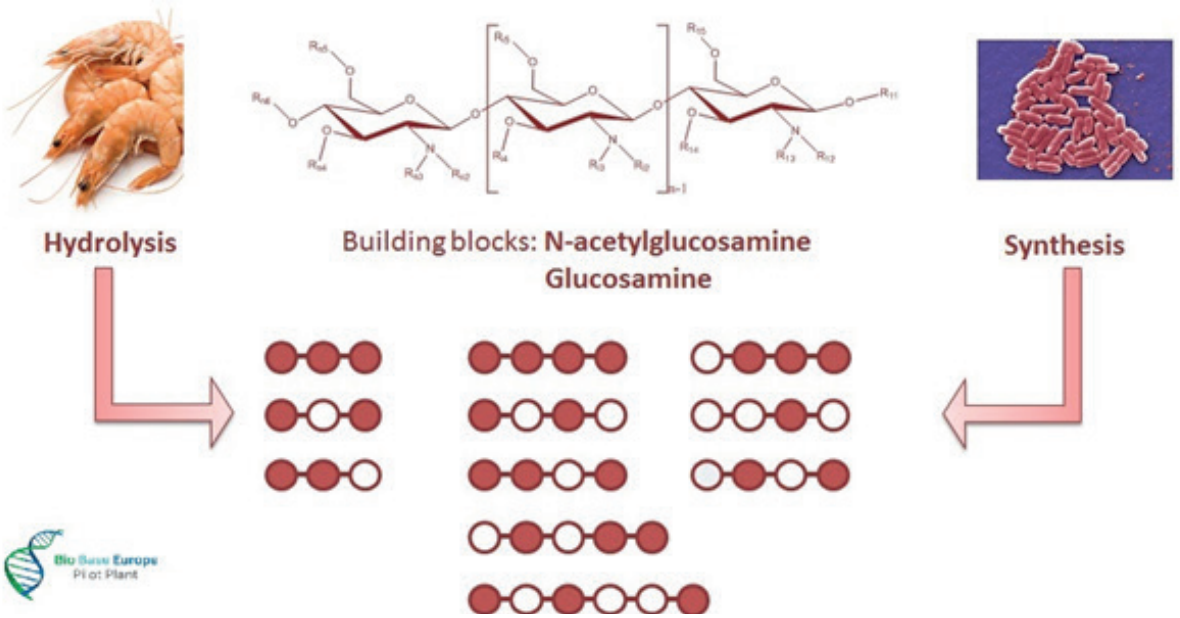

Figure 1:illustration of the two production methods: (left): the traditional chemical process starting from shrimp shell chitin, (right) the new biotechnological approach using glucose and engineered bacteria

Indeed, bearing in mind that the price for 5 mg of a well-defined chito-oligosaccharide is approximately 200 euros and that at least 50 grams are needed for a standard application test, this requires a budget of 1 million euros for just a single test! This is obviously unrealistic, particularly in view of the requirement to conduct several tests to establish reproducibility and to test different applications for different chito-oligomers. The support of ERA-IB made it possible to bring experts from different European regions together to find an alternative solution.

Thanks to the very encouraging results achieved by the project, the Chito Bioengineering partners have been able to convince other application partners to take part in the Nano3Bio project (EU Horizon 2020), in which the battery of chito-oligosaccharides will be further expanded, with the resultant molecules being tested in different fields of applications.

In addition, numerous companies have shown interest in further testing the chito-oligomers now available. Discussions have been started to determine ways in which the chito bioengineering technology can be transferred to the market. Perhaps in a few years' time your wound-healing cream will contain chito-oligomers with chito bioengineering technology? Or perhaps chicken breeders will use chito-oligomers instead of antibiotics? There is definitely more to come!

Figure 2: equipment used at the Bio Base Europe Pilot Plant to produce 1 kg of product