Industrial production of Human Milk Oligosaccharides through industrial biotechnology

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Inbiose at a glance

- Spin-off company from InBio, Centre of Industrial Biotechnology and Biocatalysis, Ghent University
- Proprietary production platform based on the biotechnological synthesis of specialty carbohydrates
- Access to production facilities (200 t/a production capacity)
- Experienced team of 30 people with industrial and academic background
- Core competence in the development and production of specialty carbohydrates through industrial (white) biotechnology
Microbes are the best carbohydrate chemists in the world

They are very productive
They are very good chemists
They are easily satisfied
They never sleep
They never complain or go on strike

The use of their synthesis potential “naturally” leads to sustainable production processes
Industrial (white) biotechnology

Why produce an HMO yourself if a bug can do it for you?

Scaleable technology

Micro-organisms can be grown in industrial fermenters for production on a very large scale.
Biotechnology Cluster in Ghent, Belgium
Carbohydrates are everywhere
Carbohydrates are everywhere

**glucose**

From the simplest form typically used as food

**Specialty carbohydrate**

To very complex structures used as an active ingredient
What are specialty carbohydrates?

- Very complex carbohydrates with unconventional structures

- Rare in nature or difficult to impossible to produce

- Price range: 10 €/kg – 1.000 €/kg (reference sugar: 0.5 €/kg)

- Quantities: 1 kg – 10.000 tpa

- High-end applications in pharmaceuticals, nutraceuticals, cosmetics, chemicals, plant protection,...
What are specialty carbohydrates?

Carbohydrates consisting of unconventional building blocks:

L-Fucose  Sialic acid  Glucosamine  Glucose  Galactose

Example: Human Milk Oligosaccharides
Disialyl-Lacto-N-Tetraose (dSLNT) as an example of a very complex specialty carbohydrate
What are specialty carbohydrates?

- Sucrose
- Glucose
- Fructose
- Lactose
- Galactose
- Xylose
- Chitin
- Deoxyribose
- FOS
- N-acetylglucosamine
- Mannose
- D-ribose
- LactoNbiose
- LactoNtetraose
- Sialic acid
- L-fucose
- L-ribose
- Lewis X
What are specialty carbohydrates?
**Specialty carbohydrate production**

**Extraction**
- Natural source required, availability problems
- High price volatility due to natural variability
- Complex extraction procedures

**Chemical synthesis**
- Very complex synthesis and costly substrates
- Low yields
- Use of toxic chemicals and high waste generation

**Enzymatic synthesis**
- Simple and cheap
- Only for simple one-step conversions
- Equilibrium can be unfavourable

**Microbial synthesis**
- Very complex carbohydrates produced in one step
- Cheap and readily available substrates
- Environmentally sustainable

Confidential
Human mother’s milk composition:
- Protein: 8 g/l
- Fat: 42 g/l
- Lactose: 70 g/l
- Human Milk Oligosaccharide: 15 g/l

Inbiose’s first target is 2’fucosyllactose as the most abundant HMO.
Metabolically engineered microbes

Fermentation

Down-stream processing

Specialty carbohydrate
Microbial synthesis of building blocks

Sucrose

Carbohydrate building blocks

Microbes at work
Microbial assembly of the building blocks

Assembly of the different monosaccharide building blocks into an oligosaccharide
Biochemical pathway assembly

Cloned genes with tunable elements such as promoters and ribosome binding sites

Assemble in an artificial chromosome
Transfer into the base strain

Sucrose

Specialty carbohydrate
Selection of best strain from the library

carbohydrate

carbohydrate

carbohydrate

carbohydrate

carbohydrate

carbohydrate

carbohydrate

carbohydrate
Integrated technology development

- Metabolic engineering of the production organism
- Development of the fermentation process
- Development of the product recovery process
Bug

- Biochemical pathway design
- Expression of suitable genes in the production host
- Strain selection for optimal producer organism

Identify the optimal biosynthesis pathway in the production host

Screen biosynthesis associated genes and proteins in DNA and protein databases

Pathway library assembly
Integrated technology development

- Development of the fermentation process
- Optimisation of yield and production rate
- Scale-up of the fermentation process
HMO strain & process improvement

Final titer (g/L)

- Strain 1: 23 mg/L
- Strain 2: 182 mg/L
- Strain 3: 2.3 g/L
- Strain 4: 5.7 g/L
- Strain 5: 16 g/L
- Strain 6: 54 g/L
Scale-up of the fermentation process
Integrated technology development

Product

- Development of down-stream processing of fermentation broth
- Product recovery and purification
- Product conditioning
Build-up of production capacity

Two new 15 m³ fermenters are operational

HMO Production capacity: 200 tonnes per year
Industrial biotechnology process hall
Industrial biotechnology process hall
Industrial biotechnology process hall
Industrial biotechnology process hall
Our technology in a nutshell

Sucrose or glucose → Cell factory → HMOs
Integrated technology development

- Synthetic Biology
- Fermentation
- Process development
- Production

Strain design → Strain development → Process optimization → Scale-up → Downstream processing → Industrial facilities

We create one strain → We develop one process → We supply one product

From Feasibility to reality

Nutraceutical Prebiotics
• Generic and scaleable technology platform for producing HMOs through industrial biotechnology
• Inbiose can produce any complex carbohydrate for which a biotechnological pathway can be designed
• Inbiose can quickly develop new processes through its base strains
• Inbiose process starts from cheap substrates and delivers high yields and productivities
• Inbiose can currently produce 200 tpa of HMOs
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