Industrial production of Human Milk Oligosaccharides through industrial biotechnology

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### Inbiose at a glance

<table>
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<tr>
<th>Core competence in the development and production of <strong>specialty carbohydrates</strong> through industrial biotechnology</th>
<th>Proprietary production platform based on <strong>fermentative synthesis</strong> of specialty carbohydrates (cell factory)</th>
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</table>
| **“Specialty carbohydrates”**  
*Started in 2013*  
*Ghent University spin-off*  
*Fast growing: >30 FTE in 2016* | |
| Access to **pilot and production facilities** | Experienced team with industrial and academic background |
| **Capacity up to 100tpa** | |
Carbohydrates are everywhere
Carbohydrates are everywhere

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?
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
Metabolically engineered microbes

Fermentation

Down-stream processing

Specialty carbohydrate
Microbial synthesis of building blocks

Sucrose

Carbohydrate building blocks
Microbial assembly of the building blocks

Assembly of the different monosaccharide building blocks into an oligosaccharide

Pathway engineering
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
Technology platform for high throughput development of specialty carbohydrate production strains

- Analytics
- Synthetic Biology & Automation
- Enzyme engineering
- Bio-informatics & Statistics
- Fermentation technology
- Bio-informatics & Statistics
Integrated technology development

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

ATGGTTGCAGTAGCCATAC
GCCTTTATAAAT
GCTATAGATCT
GATCTTCGGAG
CTGAGCTTCAA
CTATGCGGCTTA
TAATATTTATTCCAAA
TTATCCTTCTCGGAGGCTCT
AGGGGCCTTAAAATATCCCGA
GCSSDSGLYKCRRGYRSIGFG
SSESRDLFSNSEIYAAAYRRLI
GCTATAGATCGAC
FIPNSLSFSEALGALKFPNMS
GATCTTCGGAG
LKKIDPAQTESWKRLGRHYDG
CTGAGCTTCAA
VKDLAMDDLQFQDPDRKFRLS
CTATGCAGCTTAA
MAFKDMVVDVSKRNITDKTLA
AAATATCCCGA
AMLARETGKEAIAMMFGRG
TTATCCTTCTCGGAGGCTCT
TPVLVDGKDVMPGVRGVEDR

Integrated technology development
Integrated technology development

Process

- Development of the fermentation process
- Optimisation of yield and production rate
- Scale-up of the fermentation process

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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

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

Two new 15 m³ fermenters are operational

HMO Production capacity: 100 tonnes per year
Industrial biotechnology process hall
Our technology in a nutshell

Sucrose or glucose → Cell factory → HMOs

Patented
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

Value

From Feasibility to reality

Nutraceutical Prebiotics
• 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 \textbf{2’fucosyllactose} as the most abundant HMO
Human Milk Oligosaccharides

Health benefits
Human milk oligosaccharides

Prebiotics
Bifidus Growth

Antiadhesive
Antimicrobial

Modulators of Intestinal Epithelial Cell Responses

Immune modulators

Fucosyllactoses

Nutrient for neonatal brain development

Sialyllactoses

Infant milk formula

1st market

Prebiotics
 Functional foods
 Feed/Pet care

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HMOs are currently our main targets

Pubmed - human milk oligosaccharide publication count per yr

HMO Applications – Patent Families by Year
2’FL is our first HMO

Enabling
“Closest to breast milk”

Enabling Health Benefits to the infant
**Inbiose - DuPont Partnership**

**Licence and JDA**
- DuPont to commercialize 2’FL and possibly 2 other fucosylated HMO’s for Human Nutrition
- DuPont to lead up-scaling, manufacturing and sales
- Inbiose is supporting customers with samples until commercial launch by DuPont
- Parties working diligently to support

**Strategic and technology fit**

**Science**
- 50 yrs in (early life) nutrition (proteins, cultures, pre- & probiotics, hydrocolloids, enzymes)
- Leader in microbiome science

**Production**
- Strong position in large scale fermentation, carbohydrate production and DSP

**Market reach**
- Global market access and reach with local support
- Can supply customers with scale, supply security and good safety track record

**Inbiose**

**new products**

**Du Pont**

**The miracles of science**
In a nutshell

- 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 100 tpa of HMOs
Turning specialty carbohydrates into an industrial reality