NIZO: HOTSPOT FOR MICROBIOME RESEARCH

Helping you create microbiome modulators

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• Independent, private **contract research** company for food and health
  • Proteins
  • Bacteria
  • Processing

• **IP** within defined scope is for our clients

• **130 professionals**

• From lab to **practice**
  • Food-grade pilot plant
NIZO: 70 YEARS OF EXPERTISE IN MICROBIOME MODULATION

**1950**
- **Microbial Communities**: Gut & Skin & Oral
- Wine & Beer & Bread
- Cheese & Yoghurt

**2000 onwards**
- **(meta)genomics**
- Host-microbe interactomics
- Michiel Kleerebezem, Roland Siezen

**90s** molecular microbiology LAB
- Willem de Vos, Oscar Kuipers, Jeroen Hugenholtz, Eddy Smid

**NIZO Technology**
- In-vivo community analysis
- In situ screening and genomics
- In situ screening and genomics

**2020**
- Microbial Communities
  - Gut & Skin & Oral
  - Wine & Beer & Bread
  - Cheese & Yoghurt

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**60s**
- Stadhouders et al.
- Industrial upscaling
- Yoghurt production

**60s**
- Appearance of New N-Terminal Groups by the Action of Rennin on Casein

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**INNOVATING TOGETHER**

**FOR BETTER FOOD & HEALTH**
MICROBIOME MODULATORS: CHALLENGES

- Connect: find the right target for intervention
- Predict: predict biological function
- Validate: validate biological function
- Scale-up: produce your modulator (eg. Live Biotherapeutic Product)
CONNECT: MAPPING THE GUT AND SKIN

>200000 organisms
Staphylococcus
Escherichia coli
Bifidobacterium animalis supsp. Animalis
Enterococcus faecium TX1330
Homo sapiens

>12000 dermatological diseases
periarteritis nodosa
Multiple endocrine adenomatosis
Hypertrichosis
Chediak-Higashi syndrome
Atopic Dermatitis

~ 40.000 natural compounds
~ 2500 biological pathways
~ 30.000 human genes

A database of 120 million literature connections
PREDICT: BIOLOGICAL FUNCTIONS USING SAMPLE DATA

Simplified version: Biology is not linear!

\[ LCFA_{\text{breakdown}} = \sum \text{genes} + \sum \text{organisms} + \sum \text{metabolites} \]
NIZO correctly predicted active modulators for **vaginal dryness**

**histidine ammonia-lyase**

**urocanate hydratase**

**imidazolonepropionase**
VALIDATE: BIOLOGICAL FUNCTION
VALIDATE: HIGH THROUGHPUT MICROCOLON

Fecal content as inoculum for fermentations

Add different components (e.g. oligosaccharides, probiotics, pathogens, incl. relevant controls)

Sample collection: In time (t=0, t=6h, t=24h)

Monitor shifts and function in complex communities
Profiling: 16S Illumina sequencing
qPCR (bacterial or gene targets)
Metabolomics (SCFA-HPLC, NMR, LC-GCMS), Gas production

High-throughput in vitro technology for screening of novel ingredients and probiotics for their efficacy in the GIT-environment
NIZO’s high throughput Microcolon System is currently applied in a project with academic partners to study the effects of different components on *C. difficile* growth and -toxin production.
VALIDATE: IN VIVO (HUMAN)
Iron supplementation in Kenyan infants led to gut inflammation and we linked this to dysbiosis in the microbiome.

Prebiotic compounds, including a GOS, were tested for the stimulation of beneficial microbes herewith enabling the shift back to a balanced microbiome.

The subsequent clinical trial showed improved clinical outcomes on diarrhea after additional supplementation with a GOS as well as in vivo proven shifts back to a healthy state microbiome.

NIZO contributed by linking clinical sample data to earlier in vitro and in silico approaches and hereby translating such data into biological perspective.
SCALE-UP: PRODUCTION OF MICROBIOME MODULATORS
SCALE-UP: PRODUCTION FOR YOUR TRIAL

2/40 liter Inoculum

0.6/4.5 M³ bioreactors (Strict) Anaerobic

Anaerobic concentration by:
• Centrifugation
• Cross-flow filtration

Formulation:
• Liquid
  • Food products
  • Frozen sachets, tubes, vials
• Powder
  • Freeze-drying (BSL-1)
  • Spray Drying (BSL-1)

Probiotic application
# Finding the right target
- (Meta)genomics
- phylotyping
- co-, and anti-occurrence analyses

# In silico prediction
- De novo discovery
- Discover using 16S/shotgun data

# In vitro/ex vivo
- MicroColon
- MicroSkin
- Mock communities

# In vivo (human)
- Clinical POC trial
- Challenge models
- ETEC, rhinovirus, tape stripping

# Upscaled production
- Fermentation (0.5L -> 4,500L)
- DSP
- Diverse formulations

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**NIZO: HELPING YOU CREATE MICROBIOME MODULATORS**

**Connect**

**Predict**

**Validate**

**Validate**

**Scale-up**
THANKS TO

Discovery

QA, Data and chemical analytics

Production

Clinical Validation

Business support