INTEGRATED APPROACH FOR SCREENING AND
SUBSTANTIATION OF INGREDIENTS WITH A GUT HEALTH
BENEFIT

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Nutrition & Health

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FROM SCIENCE TO SOLUTIONS

- Independent, private contract research organization for infant, food, OTC, cosmetics and pharma industries
- IP is always for our clients
- We work confidentially
OUR FIELDS OF EXPERTISE

Bacteria
- Nutrition & Health
- Fermentation
- Microbiomics
- Efficient Upscaling

Proteins
- Dairy Technology
- Protein Functionality
- Flavour & Texture Interactions
- Processing Efficiency
FROM INGREDIENT TO HEALTH BENEFIT

Ingredient/strain selection

Screening for potential mechanism of action

Demonstrating health benefit in animals/humans
STRAIN SELECTION AND PRODUCTION

- Culture collection (>4000 strains)
- Robotic liquid handling system
- Many automated assays (enzymes, vitamins, volatiles, peptides, CFU.....)
- Data handling and analysis
- Microbial physiology – strains and medium optimization
- Up-scaling (up to 4500 liters)
FERMENTATION

Miniaturized
- 94-384 wells
- microL

Lab-scale
- 0.5L Multifors
- 0.1 - 5L

Bench Top / Pilot
- 20L Multifors
- 200 - 400L

Pilot / semi-industrial
- 4500L aerobic
- 4500L anaerobic
FROM INGREDIENT TO HEALTH BENEFIT

- Ingredient/strain selection
- Screening for potential mechanism of action
- Demonstrating health benefit in animals/humans
• Human Challenge Models (HCM) as Proof of Concept (PoC) to enhance efficiency of health research pipelines
• Current model: discovery $\rightarrow$ in vitro $\rightarrow$ animal models $\rightarrow$ field trials
• Gap between in-vitro/animal models and field studies creates high attrition rate
• Need for early human data and predictive models
HCM are based on “health is the ability to adapt”
Challenge = applying a stressor to healthy people and measure resilience to this stress
Examples: pathogen, exercise, or high calorie meal challenges
HCM can either replace field trials or form a sound base to design field trials and hence save time and money
WHY CHALLENGE MODELS?

- **Healthy subjects** - Health claims on foods are aimed at maintenance and improvement of health
- **Effect size** - Stress resilience is a more sensitive marker of health than steady state markers
- **High responders** - Response to challenge to identify susceptible individuals within a healthy population, for whom intervention may be most relevant
- **Limited number of study subjects**
- **Limited study duration**
- **Controlled stress exposure**

**Proof of Concept**

Challenge Models for infection resistance

<table>
<thead>
<tr>
<th>Immune function</th>
<th>Respiratory infection</th>
<th>Gut infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccination</td>
<td>Viral infection resistance</td>
<td>Bacterial infection resistance</td>
</tr>
<tr>
<td>Cholera / Hepatitis B</td>
<td>Rhinovirus</td>
<td>ETEC</td>
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Parallel, double-blind, placebo-controlled 4-weeks intervention

Habitual diet, but abstain from foods high in calcium

Healthy men/women 18-55 years, randomly assigned to experimental groups

Oral infection with E. coli vaccine (strain E1392-75-2A; 10^{10} CFU)

Stool consistency, bowel habits, frequency and severity of gastrointestinal complaints, collection of fecal and blood samples

Study outcomes
1. Infection-induced diarrhea (GSRS, BSS)
2. Fecal wet weight/% dry weight
3. Fecal ETEC excretion
4. Markers of inflammation/immunity/gut barrier
STUDY DESIGN E. COLI CHALLENGE

PERIOD I

Activities

1-9
10
11
12
13
14
15
16
17
18
19
20
21
22-27
28

- Informed consent & Screening
- Restricted intake specific medicine
- Restricted alcohol intake
- Standardized evening meal
- Overnight fast
- Infection attenuated E. coli
- Data Safety Monitoring Board
- Dietary intake (online app)
- Collection 24h fecal samples
- Collection spot fecal sample
- Collection blood sample
- Bristol stool scale (online)
- Stool frequency (online)
- Gastro-Intestinal Symptom Scale (online)
- The Gastrointestinal Quality of Life Index (online)
- Restricted diary intake
- Registration medication intake (online)
- Soy product consumption (dairy replacement)
OUTCOMES

**Primary outcomes:**
Percentage of fecal dry/wet weight

**Secondary outcomes:**
- Fecal wet weight
- Fecal ETEC excretion
- Bristol Stool Scale
- Stool frequency
- WHO-defined diarrhea
- Time to first diarrheal stool
- Gastro Intestinal Symptom Rating Scale
- Quality of life
- Adverse events

**Tertiary outcomes:**
- IgG/IgM CFAII
- C-reactive protein
- Calprotectin
- B-defensins
- Myeloperoxidase
- slgA
- Ex-vivo PBMC stimulation
- Host transcriptomics
- Cytokine profiling
Ten Bruggencate, publ. In preparation
UPPER RESPIRATORY TRACT VIRAL INFECTION:  
CHALLENGE WITH RHINOVIRUS HRV-16

- Parallel, double-blind, placebo-controlled  
  4-weeks intervention

- Healthy men/women 18-55 years, randomly assigned to experimental groups

- Challenge with HRV-16

- Study outcomes
  1. Common cold symptoms (WURSS, Jackson)
  2. RV16 antibody titer (blood sample)
  3. Viral load (PCR on nasal fluid)
  4. Cytokine profiles
  5. (Sputum of bronchoalveolar lavage: local inflammatory condition)
Effects of serostatus and gender on the HRV-16-induced local immune response

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INNOVATING TOGETHER
Parallel, double blind, placebo-controlled trial
4- to 8-weeks intervention

Healthy men/women 18-55 years, randomly assigned to experimental groups

Subjects receive a vaccination (e.g. oral cholera, Hepatitis B)

Blood, saliva and fecal samples are collected before and after vaccination

Study outcomes:
1. Specific IgG and IgA in serum
2. Specific IgA in saliva, feces
3. Inflammatory markers (e.g. cytokines, calprotectin)
4. Microbiota

SUPPORT OF IMMUNE FUNCTION:
VACCINATION CHALLENGE
FROM INGREDIENT TO HEALTH BENEFIT

Ingredient/strain selection → Screening for potential mechanism of action → Demonstrating health benefit in animals/humans
TOWARDS PREDICTIVE INTEGRATED HEALTH PIPELINES

companies competing with ingredients, not with pipelines

Proof of Concept Studies
Trusted predictive Proof of Concept studies to bridge the gap between pre clinical trials and human field trials

Integrated Databases
Structured primary data and relevant metadata to enable correlation mining and efficacy prediction

Innovations

Integrated Health Pipeline

Standardised Assays
Standardised assays to ensure quality control and comparability of pre clinical work

PEARL
Pipeline for Efficacy evaluation of Active Ingredients for Resistance and allergy
Aim of in vitro assays:

1. Help reduce large number of potential samples to few most promising ones that have highest chance of positive effects in clinical trials

2. Obtain mechanistic insights
   • To increase chance of success in clinical trials
   • To establish cause-effect relationship

3. Use minimal time, money and effort
   • Aim to define **minimal selective set** of assays / models within pipeline
FROM IN-VITRO TO HUMANS

Stage 1
Screening of food ingredients in vitro (most potent ingredient, working mechanism)

Sprong et al, Br J Nutr 2012

Stage 2
Resistance to infection in vivo

Sprong et al, Int Dairy J 1999

Stage 3
Resistance to infection in humans

Stage 4
Support in vivo results; mechanism of action

Ten Bruggencate et al, 2016
Funnel for initial selection (+ characterization)

Pathogens-specific
- Co-aggregation
- Receptor binding
- ETEC adhesion
- ETEC killing

Microbiota modulation diversity
niche occupation

TEER
- In ETEC challenge mode =

Epithelial signaling
Cytokines, anti-microbials

Phagocyte function
Phagocytosis

PBMC effectors
Pro/anti inflam, pro
Th subsets, Th2

DC assays
Pro/anti inflam, pro
Th subsets, Th2

Animal model
ETEC infection in Rats (wild type)

ETEC challenge in healthy subjects

Symptoms:
Diarrhea, Nausea, Vomiting, Abdominal pain and cramps, Flatulence, Bloating, Fever, Headache

Markers:
Faecal ETEC, fecal output, relative fecal dry weight, IgG CFAII, sIgA, Calprotectin, CRP, microbiota (?)
25 Ingredients:
• Significant positive or negative effect on clinically relevant outcome
• Covering different mechanisms
• Representing different ingredient categories:
  ➢ Probiotic, Milk ingredients, Herbal, Mineral, Polysaccharide, Fatty acid

Tested in 6 assays:
• Pathogen co-aggregation
• Pathogen decoy
• Barrier function TEER assay
• PBMC immunomodulation
• DC immunomodulation
• Epithelial signalling

➢ Data used to start building a database
SUMMARY

• Aligning in vitro assays to in vivo proof of concept – standardization and sharing data will benefit all
• Human challenge models – cost-effective methods to demonstrate benefits of foods in healthy subjects
• Controlled infections in volunteers, to demonstrate enhancement of infection resistance
• Clinical outcomes and biomarkers

• The challenge model concept is also used to evaluate benefits in other health domains (e.g. metabolic health, physical performance)
THANK YOU FOR YOUR ATTENTION

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