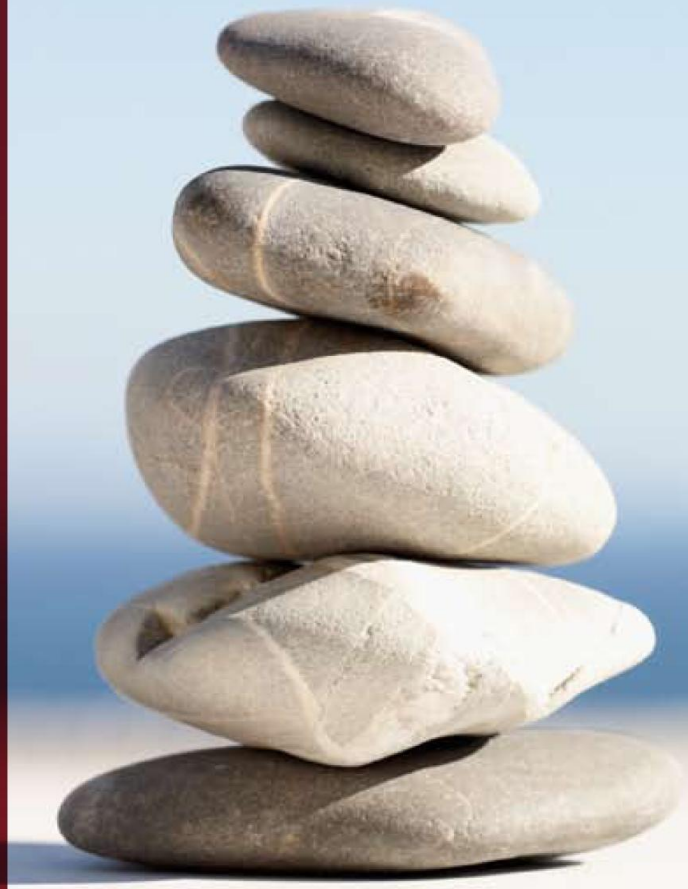
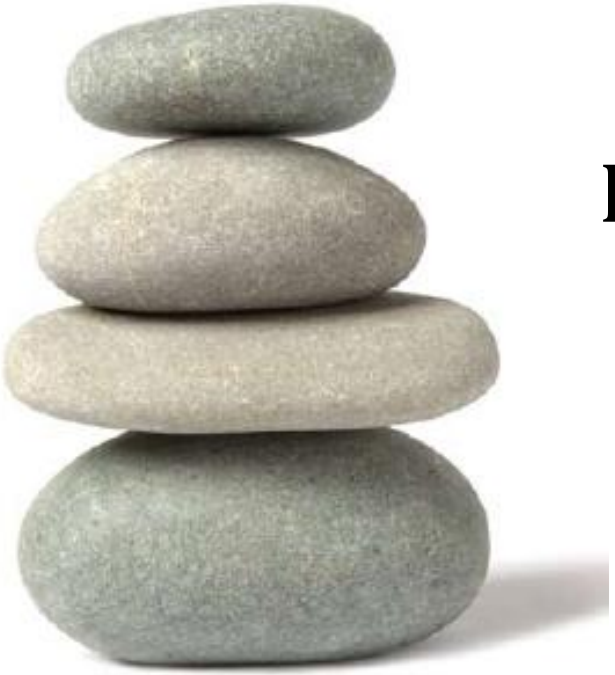


Building up your knowledge

ProDigest
Gastrointestinal Expertise

ProDigest
Gastrointestinal Expertise

Stone after stone we assist your product development



From human gastrointestinal expertise towards animal gastrointestinal expertise

ProDigest

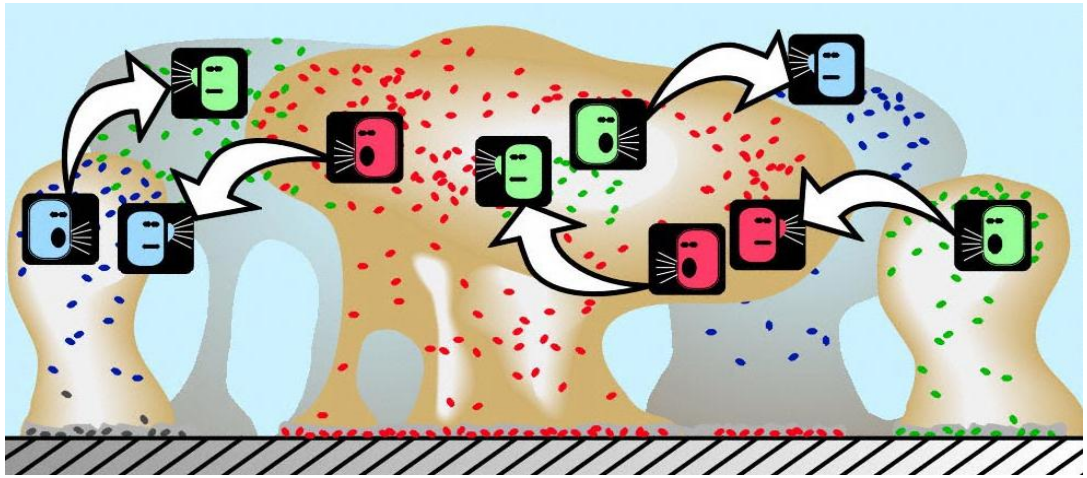
- Spin-off from Ghent University, Belgium
- LabMET: Laboratory of Microbial Ecology & Technology

- Founded by Dr. ir. Sam Possemiers, Dr. ir. Massimo Marzorati and Prof.em.Dr.ir. Willy Verstraete in 2008

- 7 FTE’s and expanding

Some history

- Laboratory of Microbial Ecology and Technology (LabMET), Ghent University
  - Head: Prof. Willy Verstraete (now emeritus)
  - Study mixed **microbial communities & interactions** to better understand ecological **processes** and use and/or **steer** the microbial community in **biotechnological** applications



Some history

- LabMET: Laboratory of Microbial Ecology & Technology
  - Study of complex microbial cultures
  - Technology development (e.g. reactor design)
  - Expertise:
  - Environmental microbiology (W. Verstraete: Top expert)
  - More recent: Gastrointestinal microbiology

Some history

- Gastrointestinal microbiology
  - Study of complex microbial cultures
  - Technology development (e.g. reactor design)

Appl Microbiol Biotechnol (1993) 39:254–258

Applied and Microbiology Biotechnology

Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem

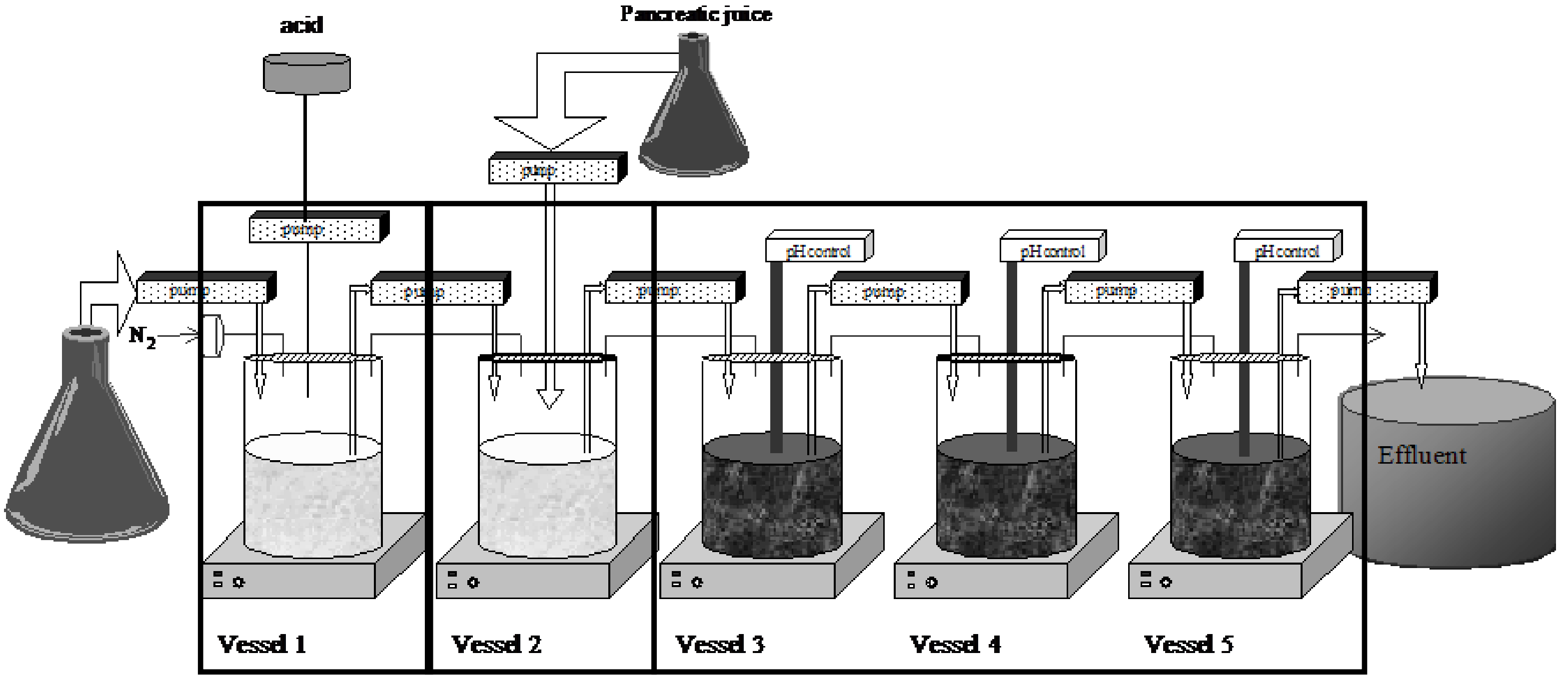
K. Molly, M. Vande Woestyne, W. Verstraete

Laboratory of Microbial Ecology, Faculty of Agricultural and Applied Biological Sciences, University of Gent, Coupure Links, 653, 9000 Gent, Belgium

Received: 3 August 1992/Accepted: 16 November 1992

Some history

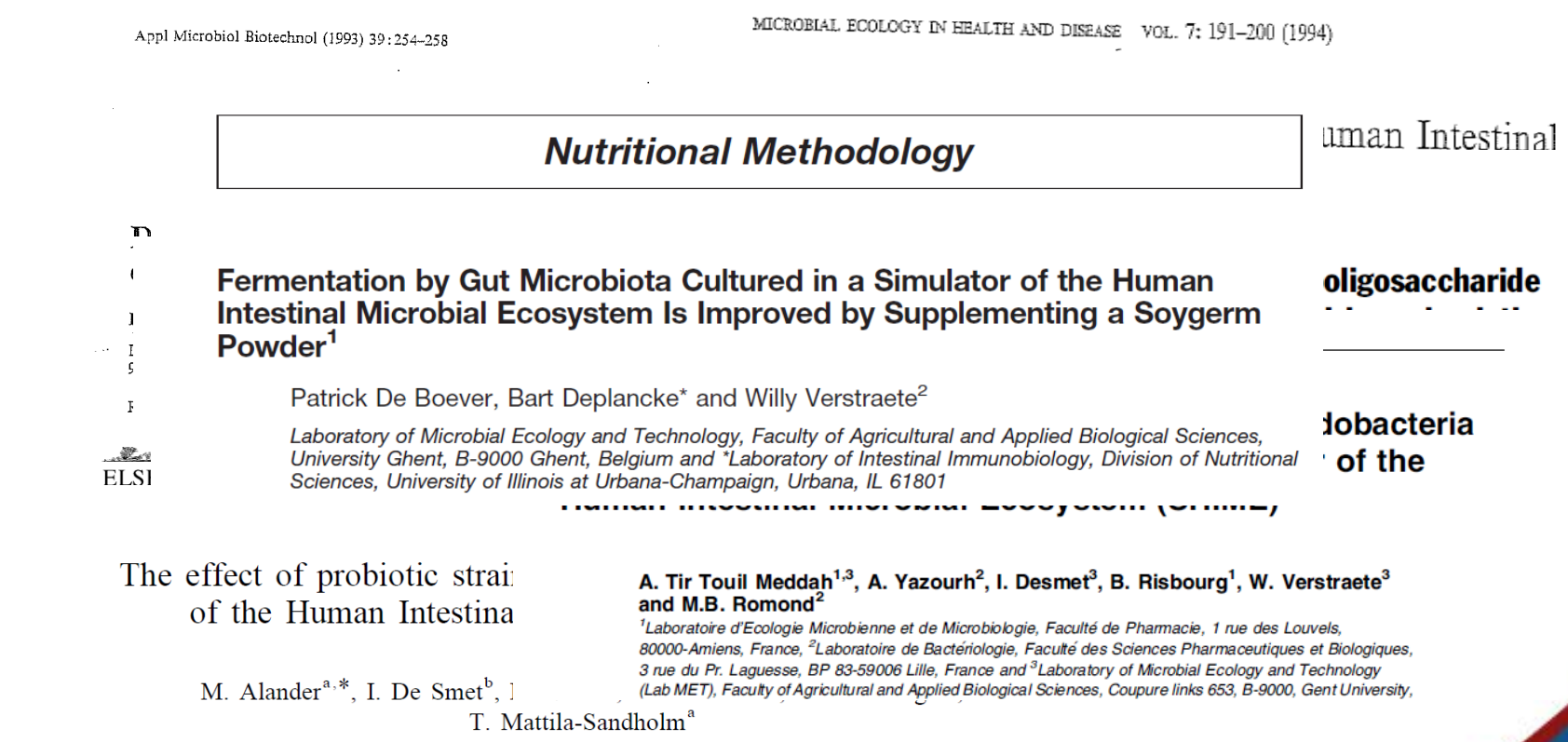
- 1993: **S**imulator of the **H**uman **I**ntestinal **M**icrobial **E**cosystem (SHIME)



•Stomach •Small intestine •Ascending, transverse and descending colon

Some history

- 1993-2003: +/- 10 SHIME publications



Appl Microbiol Biotechnol (1993) 39:254–258

MICROBIAL ECOLOGY IN HEALTH AND DISEASE VOL. 7: 191–200 (1994)

Nutritional Methodology

uman Intestinal

Fermentation by Gut Microbiota Cultured in a Simulator of the Human Intestinal Microbial Ecosystem Is Improved by Supplementing a Soygerm Powder[1]

oligosaccharide

Patrick De Boever, Bart Deplancke* and Willy Verstraete[2]

*Laboratory of Microbial Ecology and Technology, Faculty of Agricultural and Applied Biological Sciences, University Ghent, B-9000 Ghent, Belgium and *Laboratory of Intestinal Immunobiology, Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801*

dobacteria

of the

The effect of probiotic strai

of the Human Intestina

A. Tir Touil Meddah[1,3], A. Yazourh[2], I. Desmet[3], B. Risbourg[1], W. Verstraete[3] and M.B. Romond[2]

[1]Laboratoire d'Ecologie Microbienne et de Microbiologie, Faculté de Pharmacie, 1 rue des Louvels, 80000-Amiens, France, [2]Laboratoire de Bactériologie, Faculté des Sciences Pharmaceutiques et Biologiques, 3 rue du Pr. Laguesse, BP 83-59006 Lille, France and [3]Laboratory of Microbial Ecology and Technology (Lab MET), Faculty of Agricultural and Applied Biological Sciences, Coupure links 653, B-9000, Gent University,

M. Alander[a,*], I. De Smet[b], [illegible]

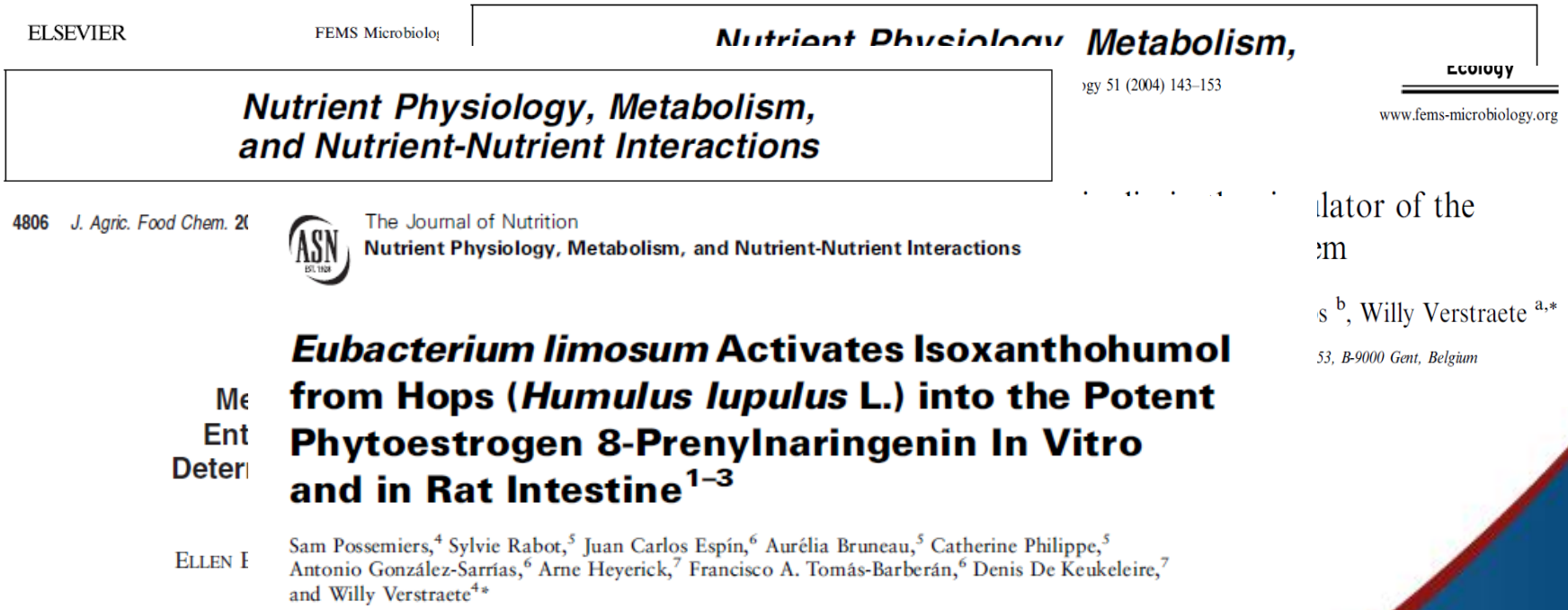
T. Mattila-Sandholm[a]

[a]VTT Biotechnology and Food Research, P.O. Box 1501, FIN-02044 VTT, Finland

[b]Laboratory of Microbial Ecology, Faculty of Agricultural and Applied Biological Sciences, University of Gent, Coupure Links 653, B-9000 Gent, Belgium

Some history

- 2003-2009: +/- 30 SHIME publications
- Master thesis + PhD Sam Possemiers: 2002-2007



ELSEVIER

FEMS Microbiolo

Nutrient Physiology, Metabolism,

ogy 51 (2004) 143–153

Ecology

www.fems-microbiology.org

Nutrient Physiology, Metabolism,
and Nutrient-Nutrient Interactions

4806 J. Agric. Food Chem. 2(

The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

lator of the
em

s [b], Willy Verstraete [a,*]

53, B-9000 Gent, Belgium

Me
Ent
Deter

Eubacterium limosum Activates Isoxanthohumol from Hops (_Humulus lupulus_ L.) into the Potent Phytoestrogen 8-Prenylnaringenin In Vitro and in Rat Intestine[1–3]

ELLEN E

Sam Possemiers,[4] Sylvie Rabot,[5] Juan Carlos Espín,[6] Aurélia Bruneau,[5] Catherine Philippe,[5] Antonio González-Sarrías,[6] Arne Heyerick,[7] Francisco A. Tomás-Barberán,[6] Denis De Keukeleire,[7] and Willy Verstraete[4]*

ProDigest
Gastrointestinal Expertise

ProDigest: a work in progress…

Our approach…

- How it started:
  - May 2007: PhD defense Sam and start postdoctoral position
  - Next step: academic or industry?
  - Solution: Let's try both…
  - Translate academic knowledge into industrial application
  - Willy Verstraete, Massimo Marzorati and Sam Possemiers

  - Concept: **SHIME as technology platform for food and pharma**

Our approach…

- Step by step approach
  - Step 1: spin-in (Q3 2007-Q3 2008)
    - Separate unit within LabMET
    - Tryout phase: active search for customers
    - Develop (initial) operational structure

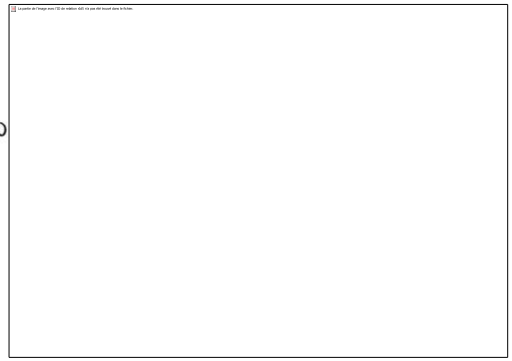
  - Step 2: preparation for launch (Q2 2008)
    - Negotiations with UGent and LabMET
    - Develop business and financial plan

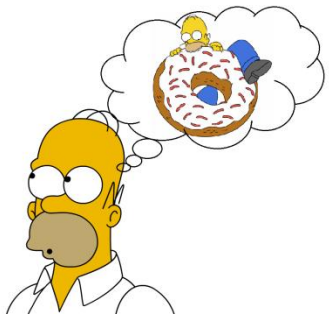
  - Step 3: Embedded spin-off
    - Launch on September 24th, 2008
    - Service company approach

  - Step 4: Growth phase…
    - Move to new facility on June 1st, 2012
  - Step 5: Development of sustainable company
    - Manage growth and development
    - Set out long-term perspective

Our approach…

- Step 1 & 2: Incubation stage
  - Transform scattered knowledge into technology platform
  - University mentality >< company reality
  - Development of initial customer portfolio
  - Agreement with UGent/LabMET:
    - Exclusive license on SHIME know-how
    - Agreement on infrastructure, equipment, bench fees, consumables
    - Right to transfer UGent contracts to ProDigest at startup
  - Development of business and financial plan





Our approach…

A baby was born…

- September 24, 2008 +/- 17h30
- Willy, Massimo and Sam founded ProDigest
- Capital input financed by founders
- Transfer of 3 contracts to ProDigest allowed short-term survival

Our approach…

- Stage 3: Growth phase
  - Initial business model: contract research for food and pharma companies
  - Strategy and aims:
    - Develop ProDigest/SHIME ‘quality label’ > awareness
    - Build customer portfolio
    - Generate positive cash flow position through consistent service work > become profitable
    - Use contract service model to allow gradual development of the company
  - Major achievements:
    - Profitable from day 1
    - Customer portfolio contains most major food industries
    - High quality staff
    - SHIME has become a well-known and respected gut model
    - 2012: Move to ProDigest labs, separate infrastructure

Our approach…

- How to get to stage 4?
  - Service model = “straightforward” entrepreneurship
    - Clear business model
    - Money spent based on money inflow
  - However:
    - Short-term vision
    - Insufficient long-term perspectives
    - No long-term value creation
  - Need for long-term strategy < Change in business model

ProDigest business model

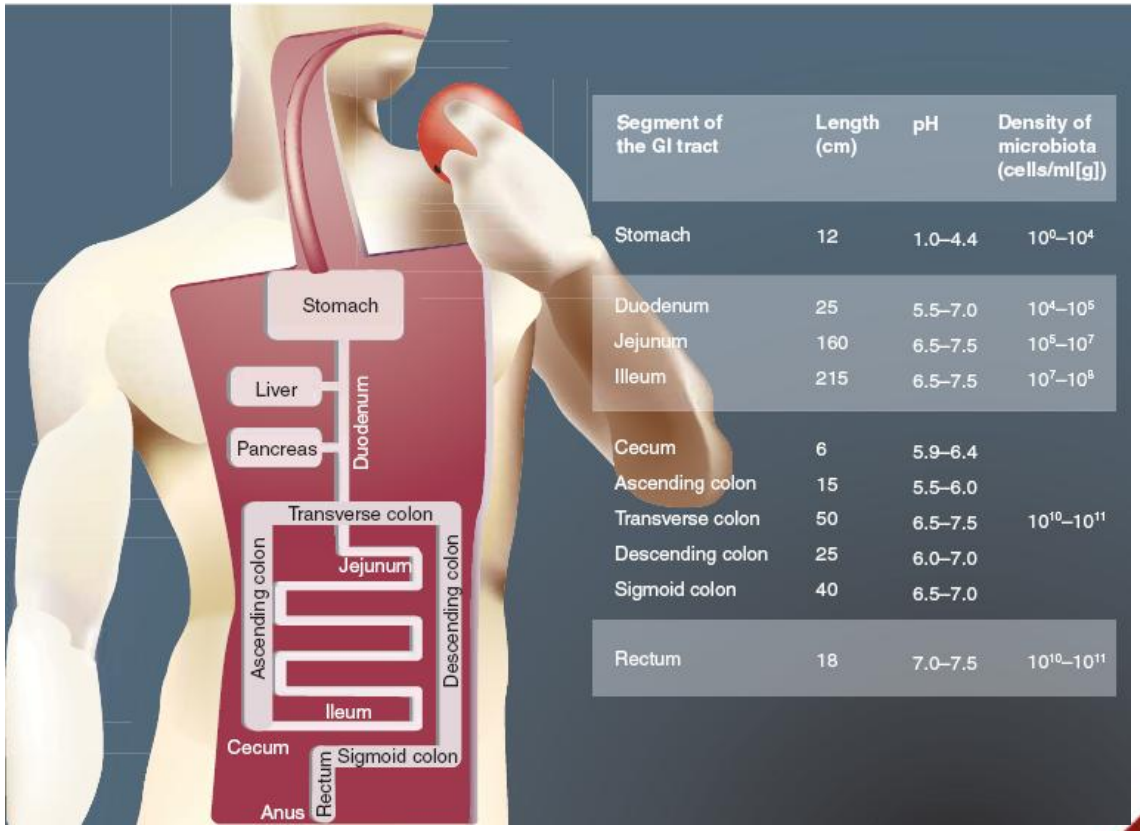
- Initial short-term business model
  - Service model
    - Generate positive cash flow to support initial growth and development
    - Specific expertise is only used for benefit of customers
    - No long-term value creation
- Medium-term business model
    - Use *excess* money inflow to develop the long-term missions of ProDigest
    - Start using specific expertise for in-house research projects
    - Create value for ProDigest!
- Long-term business model
  - Hybrid business model
    - Service arm: act as center of expertise for gastrointestinal studies
    - Project arm: Develop innovative technologies and product concepts

ProDigest
Gastrointestinal Expertise

ProDigest: Expertise

Expertise

- Study and modeling of gastrointestinal processes
- Physicochemical and enzymatic (upper intestine)
- Microbial (lower intestine)
- Host-bacteria interactions
- Relation with host health

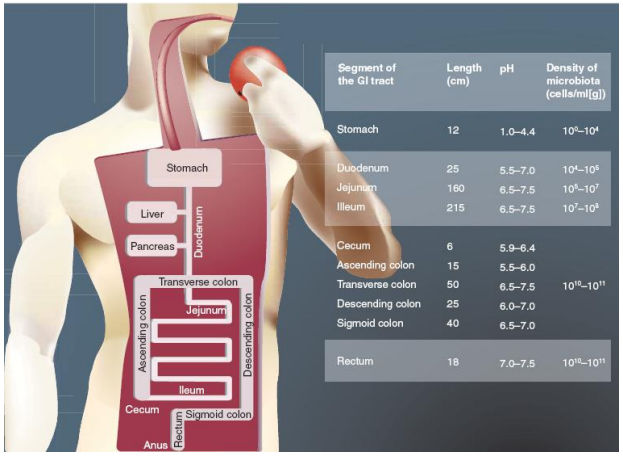


Segment of the GI tract	Length (cm)	pH	Density of microbiota (cells/ml[g])
Stomach	12	1.0–4.4	10^0–10^4
Duodenum	25	5.5–7.0	10^4–10^5
Jejunum	160	6.5–7.5	10^5–10^7
Illeum	215	6.5–7.5	10^7–10^9
Cecum	6	5.9–6.4	
Ascending colon	15	5.5–6.0	
Transverse colon	50	6.5–7.5	10^{10}–10^{11}
Descending colon	25	6.0–7.0	
Sigmoid colon	40	6.5–7.0	
Rectum	18	7.0–7.5	10^{10}–10^{11}

Step-by-step approach: from *in vitro* to clinical studies

Expertise

- Original target market: Human applications
- Recent developments:
- Expansion of expertise towards:
  - Production animals (e.g. pigs)
  - Companion animals (e.g. cats and dogs)



Segment of the GI tract	Length (cm)	pH	Density of microbiota (cells/ml[g])
Stomach	12	1.0–4.4	10^0–10^4
Duodenum	25	5.5–7.0	10^4–10^5
Jejunum	160	6.5–7.5	10^5–10^7
Ileum	215	6.5–7.5	10^7–10^8
Cecum	6	5.9–6.4	
Ascending colon	15	5.5–6.0	
Transverse colon	50	6.5–7.5	10^{10}–10^{11}
Descending colon	25	6.0–7.0	
Sigmoid colon	40	6.5–7.0	
Rectum	18	7.0–7.5	10^{10}–10^{11}

ProDigest
Gastrointestinal Expertise

Platform technology

Fate of ingested compounds

ProDigest
Gastrointestinal Expertise

- First site of contact = gastrointestinal tract!
- Oral intake of active compound or precursor:
  - Host metabolism
    - Stomach: low pH, protein degradation
    - Small intestine: bile salts, digestive enzymes
  - Microbial metabolism
    - Ileum and colon fermentation
      - Degradation
      - Active compound generation

Research methods

- Human intervention studies
- *In vivo* animal models
- *In vitro* simulation technologies
  - Advantages:
    - Easier setup and sampling
    - High reproducibility
    - Mechanistic studies possible
    - Representative to a specific process
    - No ethical constraints
    - Medium to high throughput
  - Disadvantages:
    - Absence of physiological environment
    - Clinical studies are necessary for confirmation

ProDigest
Gastrointestinal Expertise

CORE EXPERTISE:
VALIDATED GUT MODELS

Human and animal applications

Proprietary technologies

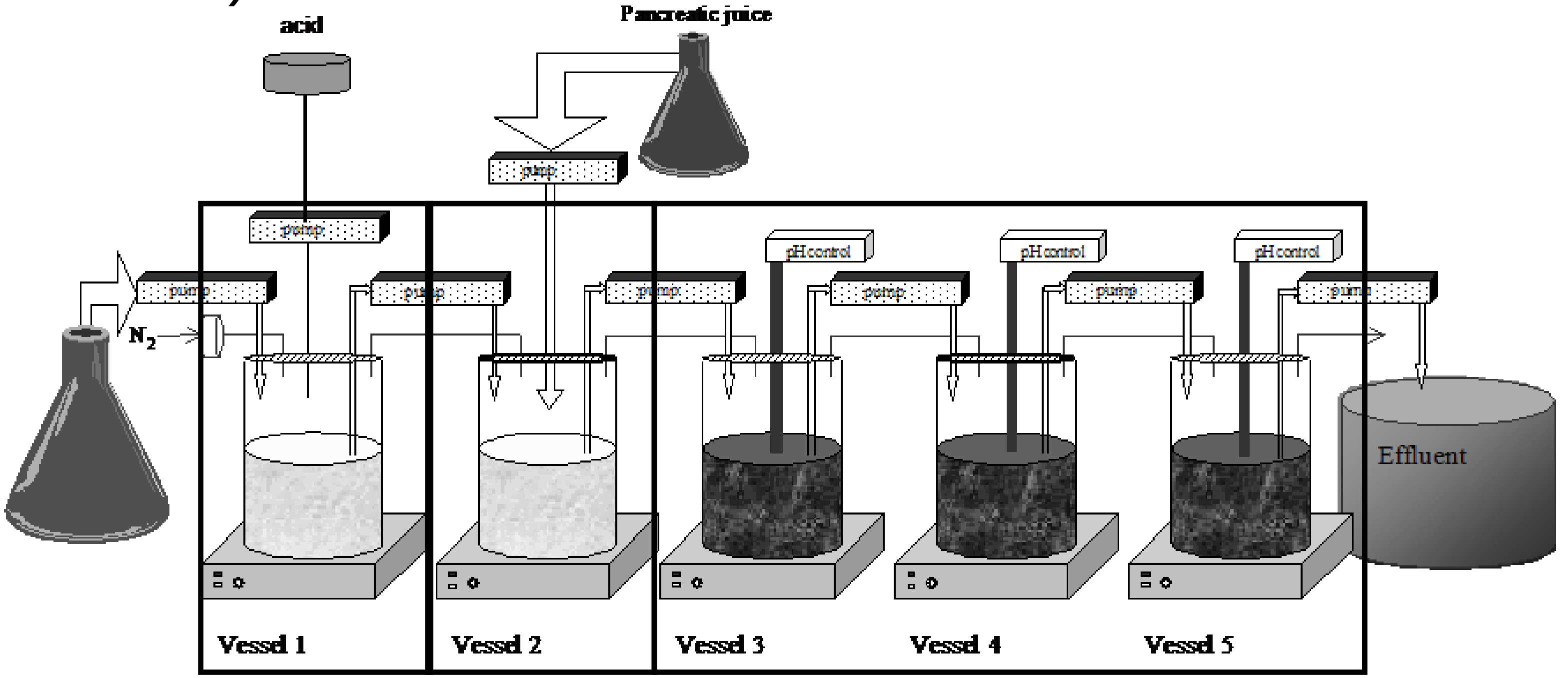
ProDigest
Gastrointestinal Expertise

Example:

Simulator of the Human Intestinal Microbial Ecosystem (SHIME®)

SHIME®

Simulator of the **H**uman **I**ntestinal **M**icrobial **E**cosystem (SHIME®)



•Stomach •Small intestine •Ascending, transverse and descending colon

SHIME®

ProDigest
Gastrointestinal Expertise

- Operational parameters:
- To operate the models, the following parameters are adjusted according to the tested human (infant, adult, elderly) or animal:
  1. Food/Feed (administered to model 3x/day)
  2. Body temperature
  3. Secretion of digestive enzymes
  4. Residence times in the different intestinal regions
  5. Intestinal pH profiles
  6. Microbial inoculum from fecal sample

- Easy sampling
- Reproducible
- Representative

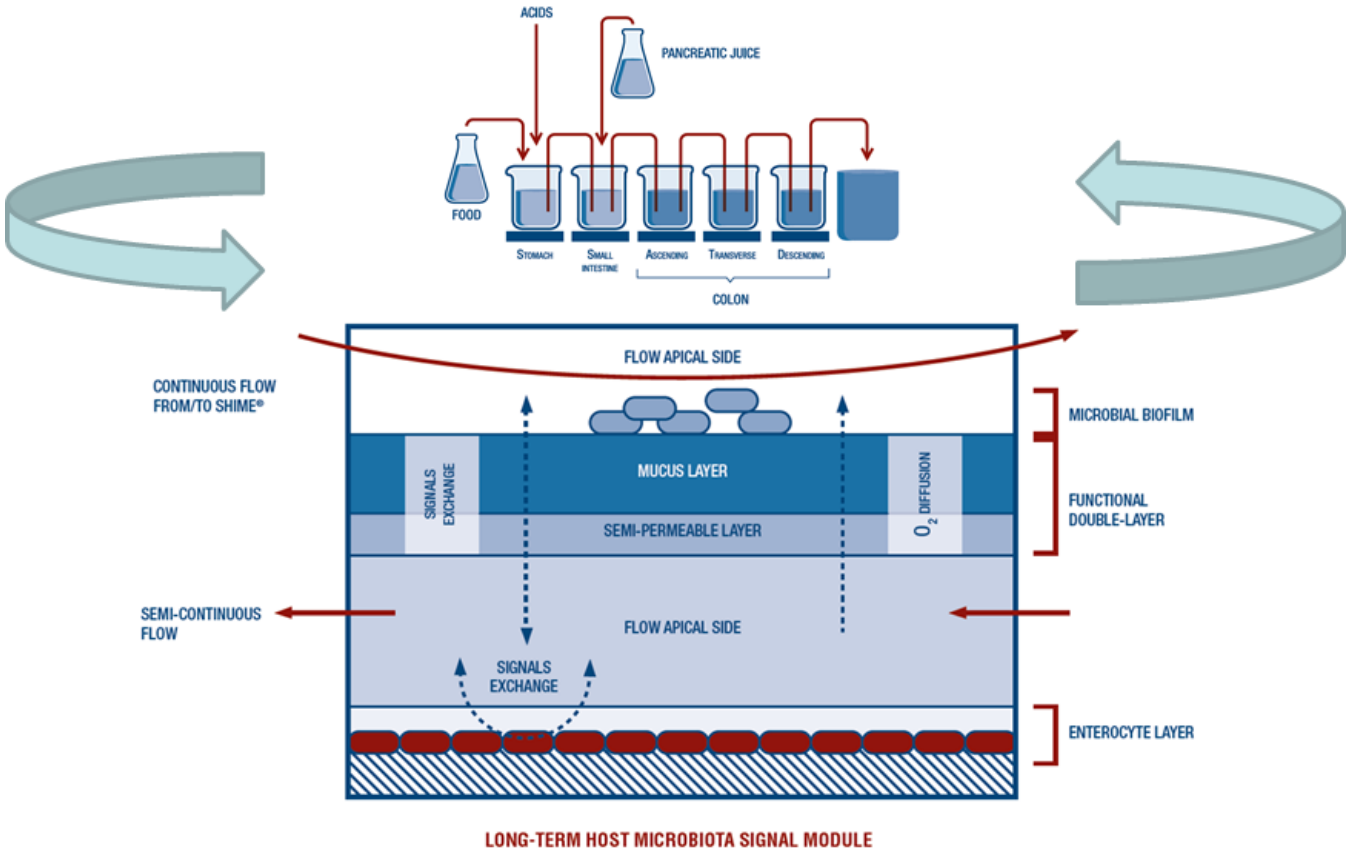
TWINSHIME® setup

ProDigest
Gastrointestinal Expertise

Parallel studies in single setup

Latest development: HMI™ module: Health effects from intestinal processes

ProDigest
Gastrointestinal Expertise



HMI: HOST-MICROBE INTERACTIONS:

- Microbial metabolites affect host cells
- Host-response affects composition (mucosal) microbiota

Patent pending ProDigest WO2010118857

ProDigest
Gastrointestinal Expertise

Activities

Overall approach

Microbial community screening, interindividual variability, stability of actives in the upper intestine, metabolism, bacteria isolation
Simulator of the Human Intestinal Microbial Ecosystem (SHIME)
Analysis of microbial community and activity, metabolite identification, formulation development
Host – Bacteria interactions
Animal validation studies
Human trials for proof-of-concept

Product testing, selection and evaluation

- Small-scale *in vitro* experiments (metabolism, effect on the microbial community, stability in upper and lower intestine)
- Absorption modeling (eg. Dialysis or Caco2-cell cultures)
- SHIME-experiments: short-and long-term evaluation of the intestinal fate of specific compounds or formulations.
- Host-microbe interaction studies using attachment models and cell cultures.
- *In vivo* validation using animal models
- Proof-of-concept validation in human trials

FOOD INDUSTRY

PHARMACEUTICAL INDUSTRY

Collaborative development of new innovative functional products (longer term strategy)

- Identification of active compounds or precursors
- Isolation of microorganisms for biotechnological applications
- Development of production strategies
- Formulation of the end product

Bioavailability of the active compound is the key focus

Stability/fate in the gastrointestinal tract (short-and long-term studies)
Degradation in the stomach and small intestine
Microbial metabolism/activation in the colon
Solubility of the formulation
Permeability/absorption of active compounds
Pharmacokinetic validation of bioavailability in laboratory animals in collaboration with third parties

Fields

- Pre- and probiotics
- Pharmaceuticals
- Dietary ingredients
- Botanicals

- Specific activity profiling
- Stability/survival in stomach and small intestine
- Efficacy in colon and localization effect
- Modulation of the microbiota
- Interindividual variability
- Host-bacteria interactions

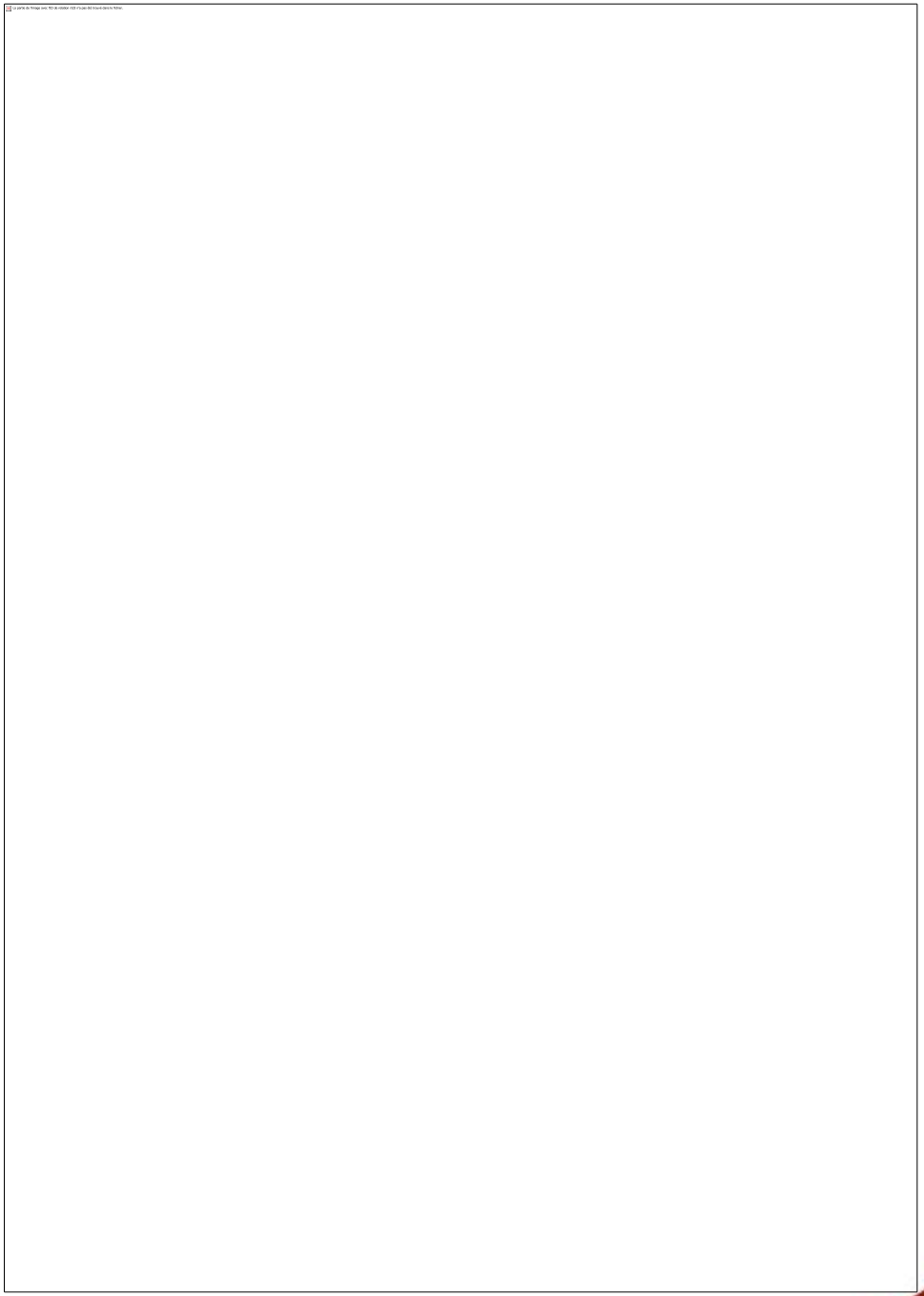
- Active compound identification
- Solubility and stability of actives in upper intestine
- Absorption modeling
- Activation of precursors
- Degradation of active compound
- Formulation and matrix effects

ProDigest
Gastrointestinal Expertise

Example:
Probiotic chocolate

ProDigest

Gastrointestinal expertise



ProDigest
Gastrointestinal Expertise

survival of probiotics

- Formulation of 2 probiotic strains in a chocolate matrix
- Research goal
  - Resistance to host environment
  - Ability to compete with indigenous bacterial community
- Approach
  - Sequential batch experiment
  - TWINSHIME setup

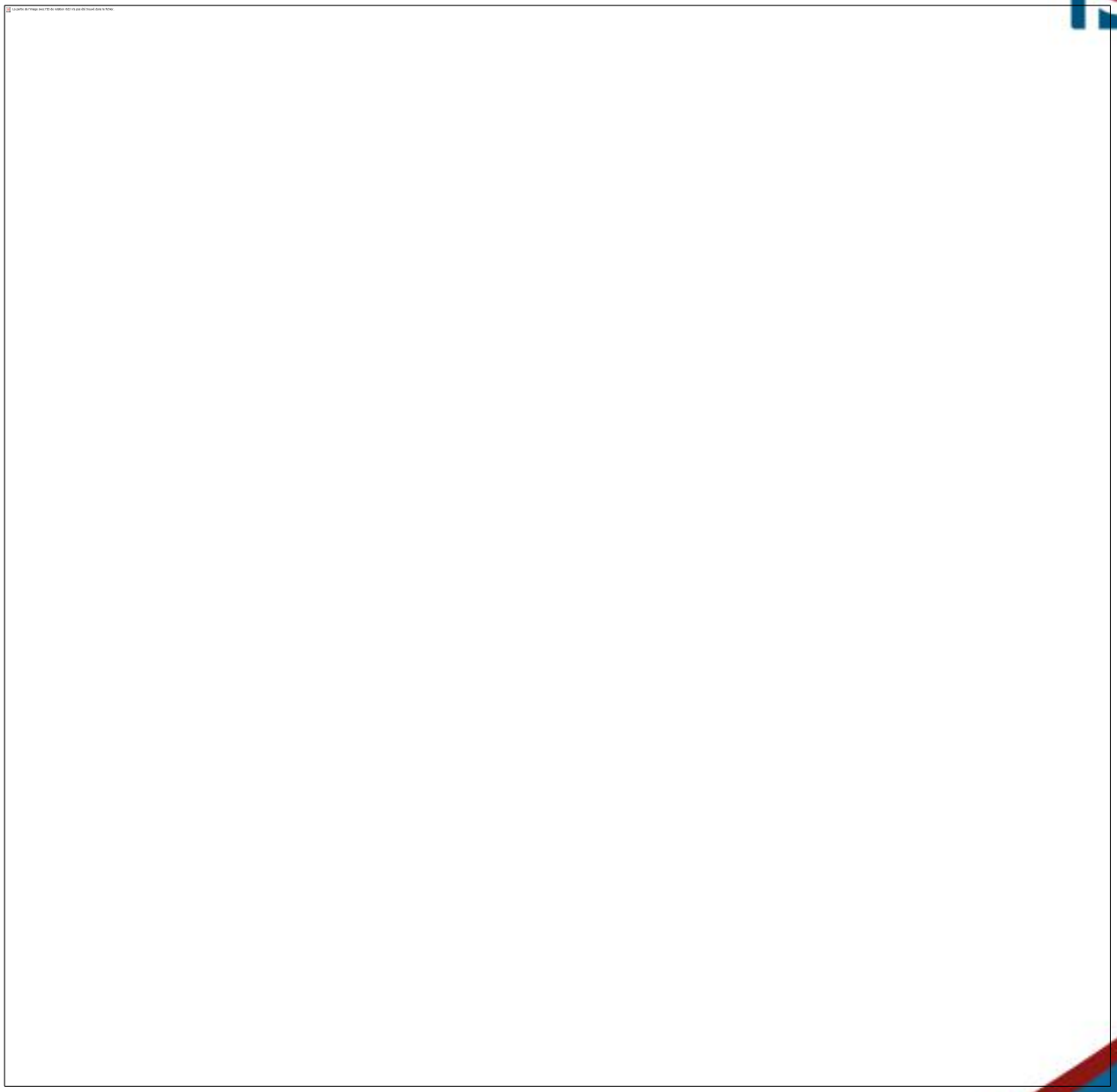
Conclusion probiotic chocolate

ProDigest
Gastrointestinal Expertise

- Results allowed Barry Callebaut to
  - Select best chocolate matrix
  - Select best probiotic
  - Commercialize product
- Take home message:
  - Happy customer=
    - Track record
    - Important commercial value!

3 pages free publicity!

ProDigest
Gastrointestinal Expertise



ProDigest
Gastrointestinal Expertise

Contact information

www.prodigest.eu

info@prodigest.eu

In collaboration with:

UNIVERSITEIT GENT

LabMET

FOOD2KNOW