Using probiotics to tackle the worldwide challenge of obesity

Gregory Lambert
Probiota Asia– October, 23rd, 2019
Historically, Asian regions had lower rates of obesity and overweight than US or Europe, however, with high rates of economic growth...

The rates of overweight & obesity are increasing fast

- As technology improves and urbanisation increases, obesity levels rise
- Asians tend to have higher amounts of abdominal fat at lower BMIs*
- Asians are more likely to develop central obesity, which is associated with higher risk of developing dyslipidaemia, impaired glucose tolerance, type 2 diabetes mellitus and CVD, as well as more adverse cardiovascular outcomes.

This increase can also negatively impact the economy

- Economic output impacted by loss of productivity of overweight and obese people
- Chronic conditions require expensive lifelong treatment

Pacific island countries and areas have a 75% adult prevalence of obesity.

In 2014, 50% of all overweight children under 5 lived in Asia.

Between 2010 and 2016, the adult obesity rate has risen by 33%.

10% Vietnam

46% Malaysia

Overweight & Obesity Prevalence

8 South-east Asian countries
TargEDys Executive Summary

- Based on **15 years of research** at Inserm (University of Rouen, France)
- Offices in Longjumeau, Paris area & Labs in Rouen, Normandy
- Financing
  - Raised 9 M€
  - Obtained 1.3 M€ in non-dilutive financing
- IP: 9 patent families and 6 trademarks
- Platform delivers both **therapeutic** and **nutraceutical** products
Reduction of food intake

Reduction in body weight

Improvement of body composition

Activation of lipolysis

Decrease of fasting glycemia ( Improvement of glucose tolerance)

Activation of central satiety pathways

ProbioSatys® benefits
The concept of molecular mimicry
ClpB / α-MSH Molecular mimicry

- Strong sequence homology between an exposed loop on the surface of ClpB and α-MSH
- Confirmation in HRM Mass Spectrometry after immunoprecipitation of enterobacteria proteins with α-MSH antibody
- ClpB is a MCR agonist

### Key Points

<table>
<thead>
<tr>
<th>Protein</th>
<th>α-MSH Mimicry</th>
<th>ClpB E.coli</th>
<th>ClpB H.alvei / ProbioSatys</th>
<th>ClpX Lactobacillus casei</th>
<th>ClpC Bifidobacterium animalis</th>
<th>ClpB Enterococcus fecalis</th>
<th>Hsp 104 Saccharomyces cerevisiae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ac-SYSEME</td>
<td>534-AEIAEVLA RW TGIPV-548</td>
<td>534-VEIAEVLA RW TGIPV-548</td>
<td>DVAEVVSQW TGIPV</td>
<td>IAEVIS QS TGIPV</td>
<td>EIAQVVG FL TGIPV</td>
<td>ISETAA RL TGIPV</td>
</tr>
<tr>
<td>α-MSH</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>ClpB</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

1. Tennoune et al., Transl Psy, 2014
2. Internal report from Biognosys, 2018
Plasma ClpB levels correlate with ClpB DNA in gut microbiota in rats

ClpB is present in human plasma

ClpB DNA in human feces inversely correlates with BMI

ClpB is endogenously present and inversely correlates to BMI

From: Breton et al., Int J Eat Dis 49: 805-808, 2016
Submitted Int. J. Obesity
Single intraperitoneal Injection (2 pmol): ClpB96 / ClpB25 / control buffer

Single injection of ClpB reduces significantly cumulative food intake

In vivo ClpB effect in mice

Cumulative food intake (g)

Time (min)

Cumulative food intake (g)

From basal level

→ Single injection of ClpB reduces significantly cumulative food intake

2-way Annova, Dunn Post-test, *p<0.05; Mann & Whitney **p<0.01
Oral gavage with *Hafnia alvei* in Genetic (ObOb) and Diet (HFD) mice obesity models.
**Hafnia alvei vs Orlistat**

**Hybrid Model HF-HSD – ob/ob**

*Hafnia alvei* reduces **food intake** and **glycemia** while Orlistat increases it.

Control of appetite is one key to achieve sustainable **weight** and **metabolic disease** management.
H. alvei 4597 resists digestion (low sensitivity to pH and enzymes)
H. alvei 4597 adheres to mucus
H. alvei 4597 grows and is metabolically active in colon
CMC Development
Industrial process - ClpB concentration

[Images of industrial equipment and graphs showing ClpB concentration in different volumes: 1L, 30L, 5000L]
ClnB content reproducibility analysed by WB with bands extinction.

- ClnB profile is similar for both 5000L batches.
Comparison of 2 GI-resistant formulations (DRCAPS™ et HPMC) in ex-vivo GI model:

- Study of the release of the bacteria
- Study of the ClpB integrity

<table>
<thead>
<tr>
<th>% cells released in the medium at each digestion stage</th>
<th>ClpB content - Ileal phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>end of gastric phase</td>
<td>HPMC</td>
</tr>
<tr>
<td>end of duodenal phase</td>
<td>DRCAPS™</td>
</tr>
<tr>
<td>end of ileal phase</td>
<td>DRCAPS™</td>
</tr>
</tbody>
</table>

DRCAPS™ provide a delayed bacteria release & protection of the ClpB from degradation.
Finished product
Formulation & control

Strain

Petri dish cell count (CFU)
Flow cytometry
  - Total cell count (bacteria)
  - Bacteria in intermediate stage
  - Cultivable bacteria

All non-lysed bacteria alive or not Contain and protects ClpB.

Protein and fragments characterization

ELISA to quantify $\alpha$-MSH pharmacophore
Western Blot to characterize degradation

Molecular mimicry is present even after partial degradation

Stability data on the first commercial batch at 30°C / 65% HR
Regulate your appetite via your microbiome!

- *Hafnia alvei* 4597, THE connected strain
- Zinc & Chromium to rebalance your metabolism
- The only probiotic with a validated molecular level mechanism of action
- Proven efficacy
- Gastro-resistant capsules
- 2 capsules per day (breakfast and lunch)
- Feel satiety after only one week
- Reduce body weight after one month

### Ingredients

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Defined Daily Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hafnia alvei</em> 4597</td>
<td>50 million CFU's</td>
</tr>
<tr>
<td>Zinc (bisglycinate)</td>
<td>5 mg or 50% of ARs</td>
</tr>
<tr>
<td>Chromium (picolinate)</td>
<td>20 μg or 50% of the ARs</td>
</tr>
</tbody>
</table>
ProbioSatys®

PET CARE
- Pet Food/supplement

NUTRA
- Food supplement
- Looking for partners

PHARMA
- Ingredient
- LBP
- ClpB

Nutraceutic based approaches
- Cheese strain = food
- Self affirmed GRAS in the USA
- Dietary supplement
- Functional foods

Therapeutic based approaches
- The ClpB or ClpB fragments as API
- Life Biotherapeutic with Hafnia alvei
Ongoing Clinical Trial

- Double-blind, randomised, placebo-controlled study to evaluate benefit of ProbioSatys® on weight reduction in overweight subjects
- Probiotic strain & dosage: *Hafnia alvei* 4597 – 1.10^{11} cells/day
- 12 weeks of treatment, 2 capsules/day – 5 visits
- 3 centres in Germany
- 2 arms / 120 subjects per arm - BMI: 25 kg/m² – 29.9 kg/m²
- Main endpoints:
  - Body weight (kg and %)
  - Body fat and fat free mass
  - Waist and hip circumference
  - Lipid metabolism parameters
  - Glucose blood parameters
  - Feeling of satiety
  - General well-being parameters
  - Safety

A Human POC for ProbioSatys® Nutra and Therapeutic products
EnteroSatys® consumer study

METHODOLOGY
- Consumer experience study after 1 and 3 months of EnteroSatys® use
- Questionnaire via Google Forms or by phone (10-15 min)
- Inclusion criteria: people who bought at least 2 boxes
- Based on a voluntary decision to enter the study
- Reward: 1 free box per questionnaire

POSSIBLE LIMITATIONS
- Sample – total n=61
  - People included bought the product on trade-shows or on the website, so sample is slightly different from people who would by in pharmacies)
  - Sample is probably more motivated, educated and experienced than the common population
  - Sample size for Q3 after 3 months is limited for the moment (n=27)
- Self-assessment
- Incentive box
Profile of a typical participant in the survey

- Woman (80%)
- Average age: 48 years-old
- Overweight or obese (BMI >25 = 70%)
- Exercising at least 1x per week
- Feels hungry especially in the evening
- 93% Has some bad eating habits
  - Mostly refill or snacking
- Tried restrictive dieting in the past
- Primary objective: to lose weight

Study results September 2019; n=61
Study results
Eating behaviour

- 83% are responders*
- 77% have reported at least 1 effect on their eating behaviour
- Majority of people have reported their first effects within first 10 days

**% of people who reported different impacts on their eating behaviour**

- **65%** Decreased Appetite
- **56%** Less Sweet Cravings
- **58%** Less Snacking
- **55%** Smaller Portions

**Lead time to first reported effects**

*reported effect either on eating behaviour or weight loss or both
Weight loss

After 1 month
Average loss: - 2.7%
35% have lost more than 3%
18% have lost more than 5%

After 3 months
Average loss: - 6.3%
65% have lost more than 3%
38% have lost more than 5%

Average (kg)
After 1 month - 2.2kg
After 3 months - 5.04kg

Consistent with preclinical data
Study results
Other results and satisfaction

Other improvements reported by responders
- Waist circumference* (63%)
- Digestive comfort (59%)
- Feeling better in their body (73%)
- Improved relation with food (69%)
- Vitality (41%)

* Tightened their belt at least by one notch (2 cm)

84% WOULD RECOMMEND THE PRODUCT AFTER THE FIRST MONTH

Compliance
- 88% report to be compliant during the 1st month
- 60% report to be compliant during the 3rd month

96% WOULD RECOMMEND THE PRODUCT AFTER 3 MONTHS
Grégory Lambert
CEO and VP R&D
Gsm: +33.6.79.39.41.16
Mail: glambert@targedys.com

TargEDys
Faculté de Médecine et Pharmacie
22 boulevard Gambetta
76000 Rouen

TargEDys
Parc Nativelle
1 chemin de Saulxier
91160 Longjumeau

Contact information

TargEDys
Parc Nativelle
1 chemin de Saulxier
91160 Longjumeau

Grégory Lambert
CEO and VP R&D
Gsm: +33.6.79.39.41.16
Mail: glambert@targedys.com

TargEDys
Faculté de Médecine et Pharmacie
22 boulevard Gambetta
76000 Rouen

www.targedys.com