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22 – 23 FEBRUARY 2017
MADRID - SPAIN

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"Latest clinical Evidences showing that a proprietary *Lactobacillus reuteri* Strain can reduce the Symptoms associated with a *Helicobacter pylori* Infection"

Gilles Jequier

Commercial Director
Organobalance, a Novozymes Company

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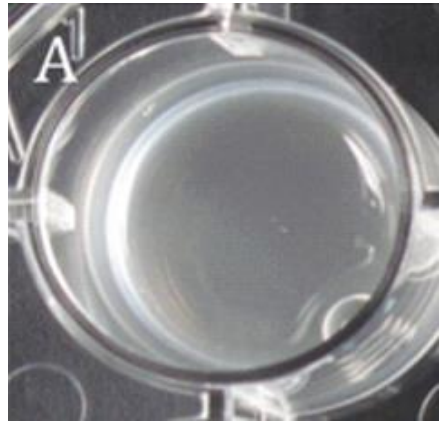
What is Pylopass™?

- *Helicobacter pylo*ri is the main cause for developing gastritis and ulcers
- Thanks to a unique mode of action Pylopass™ can reduce the *Helicobacter pylori* load of the stomach thus reducing the risk of developing gastritis and gastric ulcers
- Pylopass™ is obtained through fermentation of a unique and patented probiotic strain of *Lactobacillus reuteri* DSM 17648
- Pylopass™ is comprised of inactivated cells and is therefore stable at room temperature.



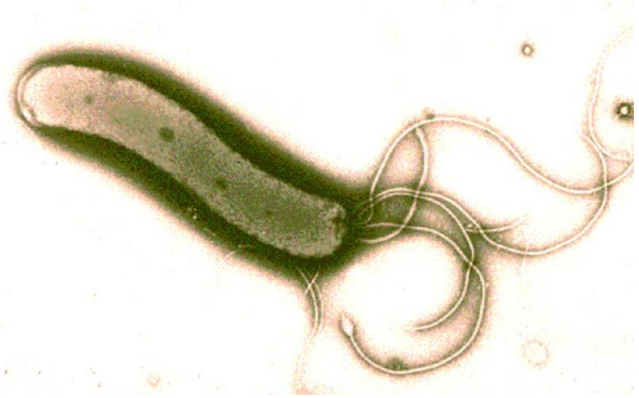
Pylopass™

Helicobacter pylori – A Recent Discovery

- In 1982, two Australian scientists, Dr. Barry Marshall and Dr. Robin Warren, discovered that *Helicobacter pylori* is the main cause of gastritis and gastric ulcers

- Up to then it was thought that no bacteria could survive in the acidic conditions of the stomach and that ulcers were caused by lifestyle

- This groundbreaking discovery was awarded with the Nobel Prize for Medicine and Physiology in 2005



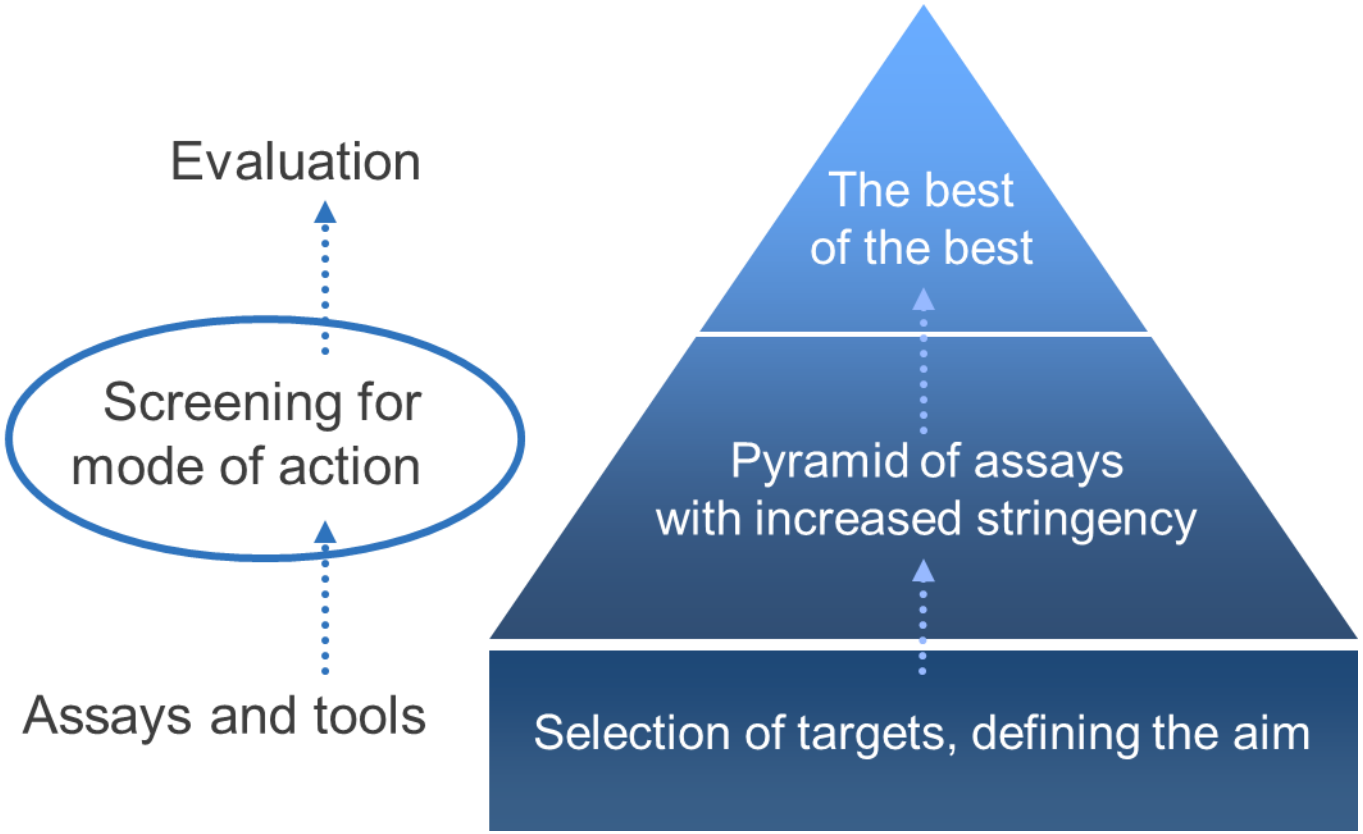
H. pylori – Conventional Treatment

- Eradication with 2-3 antibiotics and a proton pump inhibitor (PPI)

- There is no global or country specific total eradication programs for *H. pylori* as there are several issues with the pharmaceutical approach:

1. **Increased resistance against antibiotics** (even when combined, success rate decreased between 90% to 75%) and **high risk of re-infection**
2. **Severe side-effects with antibiotics** such as nausea, vomiting, digestive disorders and headache are observed
3. **Exposure to antibiotics results in dysbiosis:** beneficial bacteria are eliminated and that can lead to an imbalance of the microbiota
4. **PPIs are addictive**: increased gastric acid production at the end of the treatment make it hard to stop them (rebound effect)
5. **Side-effects of PPIs** such as increased risk of osteoporosis and magnesium deficiency leading to cardiac arrhythmia

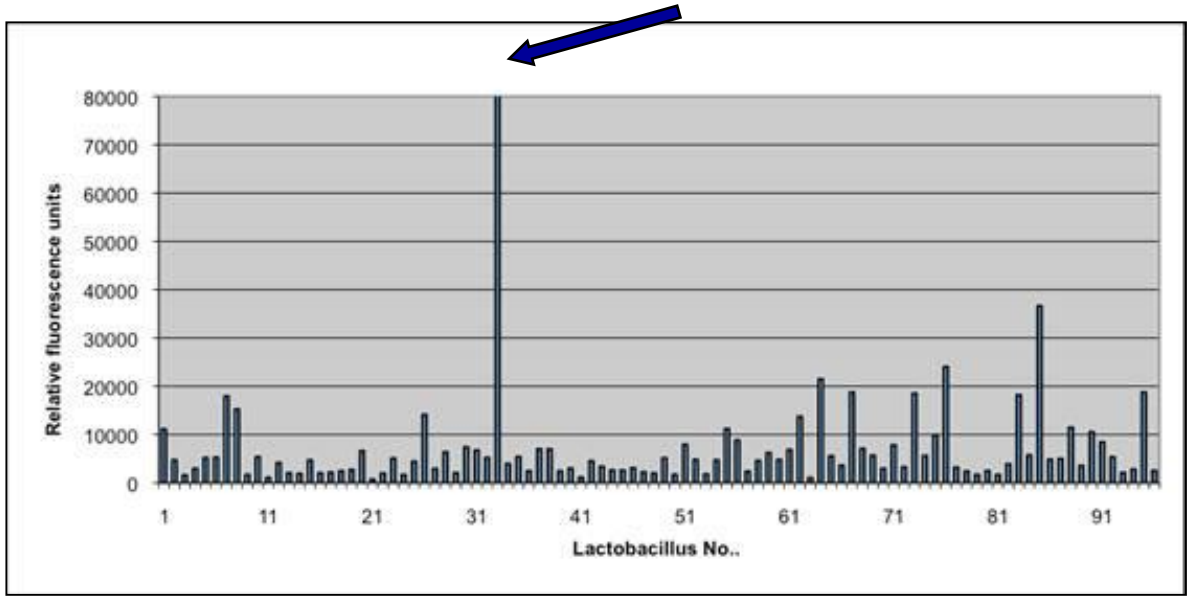
Pylopass™ Strain Screening



Selecting the most-effective Bacteria

Pylopass™ contains a specifically acting bacterium which co-aggregates *Helicobacter pylori* and thus reduces *Helicobacter* bacteria in the stomach.

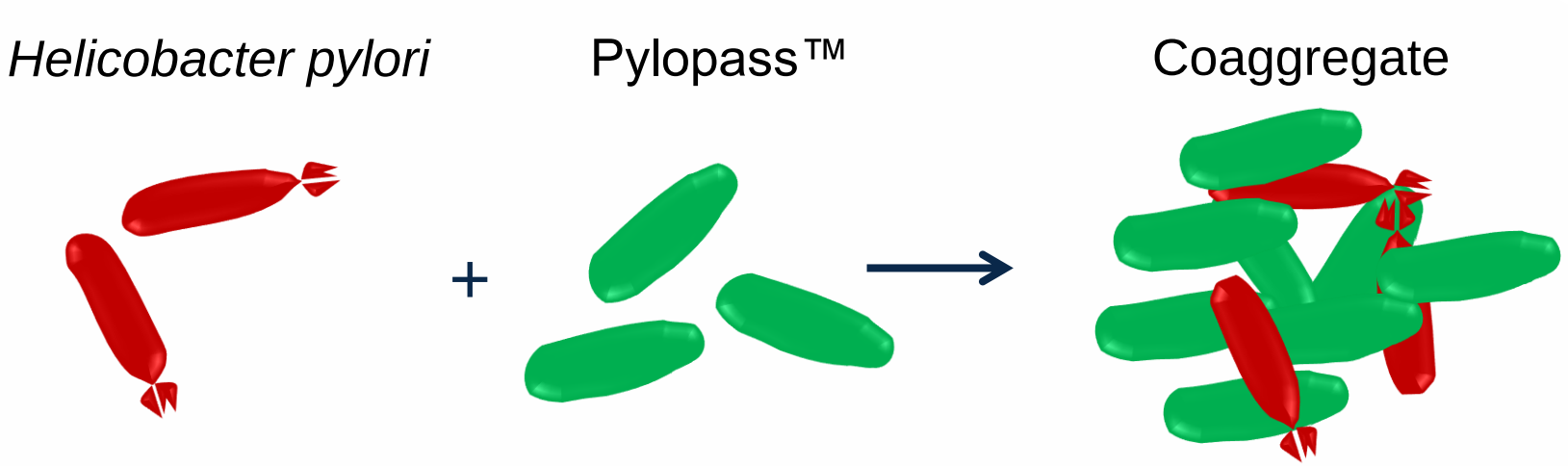
Example:
1 out of 96 *Lactobacillus* strains is tightly bound to immobilized *H.pylori* (read-out: high fluorescence of binding labelled lactobacilli).



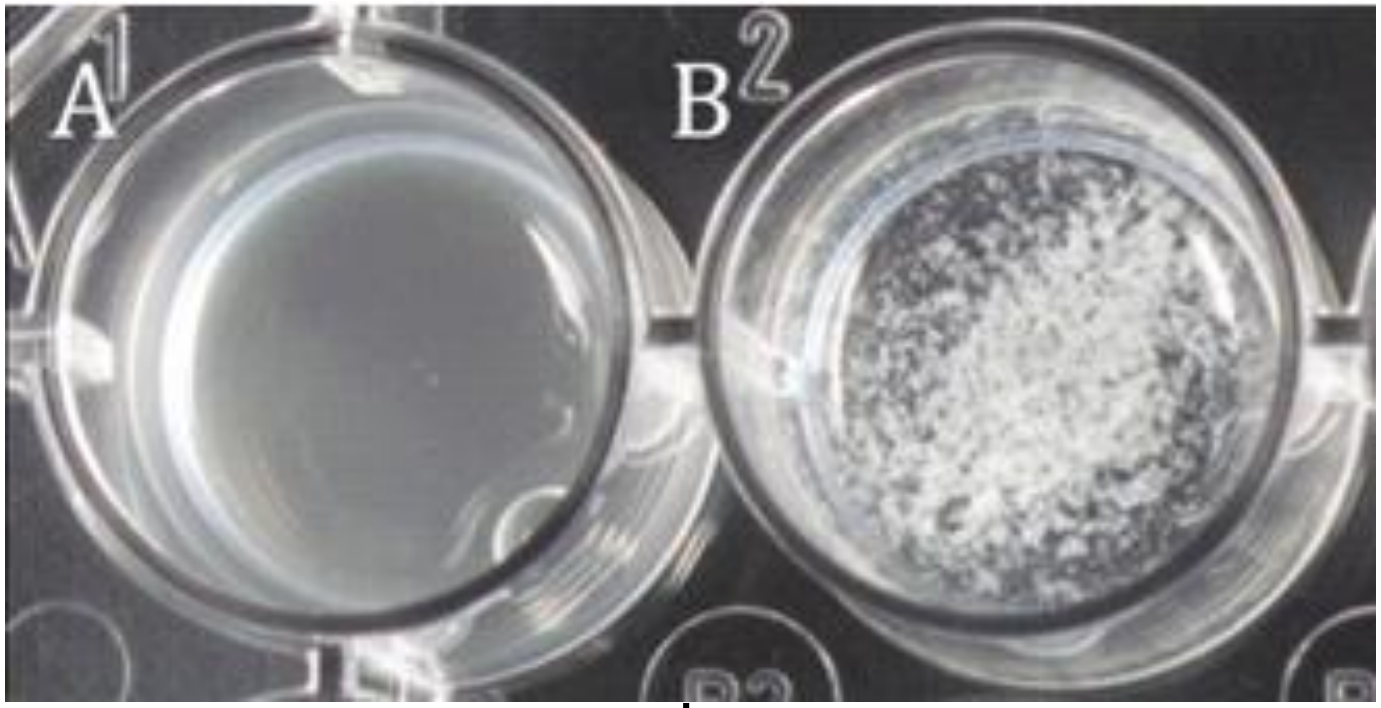
Screening among 700 *Lactobacillus* strains of the ORGANOBALANCE strain collection reveals specifically binding *Lactobacillus* antagonists to *H. pylori*.

Pylopass™ - Unique Mode of Action

- Pylopass™ is able to recognize surface structures on *Helicobacter pylori* and to form co-aggregates
- Co-aggregates are eliminated from the organism through the gastrointestinal tract
- This leads to a reduction of *Helicobacter pylori* load in the stomach



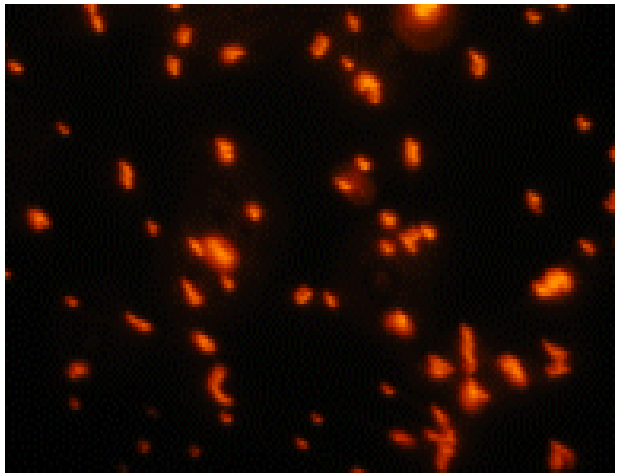
Pylopass™ in vitro Co-aggregation with *H. pylori*



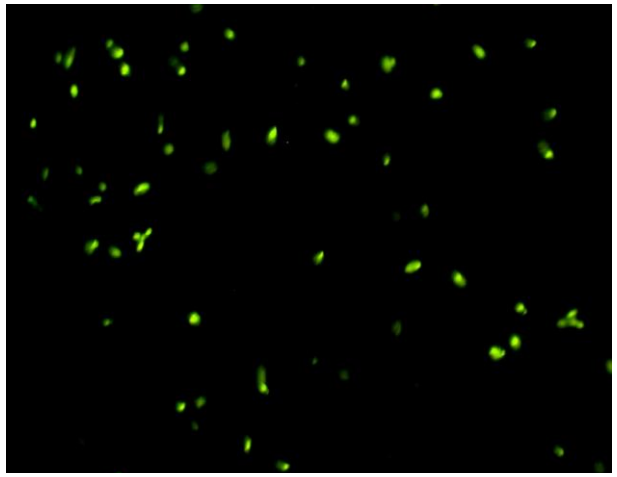
H. pylori **+ other lactobacillus = no co-aggregation**	***H. pylori*** **+ Pylopass™ = co-aggregation**

Pylopass™ specifically aggregates *H. pylori*.

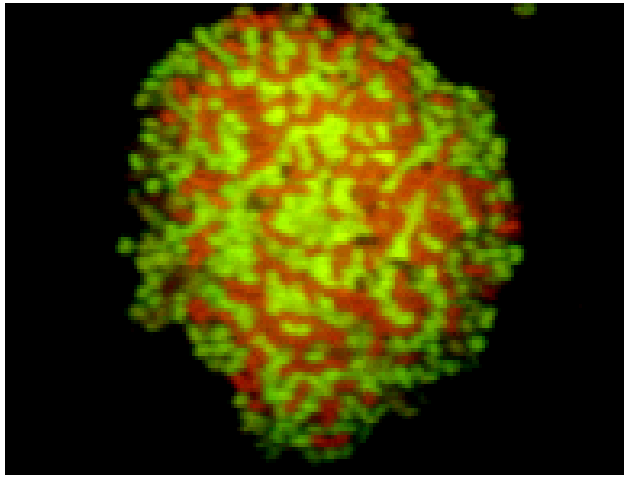
Pylopass™ in vitro Co-aggregation with *H. pylori*



Helicobacter pylori



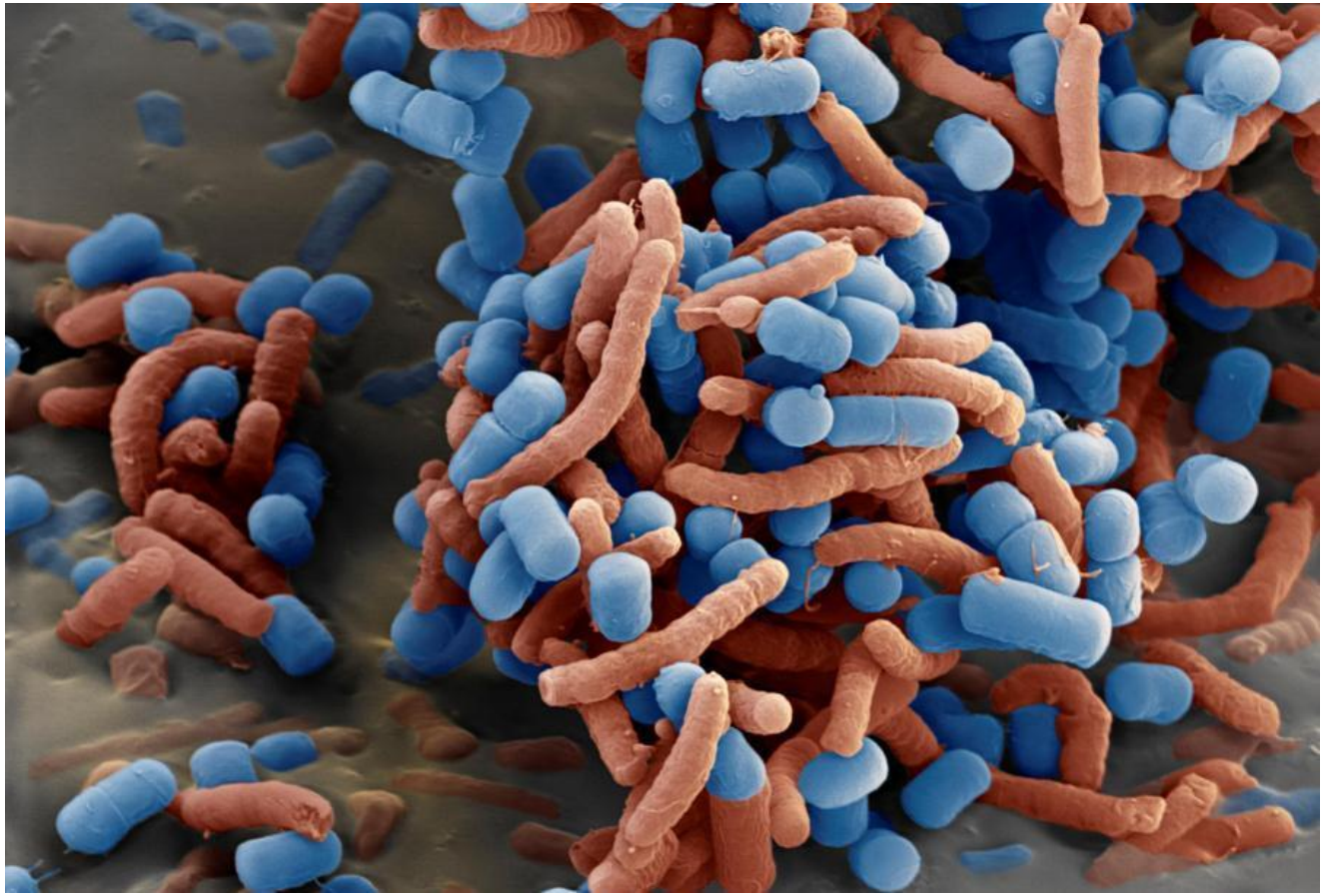
Pylopass™



Co-aggregation of *H. pylori* and Pylopass™

Pylopass™ specifically aggregates *H. pylori*.

Pylopass™ and *H. pylori* co-aggregates seen under SEM



Pylopass™ = blue | *H. pylori* = red | magnification = 13,000x
SEM: scanning electronic microscope

Urea Breath Test (UBT): non-invasive test to measure *H. pylori*

Pylopass™

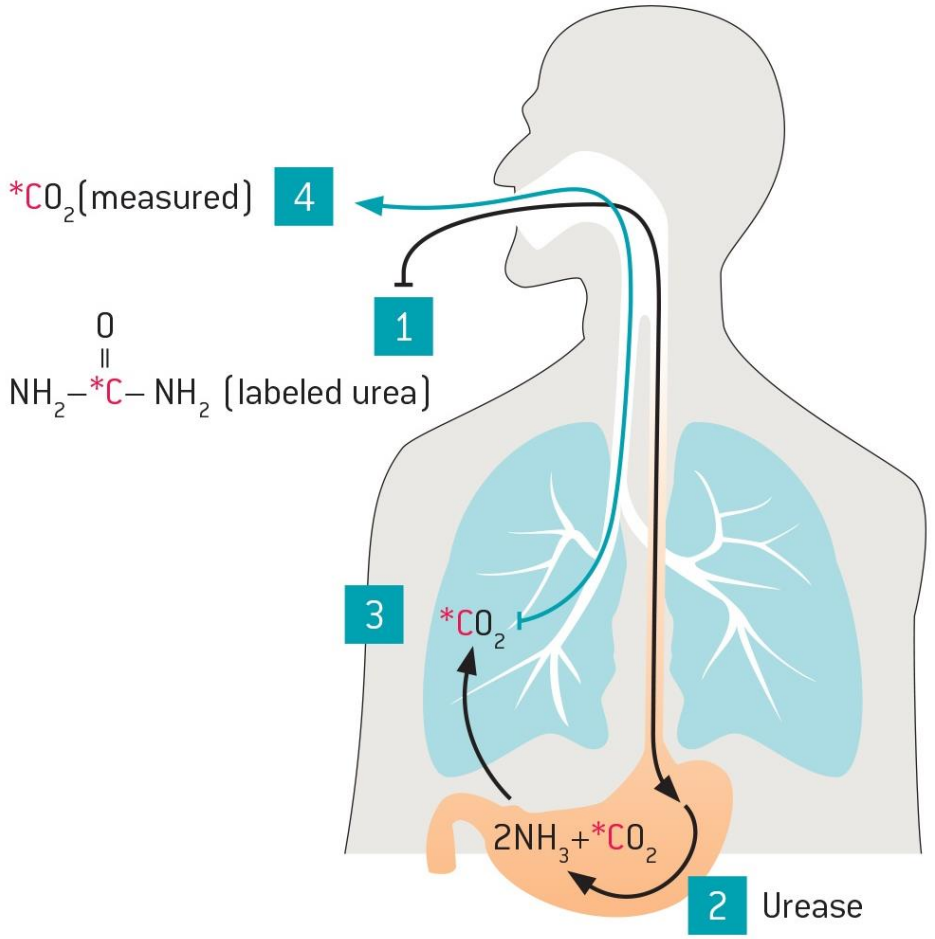
Urea (CH_4N_2O) is not metabolized in the body. Helicobacter pylori produces urease, the enzyme is able to hydrolyze urea

1. Ingest known amount of labeled urea

2. Due to the enzyme urease produced by *H. pylori*, the urea is converted to ammonia and carbon dioxide in the stomach

3. The labeled carbon dioxide is absorbed into the blood stream and travels to the lungs

4. A breath sample is taken and the amount of carbon dioxide is measured



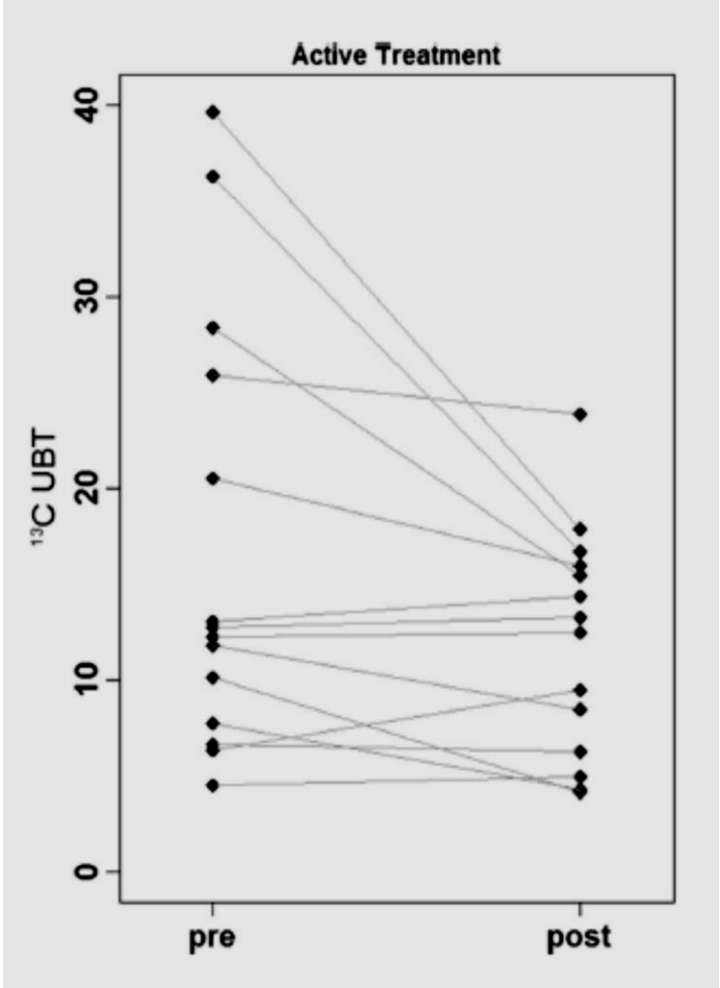
H. pylori reduction confirmed *in vivo*

Design: single-blinded, randomized, placebo-controlled, cross-over study

n = 24 *H. pylori* positive, asymptomatic adults (> 18)

Treatment: $2x10^{10}$ bacteria cells/day
2 tablets with $5x10^{9}$ bacteria cells, after breakfast and dinner.

Primary outcome: *H. pylori* load after 2 week Pylopass™ supplementation as measured by urea breath test (UBT)



- **Reduction of *H. pylori* load in 60% of the subjects in only 2 weeks**
- **Response significantly higher with increased basal *H. pylori* level**

Holz C. et al (2014). **Significant Reduction in Helicobacter pylori Load in Humans with Non-viable Lactobacillus reuteri DSM17648: A Pilot Study.** *Probiotics & Antimicro. Prot.*

Tyndallized bacteria show same efficacy

Pylopass™ pilot study conducted in Berlin, Germany

Design: Single-blinded, randomized, placebo-controlled, cross-over study

n = 22 *H. pylori* positive, asymptomatic adults (UBT> 12; mean UBT= 20)

Treatment: 200 mg Pylopass™/day in two servings

Primary outcome: *H. pylori* load after 2 week Pylopass™ supplementation as measured by urea breath test (UBT)

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Article

Non-Viable *Lactobacillus reuteri* DSMZ 17648 (Pylopass™) as a New Approach to *Helicobacter pylori* Control in Humans

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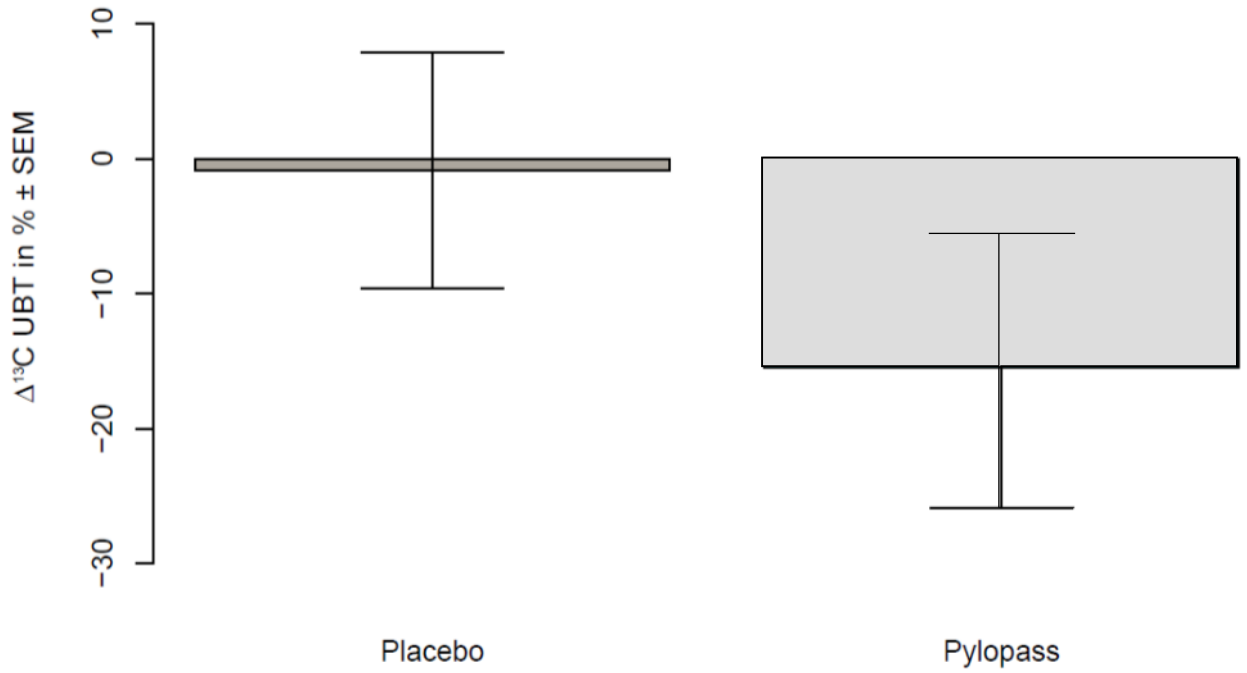
Abstract: Prevalence of infections by *Helicobacter pylori*, a pathogen involved in a number of gastrointestinal diseases, remains high in developing countries. Management of infections by eradication is not always an option. *Lactobacillus reuteri* (*L. reuteri*) DSMZ17648 (Pylopass™/Lonza) specifically co-aggregates *H. pylori in vitro* and was shown to reduce ^{13}C urea breath test *in vivo*. In this pilot study, we tried to replicate previous findings in an independent sample and to evaluate effects of spray-drying *vs.* freeze-drying of cultures. A single-blinded, placebo-controlled study was done in 22 *H. pylori* positive, asymptomatic adults. *H. pylori* levels were determined by ^{13}C-urea-breath method after 14 days of supplementation, as well as after 6, 12, and 24 weeks follow-up. In the test group, but not in the placebo group, a significant reduction of *H. pylori* was observed. For the first time, spray-dried cells of *L. reuteri* DSMZ17648 have been used in a human study and results are in line with the first study results, supplementing with freeze-dried material. This is of special interest as spray-drying results in dead cell material, meaning that the effect of *L. reuteri* must be independent of its probiotic activity. These results confirm the potential of Pylopass™ as a novel way to reduce the load of *H. pylori*.

Keywords: *Helicobacter pylori*; *Lactobacillus reuteri*; urea breath test

2 weeks of placebo — **2 weeks of Pylopass™**

Baseline UBT — **Placebo UBT** — **Pylopass™ UBT**

Confirmation that Pylopass™ has significant impact on UBT mean value



- Placebo: 3% change in UBT from baseline
- Pylopass™ : 16% decrease in UBT from baseline

Mehling H et al (2013). **Non-Viable *Lactobacillus reuteri* DSMZ 17648 (Pylopass™) as a New Approach to *Helicobacter pylori* Control in Humans.** *Nutrients 5*, 3062-3073

Human Pilot Study with higher Dosage and longer Treatment

Pylopass™ study conducted at the Beijing Hospital 301

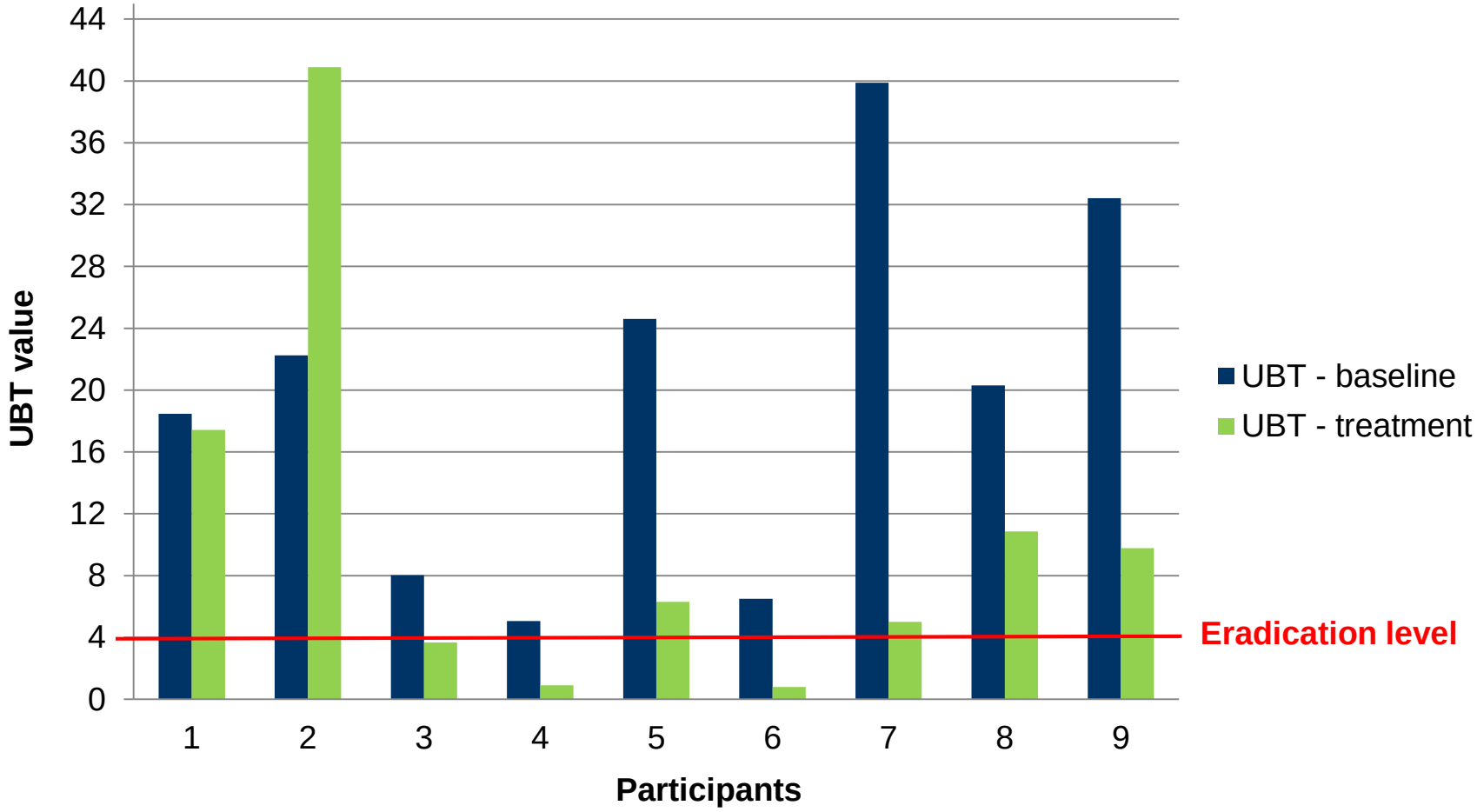
Design: unblinded trial

n = 9 *H. pylori* positive adults (UBT> 4; mean UBT = 20)

Treatment: 400 mg Pylopass™/day;
1 sachet after breakfast and dinner for 4 weeks.
2 g sachet: 200 mg Pylopass™ , 1000 mg dietary fiber, 800 mg maltodextrin

Primary outcome: *H. pylori* load after 4 weeks Pylopass™ supplementation as measured by urea breath test (UBT)

First Study showing *H. pylori* Eradication Potential



- Reduction of *H. pylori* Load in 90% of the subjects
- UBT value reduced by 70%
- Eradication (UBT<4) in 33 % of the subjects

Potential Benefits in Patients showing Symptoms associated with Gastritis

Pylopass™ study conducted at the Central Research Institute of Gastroenterology in Moscow, Russia

Design: open; efficacy and safety study

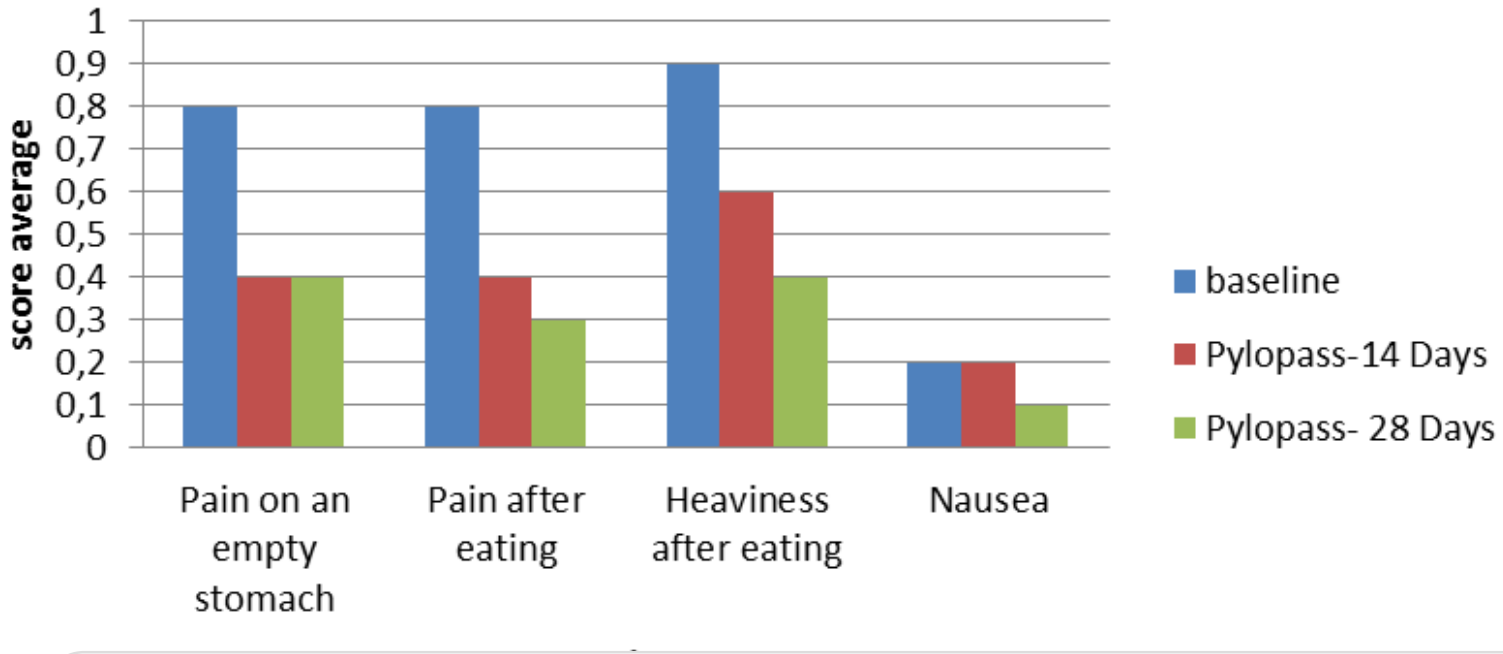
n = 30 enrolled- *H. pylori* positive adults without indication for eradication therapy

Treatment: 200 mg Pylopass™ /day for 4 weeks.

Objectives:
- Reduction in severity of main patients' complaints - clinical efficacy
- Decreased *Helicobacter pylori* load - microbiological efficacy
- Positive dynamics of morphological changes (OLGA) - morphological efficacy.

Borodin et al (2015). **Efficiency and safety of probiotic bacteria Lactobacillus reuteri DSMZ17648 in patients infected with *Helicobacter pylori* who haven't absolute indications for eradication therapy: the study outcomes.** http://www.lvrach.ru/2015/08/15436273/

Pylopass™ helps to decrease Symptoms associated with a Gastritis after 14 days



- *H. pylori* load reduction leads to morphological improvement (OLGA)
- Decrease of the severity of the symptoms on a 3 points scale: improvement of quality of life

Children Study in Russia

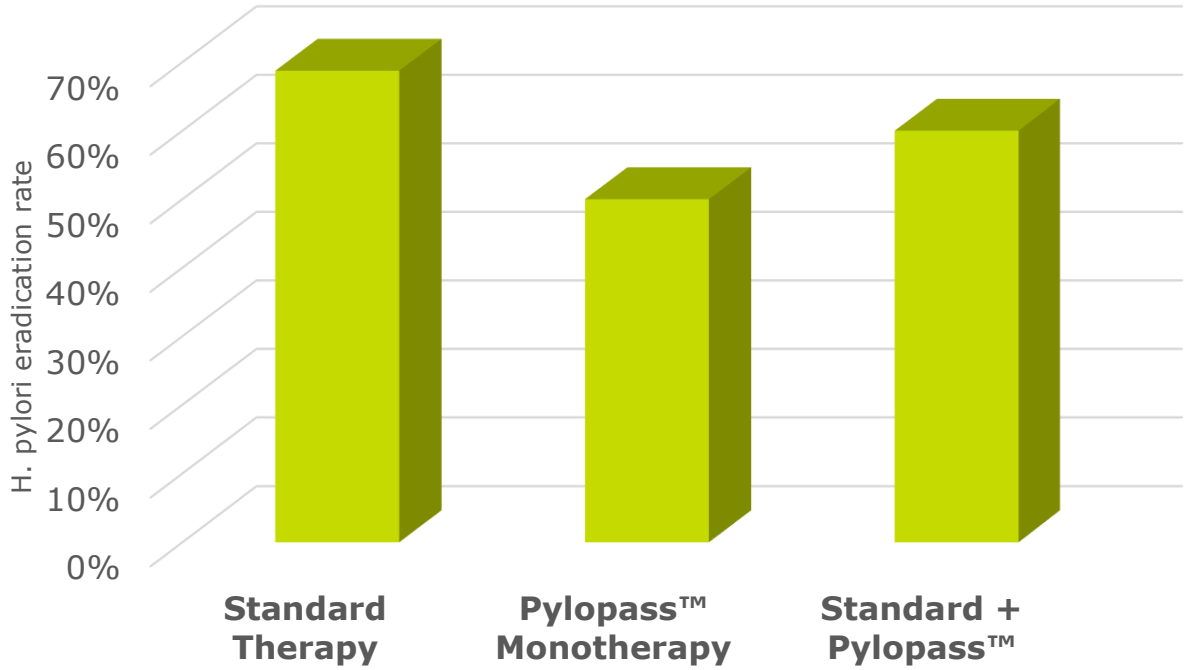
Clinical study with 49 children aged 9-17 suffering from chronic *H. pylori* associated gastroduenal diseases

- group 1: n= 17, 200mg Pylopass™ per day for 28 days
- group 2: n=16 triple therapy (amoxicillin + metronidazole + omeprazole + bismuth) for 10 days
- group 3: Triple therapy in combination with 200 mg Pylopass/day, 10 days, followed by 18 days with only 200 mg Pylopass

Efficacy tested both by UBT and by endoscopy

Parolova et al (2015). **An innovative approach in the treatment of H. pylori infection in children.** PMX 2015, No 22, C. 1339-1340.

Monotherapy of Pylopass™ can lead to an eradication of *H. pylori*



- Pylopass™ supplementation led to less adverse drug reactions and to a decrease in inflammation
- No significant difference in eradication rate could be observed due to small and heterogenous arms

Pylopass™ to increase Efficacy of Eradication Therapy

Clinical study with 60 patients suffering from peptic ulcer disease and duodenal ulcer associated with *H. pylori* infection

3 arms:
- group1: n=20, antibiotics, PPI and bismuth for 10 days

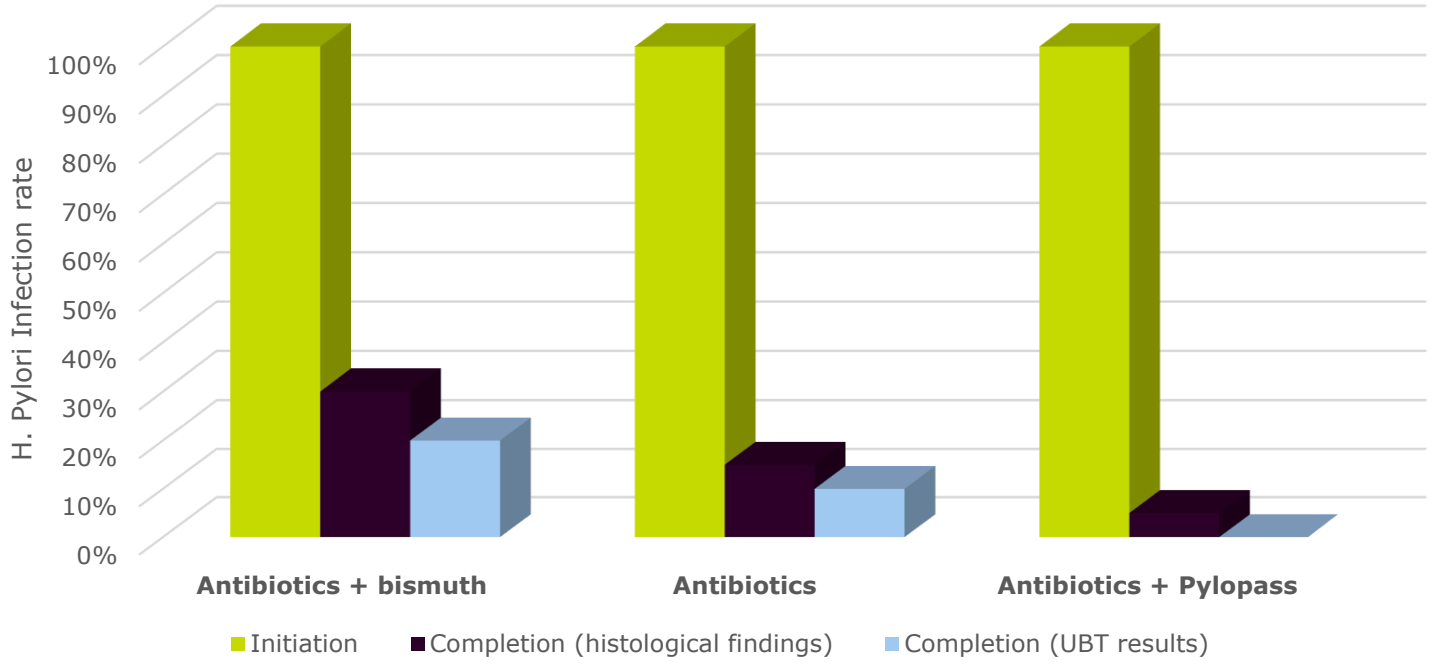
- group 2: n=20, antibiotics and PPI for 10 days

- group 3: n=20, antibiotics and PPI for 10 days and 2x200 mg Pylopass™ for 28 days

Antibiotics: 500 mg clarithromycin, 2 times a day and 1000 mg amoxicillin, 2 times a day
PPI: 20 mg omeprazole, 2 times a day
Bismuth: 240 mg de-nol, 2 times a day

Uspienskiy et al (2016). **Evolution in eradication therapy of HP – associated diseases: beyond the standards?** Gastroenterology 2016 No 17

Pylopass™ can increase the Efficacy of Antibiotics Treatment



- Pylopass™ supplementation results in positive effect on clinical picture and relief from abdominal pain
- Improvement of quality of life may explain the increased eradication rate

Clinical Evidences supporting Pylopass™

Country	Design	Outcome	Output
Germany	• Placebo controlled study • 22 subjects • 200 mg daily for 2 weeks	• Significant *H. pylori* reduction	Mehling and Busjahn 2013 publication
	• Pilot study • 27 subjects • 200 mg for 2 weeks	• Strain selection, assay, safety and significant results	Holz et al. 2014 publication
China	• Pilot study • 9 subjects • 400 mg for 4 weeks	• Reduction in 90% and eradication in 33% of the cases	Study report
Romania	• Post-marketing study • 37 subjects • 150 mg for 4 weeks	• Significant condition improvement, also observed 3 months after end of treatment	Product promotion
Ireland	• Clinical study • 24 subjects • 200 mg for 4 weeks	• Reduction of H. pylori infection load and improvement of the abdominal symptoms	Under publication
Russia	• Clinical study • 30 patients • 200 mg for 4 weeks	• Statistically significant *H. pylori* reduction • Degree of inflammation decreased in 25% of cases • Positive dynamics of dyspeptic symptoms	Bordin et al., 2015 publication
	• Clinical study • 49 children aged 9-17 • 200 mg for 4 weeks	• Eradication rate of 50%, increased to 60% when combined with antibiotics and reduced side effects and symptoms	Paralova et al., 2015 publication
	• 60 patients • Eradication therapy w and w/o 2x200 mg	• Improvement of quality of life indicators when Pylopass added to eradication therapy • Eradication rate 10% higher with Pylopass	Uspensky et al., 2016 publication

Efficacy and safety confirmed by different studies in different countries:

- **Reduction of *H. pylori* load in all cases**
- **Eradication between 10 % and 50 % of the subjects**
- **Improvement of the symptoms**

Possible Product Positioning based on Clinical Evidences

Pylopass™

Target Population	• 85% of the *H. pylori* infected population who show no symptoms • To prevent an infection e.g. when traveling	• People looking for gastric relief • As a first line of defense against *H. pylori* symptoms	• Patients undergoing an *H. pylori* eradication therapy
Clinical Evidences	• Significant *H. pylori* reduction • Selective co-aggregation of *H. pylori* with no negative impact on healthy microbiota	• Significant *H. pylori* reduction • Significant improvement of the quality of life indicators	• Significant improvement of the quality of life indicators • Increases the *H. pylori* eradication rate • Higher efficacy than bismuth when combined to an antibiotics therapy
Examples			
Concept	**Balancing a healthy microbiota**	**Gastric well-being**	**In combination with eradication therapy**

Pylopass™ a clinically proven Ingredient for Stomach Wellbeing

- Microbiome-based, unique and specific mode of action to prevent gastritis and gastric ulcer
- *Helicobacter pylori* control clinically tested in human studies
- Increasing number of reasons supporting *H. pylori* control rather than eradication for asymptomatic people
- Proven benefits without any side effects associated with the drugs treatment

novozymes®
Rethink Tomorrow

Key messages

- Better understanding from the microbiota is key to develop more targeted products:
  - strain specific
  - well-defined mode of action
  - specific health benefits

- Good products result from screening a good strain collection:
  - high natural diversity
  - well characterized
  - safe and approved

- With increasing knowledge we expect many more innovative products

Thank you

novozymes®