New probiotics with novel functional effects against infection by *Helicobacter pylori* and celiac disease

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GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
MEDICAMENTOS INNOVADORES
Plataforma Tecnológica Española
farmaindustria

CONTENT

1. **The Company**
2. **The Products**
   a) Target Indications
   b) Innovative mechanisms of action
   c) Differential features facing the market
   d) Current status of development
   e) IPR protection
   f) Pitfalls & Risks to be considered
3. **Partnering Opportunities**

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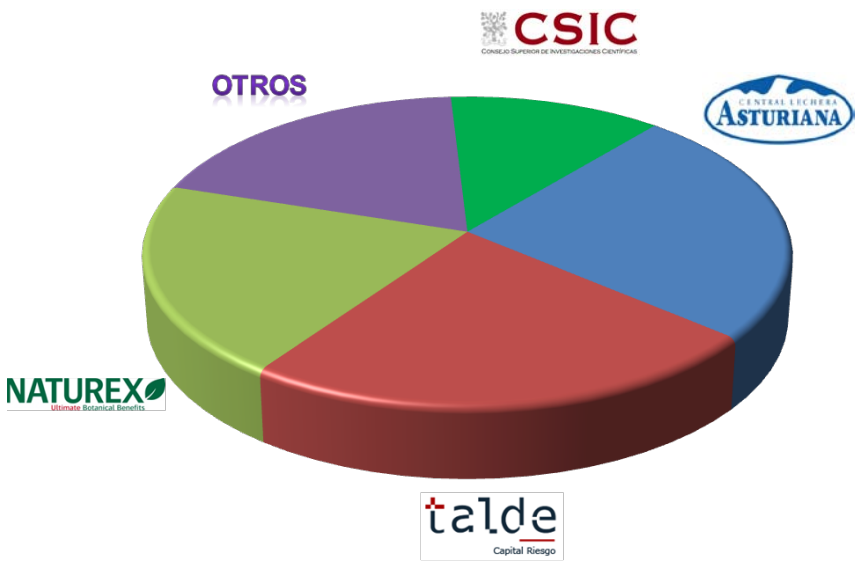
General data



CAPSA	24,99%
Talde	23,74%
Naturex	20,07%
Otros	19,15%
CSIC	12,04%

- Biopolis SL is a biotech company founded in 2003 as a spin-off of the National Spanish Research Council (CSIC)
- Biopolis SL offers its customers a full array of R&D services ranging from the molecular screening and identification of any microbial organism and/or metabolite to its validation and production
- Biopolis SL currently provides these services to a portfolio of domestic and foreign clients, both in the food and feed industry and the chemical-pharmaceutical sector

The mission of Biopolis

- The mission of Biopolis SL is to develop tailor-made biotechnological solutions to solve their clients' needs
- Biopolis SL scientists' work is based on direct contact with their clients within the strictest confidentiality
- When the solution has been found (usually a microorganism, a metabolite produced by a microorganism or a fermentation process) at laboratory scale, Biopolis SL offers their clients the scale-up and industrial production of the microorganism, the microbial metabolite or the fermentation process.
- This "transversal and global" offer is the main asset of the company

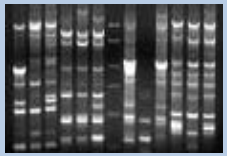
Our tools: a range of platforms

Biochemistry

Cell biology

Microbiology

Pre-clinical trials



Molecular biology

Scale-up

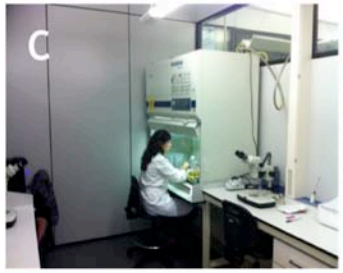
Industrial production

Genomics

Metabolomics

Murine models

The facilities



- Biopolis SL has several laboratories and two different production plants at its headquarters of Valencia
- It is important to notice that the Biopolis group works in strict compliance with all environmental regulations
- For that reason, all the facilities have been inspected and approved by the Spanish National Bio-Safety Committee

The key point: Biopoli's staff

- Currently, 41 people are working at the Biopolis group (34 people in Biopolis SL and 7 in Lifesequencing SL)
- Most of them are PhD holders (17) or BSc (16); the rest are highly qualified technicians (8)
- The staff of Biopolis includes agronomic, chemical and industrial engineers, biologists, bioinformaticians, chemists, economists, lawyers and food technologists (pluridisciplinary approaches)
- It is noteworthy that there is a gender imbalance in that 28 of the 41 employees of the group are women; many of them occupy senior positions in the company (8 of 9 laboratory managers are women)

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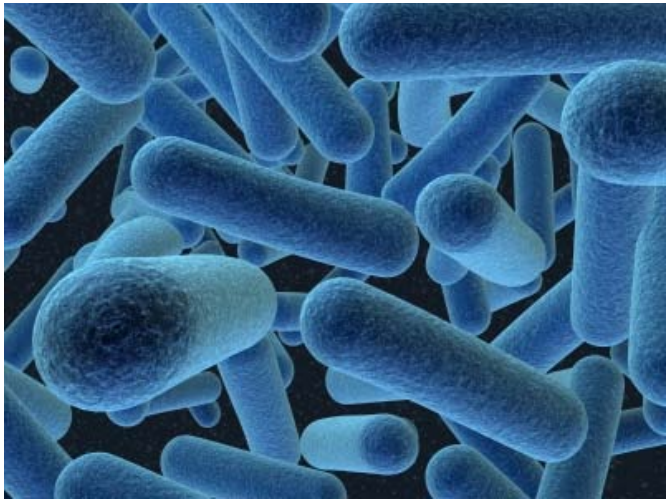
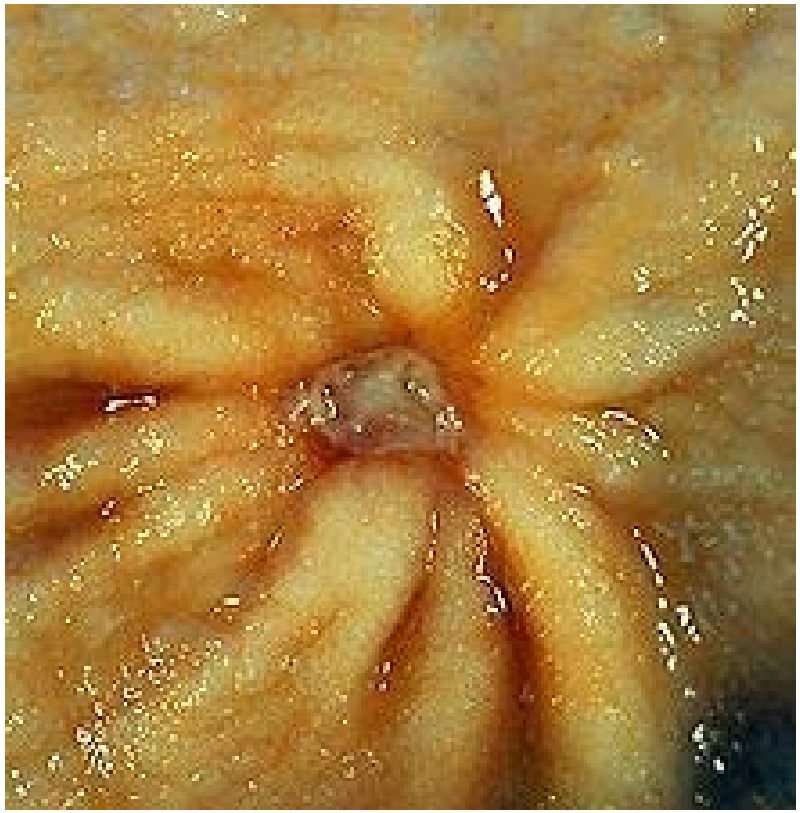
The products

Helicobacter pylori
(*Bifidobacterium longum* CECT 7366)



Celiac disease
(*Bifidobacterium bifidu*m CECT 7347)

Peptic ulcer and *Helicobacter pylori*



- Peptic ulcer is the most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful
- As many as 70-90% of such ulcers are associated with the presence of the bacterium *Helicobacter pylori*
- The incidence ranges from 15-40% in developing countries and up to 80-90% in non-developing countries
- In USA about 4 million people have active peptic ulcers and about 350000 new cases are diagnosed each year

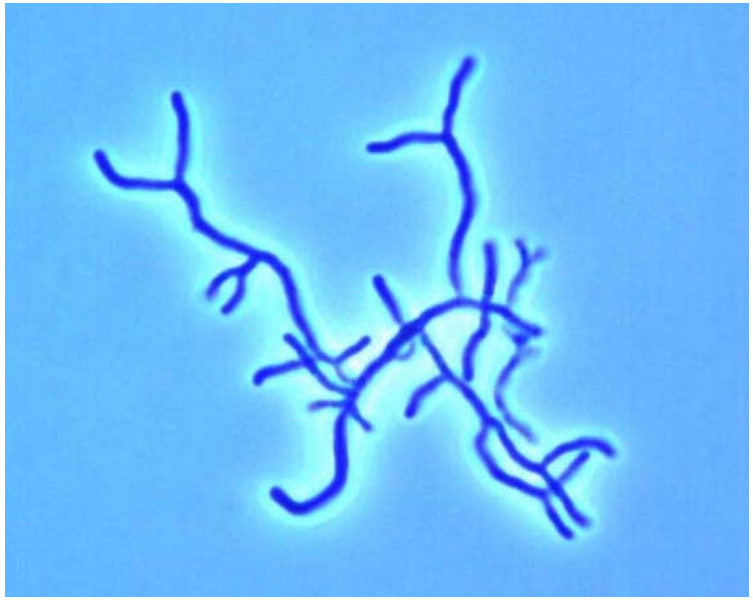
Therapeutic approaches

- When *H. pylori* infection is present, the most effective treatments are combinations of two or three antibiotics (e.g. amoxicillin, chlarithromycin, metronidazole and tetracycline) and one proton pump inhibitor, sometimes together with a bismuth compound

- The 3-year recurrence rate for gastric and duodenal ulcers is less than 10% when *H. pylori* is successfully eradicated but more than 50% when it is not

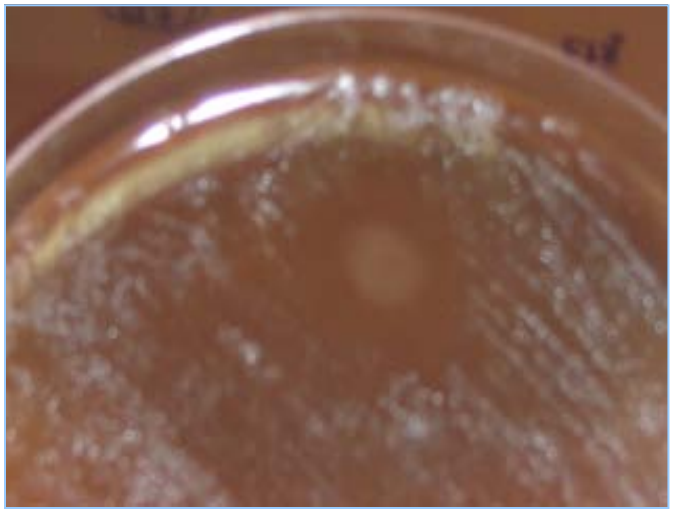
- Improved treatments and alternative therapies are still needed; in this sense, the use of probiotics is a potentially promising tool to prevent *H. pylori* infections

Probiotic strain from Biopolis SL



- A *Bifidobacterium bifidum* strain (CECT 7366) which proved active *in vitro* against *H. pylori* has been isolated, with inhibition levels reaching 81.94% in the case of supernatant
- The strain is stable under conditions of gastrointestinal stress (acid pH and high concentration of bile)
- This bacterial strain survives transit in rats following oral administration
- The genome of the strains is fully sequenced
- This novel strain fulfills the main properties required of a probiotic

Mechanisms of action

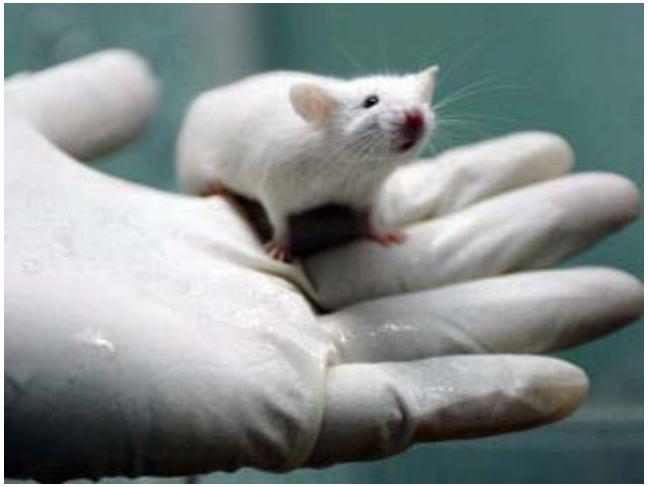


- There are two different mechanisms responsible for the effect
- The first one is direct ecological competence between the probiotic strain and the *H. pylori* cells.
- The second one is mediated by a peptide fraction with molecular masses below 3,000 Da produced by the probiotic
- This peptide has been identified at the amino acid sequence level

Safety assessment & scale-up production

- Furthermore, the absence of undesirable metabolites has been demonstrated
- Its food safety status confirmed by following the FAO and WHO guidelines
- The absence of undesirable metabolites has been demonstrated, and its food safety status has been confirmed by acute ingestion studies in mice; probiotic administration did not lead to bifidobacterial loads in organs or changes in histomorphology.
- Scale-up production of the strain has been accomplished at 3000

Preclinical trials



- *In vivo* studies using a BALB/c mouse model have been done
- Results indicated there were no signs of infection in spleen or in the mesenteric ganglion of those mice treated with the probiotic strain
- The group treated with vehicle developed more ulcers than the group treated with the probiotic
- Furthermore, *in vivo* assays have demonstrated that it partially relieves damage to gastric tissues caused by the pathogen

Publications & IPR

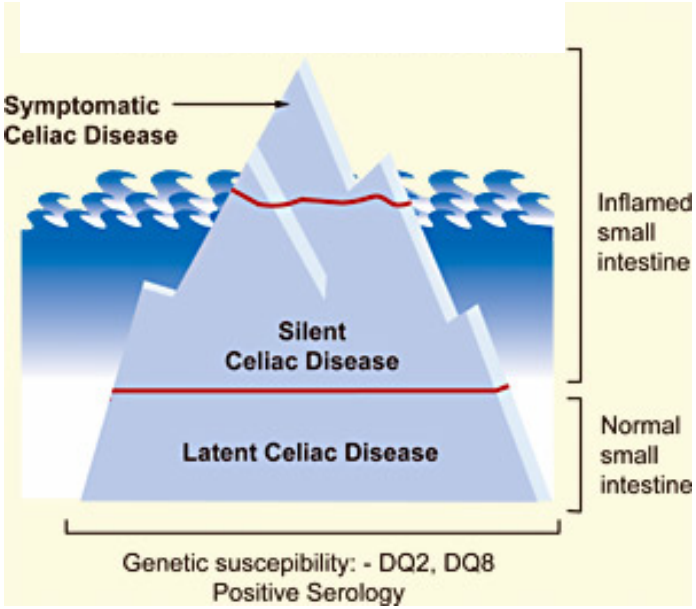
- Chenoll E, Casinos B, Bataller E, Astals P, Echevarria J, Iglesias JR, Balbarie P, Ramón D, Genovés S. (2011). Novel probiotic *Bifidobacterium bifidum* CECT 7366 strain active against the pathogenic bacterium *Helicobacter pylo*ri. Applied Environmental Microbiology 77: 1335-1343

COUNTRY	PATENT CODE
EU	EP09735168

Celiac disease (CD)

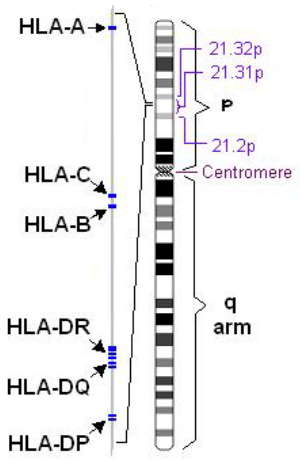
- An autoimmune disorder triggered by ingestion of gluten, a major protein in wheat, or of related proteins in other grains
- Research into the root causes indicates the disorder develops when a person exposed to gluten is also genetically predisposed to CD and has an unusually permeable intestinal wall
- Surprisingly, the same trio (an environmental trigger, genetic susceptibility and a “leaky gut”) seems to underlie other autoimmune disorders
- This finding indicates that new treatments for CD may also ameliorate other conditions

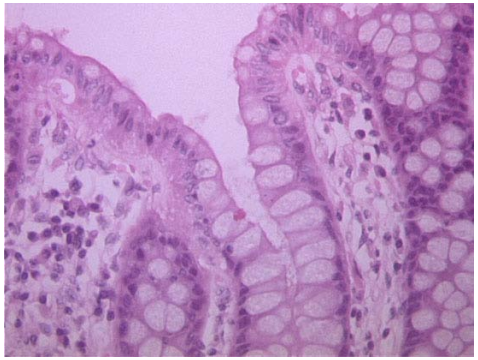
The celiac iceberg



- For many years CD was considered a rare disease in Europe
- In USA symptoms were detected in less than one person in 10,000 then in 2003 Dr. Fasano's team published a clinical study with 13,000 individuals showing that one in every 133 apparently healthy people was affected
- Noticeable symptoms of CD (permanent indigestion and chronic diarrhea) occur only when large areas of the small intestine are seriously damaged
- If the dysfunction affects only a small segment of bowel or inflammation is mild, the symptoms may be less severe or atypical

The celiac trio





PRESENCE OF GLUTEN AND GLUTEN PEPTIDES

GENETIC FACTORS (HLA-DQ2 OR HLA-DQ8)

CHANGES IN THE INTESTINAL PERMEABILTY AND MICROFLORA

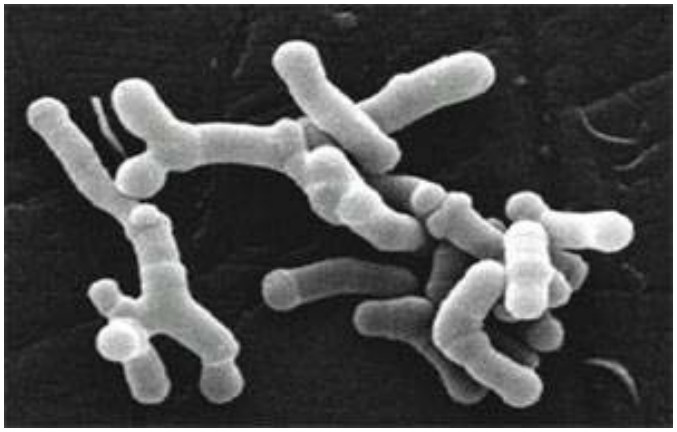
Therapeutic approaches

THERAPY	PRODUCT
Avoid gluten in diet during the first year of life	Diet
Degradation of gluten peptides	Enzymes ALV003 and AN-PEP(VU University); clinical trials at The Netherlands
Avoid zonulin permeability	Larazotide (Alba Therapeutics); clinical trials
Avoid transglutaminase modification	Numerate (Univ. Stanford)
Avoid binding of HLA-DQ2 or HLA-DQ8 to gluten peptides	Gluten-related molecules (Univ. Leive, Univ. Stanford)
Vaccine	Nexvax2 (Nexpep Australia); clinical trials
Avoid the presence of killer T cells	CCX282-B (Chemocentryx); clinical trials
Ancylostomosis by Anclystoma infection	Anclystoma larvae (Princess Alexandre Hospital in Australia); clinical trial

Gluten-free diet

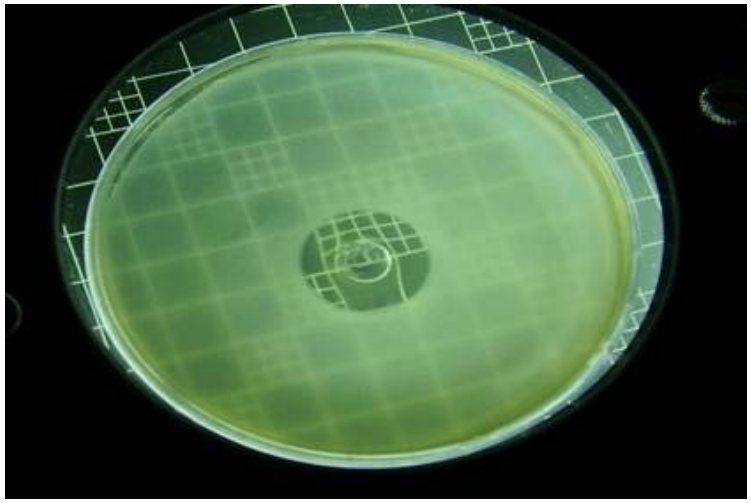
- At present, the only effective treatment for CD is a life-long gluten-free diet
- Dietitians inform CD patients which foods contain gluten, which are safe, and how to get a balanced diet despite the limitations
- Failure to comply with the diet may cause relapse
- The exact level at which gluten is harmless is uncertain and controversial
- Gluten-free products and diet are often low in vitamin B and D, calcium, iron, zinc, magnesium, and fiber

Probiotic strain from Biopolis SL



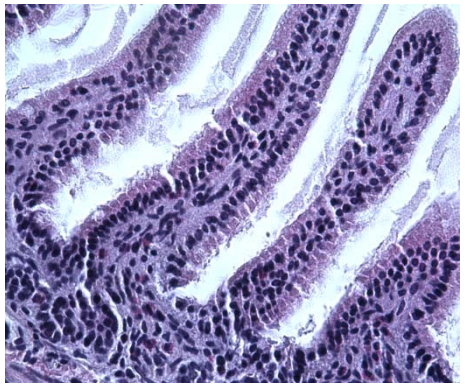
- Biopolis SL has the exclusive license of the CSIC strain *Bifidobacterium longum* ES1 (CECT 7347)
- This strain has immunomodulatory properties capable of regulating the Th1-type pro-inflammatory responses characteristic of CD and related diseases (multiple sclerosis, diabetes, ataxia, etc.), as well as the Th2-type immune responses characteristic of IgE-mediated dietary protein allergies, linked to ingestion of wheat and other cereal proteins
- The strain inhibits production of Th1 cytokine IFN-λ and pro-inflammatory cytokine IL-1, and boosts regulatory cytokines IL-10 and TGF-β production in peripheral blood mononuclear cells (PBMCs)

Other properties of the strain

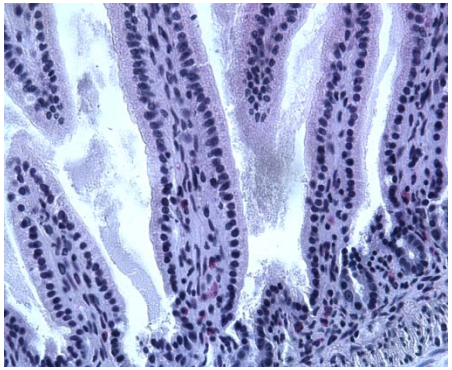


- The strain can adhere to mucin (1-4%) and is stable under conditions of gastrointestinal stress (acid pH and high concentration of bile)
- *In vivo* it survives transit in humans following oral administration
- It can also inhibit pathogenic bacteria with pro-inflammatory potential and virulence factors (isolated from the intestinal microbiota of celiac patients) helping to reestablish the intestinal balance

Preclinical work



Placebo



B. longum ES1

- *In vivo* studies using a mouse model prove this strain partially relieves CD related damage
- Ingestion of the strain downregulates expression of the gene encoding the pro-inflammatory cytokine TNF-α and upregulates expression of the gene coding for the anti-inflammatory cytokine IL-10
- At the cellular level, ingestion of this probiotic induces an increase in CD8+ cells while significantly reducing the percentage of CD4+ and CD4+/Foxp3; an increase in the latter is characteristic of celiac disease
- Finally, CD related histological damage improves in animals fed on this strain

Safety assessment & scale-up production

- This novel strain fulfills the main properties required of a probiotic
- Furthermore, the absence of undesirable metabolites has been demonstrated, and its food safety status confirmed by acute ingestion studies in mice following the FAO and WHO guidelines
- A clinical trial with celiac infants is now underway
- The genome of the strain is fully sequenced
- Scale-up production of the strain has been accomplished at 3000 L

Publications & IPR

- Izquierdo E, Medina M, Ennahar S, Marchioni E, Sanz Y. (2008). Resistance to simulated gastrointestinal conditions and adhesion to mucus as probiotic criteria for *Bifidobacterium longum* strains. Current Microbiology 56: 613-618
- Laparra JM, Sanz Y. (2010). Bifidobacteria inhibit the inflammatory response induced by gliadins in intestinal epithelial cells via modifications of toxic peptide generation during digestion. Journal of Cell Biochemistry 109: 801-807
- Laparra JM, Olivares M, Gallina O, Sanz Y. (2012). *Bifidobacterium longum* CECT7347 modulates immune responses in a gliadin-induced enteropathy animal model. PLoS ONE 7: e30744
- Medina M, de Palma G, Ribes-Koninckx C, Calabuig M, Sanz Y. (2008). *Bifidobacterium* strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. Journal of Inflammation 3: 5-19
- Olivares M, Laparra M, Sanz Y. (2011). Influence of *Bifidobacterium longum* CECT 7347 and gliadin peptides on intestinal epithelial cell proteome. Journal of Agricultural and Food Chemistry 59: 7666-7671

COUNTRY	PATENT CODE
Australia	AU2008341708
Brazil	PI0819533-1
Canada	CA2710666
China	CN101983237
European Union	EP2236598
Japan	JP2011507540
Mexico	MX2010007038
South Korea	KR20100110341
Spain	ES2343499
USA	US2010310520

Pitfalls & Risks

- More human clinical trials are necessary, mainly in the case of the probiotic against *H. pylori*
- Lack of efficiency in clinical trials
- Commercial inconsistency

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Partnering opportunities

•Licensing-out of probiotic strains

- Exclusivity for:
  - ✓Country or geographical area
  - ✓Sector
  - ✓Format

•The process involves:
  - ✓Clinical study
  - ✓Up-front payment for license exclusivity
  - ✓Royalties
  - ✓Support and preparation of EFSA dossier

- Contract R&D using proprietary screening and validation technological platform for develop new probiotics with novel functional effects

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