Biopolis SL

1. A brief overview of Biopolis SL
2. Probiotics portfolio
3. ES1: a probiotic against celiac disease
Biopolis SL

1. A brief overview of Biopolis SL
2. Probiotics portfolio
3. ES1: a probiotic against celiac disease
Origin and mission

- Biopolis is a biotech company, founded in 2003 as a spin-off of the National Spanish Research Council (CSIC)
- Our mission is to develop biotech solutions to cater to our clients’ needs (*tailor-made biotechnology*)
- We offer R&D services, industrial production of microorganisms and we out-license our own products
- All our services are provided promptly and in the strictest confidentiality
- Our global and transversal services aim to develop and launch marketable products
Biopolis R&D Centre

- Biopolis R&D Centre is located in a building measuring 1500 m² in the Scientific Park, Universitat de València, Spain
- Our facilities house eleven laboratories with several platforms and two production plants (non-GMO and GMO)
- All our facilities have been approved by the Spanish National Biosafety Committee
Platforms at Biopolis SL

- Biochemistry
- Cell culture
- Microbiology
- Model organisms
- Molecular biology
- Scale up
- Fermentation
- Genomics
- Metabolomics
- Murine models
Biopolis staff: our greatest asset

- Currently, 43 people work at Biopolis; most are PhD holders (18) or BSc (15); the rest are highly qualified technicians (10)
- Biopolis employs experts in: biology, biotechnology, pharmacy, food technology, agricultural and chemical engineering, IT and bioinformatics, economy and law
Services provided by Biopolis SL

- Food and feed
  - Functionality Assessment
  - Probiotics

- Fine chemistry and pharmaceuticals
  - Waste Recovery
  - API Synthesis
C. elegans assays run at Biopolis SL

- Antioxidant effect
- Longevity
- Inflammation
- Obesity
- Alzheimer
- Viral replication
- Bacterial colonization

- Isolated molecules
- Purification fractions
- Plant extracts
- Probiotics
- Beer
- Soft fizzy drinks
- Yoghurt
- Fruit juice
- Solid powdered products
Metagenomics and probiotics at Biopolis SL

- Samples can come from any source: feces, vaginal swab, oral swab, soil, air, etc.
- The v3, v5 region of 16S rRNA gene is isolated
- High-throughput sequencing is performed on the 454 FLX+
- Generated sequences are sorted based on quality criteria (Q20 threshold value) and sequence length
- Rarefaction analysis is performed
- Finally comparative bioinformatics identifies main genera and phyla, and main species (sequence count value above 1%)
Biopolis SL

1. A brief overview of Biopolis SL
2. Probiotics portfolio
3. ES1: a probiotic against celiac disease
Conventional *versus* specific probiotics

**Conventional probiotics**

- Nutritional supplements or added to a food matrix
- Sold in supermarkets, hypermarkets, parapharma or herb stores
- Typically mixtures of two or more probiotics are sold
- Require neither claims nor clinical trials
- Help recover intestinal flora after antibiotic treatment, improve intestinal tract and comfort, inhibit growth of spoilage or pathogenic microorganisms, maintain intestinal homeostasis and strengthen the body's natural defenses

**Specific probiotics**

- Nutritional supplements or added to a food matrix
- Sold in parapharma and drug stores/chemists
- Usually sold as a single strain
- Normally make claims and require clinical trials
- Besides the benefits of generics, specific probiotics target particular diseases, e.g., allergies, cholesterol, dermatitis, diarrhea, bacterial and viral pathogens, intestinal health, immune system or bacterial vaginosis
Our philosophy at Biopolis SL

- Biopolis SL has expertise in probiotic research and production
- Our premises are fully equipped for selection, identification, validation, food-safety assessment, scale-up and production
- We can isolate novel *a-la-carte* probiotics for our clients
- Our own culture collection holds specific and generic probiotic strains
- Our specific probiotics come with a robust scientific dossier
- Production process optimization means we can sell at low cost and ensure long shelf-life, adapting to customer demands
Collection of conventional probiotics

Selection
- From human breast milk or from feces of infants under three months old

Identification
- Molecular taxonomy by sequencing the variable region of the 16S ribosomal repeat unit

Safety
- Food safety assays, in vitro and in vivo, following WHO and FAO guidelines

Scale-up
- Production scale-up and optimization (1, 20 and 300 L)

Production
- Optimization of industrial production and drying; stability assays (under different conditions)

Bifidobacterium animalis subsp. lactis BPL1
Bifidobacterium breve BPL61
Bifidobacterium longum BPL65
Lactobacillus paracasei BPL62
Lactobacillus rhamnosus BPL15
Collection of specific probiotics

- Bowl inflammation: *Bifidobacterium longum* ES1
- Rotavirus: *Bifidobacterium longum* subsp. *infantis* CECT 7210
- *Helicobacter pylori*: *Bifidobacterium bifidum* CECT 7366
- Metabolic syndrome: *Bifidobacterium animalis* subsp. *lactis* CECT 8145
- Vaginosis: *Lactobacillus rhamnosus*
1. A brief overview of Biopolis SL

2. Probiotics portfolio

3. ES1: a probiotic against celiac disease
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

Gluten and gluten peptides

Genetic factors (HLA-DQ2/DQ8)

Changes in microbiota
Gluten and gluten peptides

- The majority of the CD related proteins are prolamines from wheat gluten

- Prolamins are storage proteins rich in proline and glutamine, partially resistant to protease and peptidase degradation in the human gut. Consequently prolamins release small peptides rich in both amino acids in the intestinal tract.

- The strongest and most common adaptative response to gliadin is directed toward an α-gliadin fragment of 33 amino acids in length named 33mer

- There are other CD related gliadin peptides and also other prolamins such as hordein from barley or secalin from rye, which release small peptides causing CD.
Genetic factors

- Most CD patients (95%) have one or two isoforms of HLA-DQ variants (HLA-DQ2 or HLA-DQ8), while in non-celiac populations the rate is around 30-40%.

- HLA-DQ2 is more frequent in peoples of North and Western Europe (highest frequencies Basque Country and Ireland) and parts of Africa and India but is not found along West Pacific rim.

- HLA-DQ8 is more widespread and is more prevalent in South and Central America.

- In CD patients tissue transglutaminase modifies gluten peptides by deamidation or transmaidation into a form that increases its binding to HLA-DQ receptors.

- HLA-DQ2 and HLA-DQ8 genes encode receptors that bind gliadin peptides more tightly than the other HLA-DQ variants.

- As a result, T-cell activation is more effective and triggers the autoimmune process.
Intestinal changes

- Biopsies show blunting of villi, crypt hyperplasia and lymphocyte infiltration of crypts
- At molecular level, there is a strong secretion of zonulin in the intestine. Consequently, intestinal permeability increases, facilitating the absorption of the gliadin peptides
- There are changes in intestinal microflora: more Gram negative bacteria (Bacteroides and E. coli) and less beneficial bacteria (Bifidobacterium)
- Increase in virulence factors in enterobacteria colonizing the intestine. Also *in vitro* tests indicate microbial flora stimulates pro-inflammatory cytokines (IFN-γ and TNF-α) more effectively but anti-inflammatory cytokines (IL-10) less effectively
Disbyosis (*Bacteroides*)

- *Bacteroides fragilis* group
  - *Faeces*
  - *Duodenal biopsies*

- **Faeces**
  - Active CD: 10, Non-active CD: 9, Healthy: 8
  - *P*=0.026

- **Duodenal biopsies**
  - Active CD: 7, Non-active CD: 6, Healthy: 5
  - *P*<0.014

- *Bacteroides fragilis* group
  - Active CD: 11, Non-active CD: 10, Healthy: 9
  - *P*<0.002

- *Bacteroides fragilis* group
  - Active CD: 8, Non-active CD: 7, Healthy: 6
  - *P*=0.038

Images of *Bacteroides* cells are also shown.
Disbyosis (Enterobacteriacea)

Faeces

Duodenal biopsies

P = 0.006

P = 0.030
Disbyosis (*Bifidobacterium*)

Faeces

Duodenal biopsies
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
The origin of ES1

- Strain ES1 was isolated by Dr. Yolanda Sanz’s team at the Institute of Agrochemistry and Food Technology (National Research Council) Valencia.
- It was isolated from the feces of a healthy breastfed child, under three months old.
- It was classified as a member of *Bifidobacterium longum* by molecular taxonomy (16S rRNA gene sequencing).
- This species is listed as GRAS and QPS by the Food and Drug Administration and the European Food Safety Agency, respectively.
- The strain is patented and deposited at the Spanish Type Culture Collection as CECT 7347, licensed exclusively to Biopolis SL.

Anti-inflammatory response in PBMC cells

- We performed a study with feces of celiac children on a gluten-free diet and feces from healthy children.
- Feces were coincubated with strain ES1 and strainless negative controls were designed. Samples were then used to stimulate peripheral blood mononuclear cells (PBMC) from healthy adults.
- Feces from celiac subjects boosted TNFα production and CD86 expression, and lowered IL-10 production and CD4 expression.
- Feces co-incubated with ES1 strain suppressed this pro-inflammatory response and increased IL-10 production.
Anti-inflammatory response in Caco-2 cells

- We also measured transcription levels of genes encoding the CXCR3, NFκβ and TNFα receptors.

- We analyzed levels of the corresponding proteins and of IL-1β and p50 by ELISA.

- Cell cultures coincubated with strain ES1 had lower transcription levels and, consequently, IL-1β, NFκβ, p50 and TNFα cell concentrations.
Anti-inflammatory response in dendritic cells

- We investigated the interaction of strain ES1 with monocyte-derived dendritic cells (MDDC) in combination with gliadin and Caco-2 cells.
- Unlike some pathogenic strains (*Escherichia coli* or *Shigella* CBD8), ES1 induced minor MDDC morphological changes and activated adhesion, spreading and inflammatory cytokine production to a lesser extent.
- Moreover, ES1 induced lower CD86 and CD40 expression, and reduced gliadin-induced IFN-γ production, but increased IL-10 secretion.
Preclinical trial in rats

• The effect of ES1 intake was evaluated in a gliadin-induced enteropathy model using newborn rats sensitized with IFN-γ and fed either ES1 or a placebo.

• Rats fed ES1 showed significant changes in intestinal epithelium morphology.

• Specifically, enterocyte height—a characteristic of celiac disease—was restored in the ES1-fed rats.
Anti-inflammatory response in animals

- We also analyzed immune system components and studied stool microbiota in both groups.

- The jejunums of ES1-fed rats contained less TNF-α and more IL-10.

- The gene encoding the NFκβ factor was upregulated in this organ, leading to an anti-inflammatory regulatory signaling response.
Food safety assessment *in vitro*

- Food safety assessment of ES1 followed FAO and WHO guidelines
- No problems were found related to undesirable metabolites (biogenic amine production, D/L-lactic acid ratio or deconjugation of bile salts)
- Break points for some 20 clinical antibiotics were defined without finding significant resistance
- Regarding toxicity, acute ingestion studies were done in mice (normal and artificially immunosuppressed)
Genome sequencing

- The ES1 genome was sequenced with high-throughput pyrosequencing technology
- ES1 genome size is around 2.5 Mb
- We have almost 75 Mb of information (approximately 30X genome sequence)
- ES1 does not contain genes related with pathogenicity, virulence or resistance to antibiotics
Production scale-up

- ES1 production was scaled up from the laboratory to the pilot plant, and from the pilot plant to industrial production
- Fully optimized fermentation process
- Scale up and optimization of drying and freeze-drying processes
A randomized, double-blind trial was done with 12 human volunteers (30.3 years, from 23 to 40 years) using the ES1 strain and a placebo.

For two weeks, participants received $10^9$ cfu of ES1 or placebo daily, then underwent a 2-week washing period, then a further period with a similar intake for 3 more weeks.

No adverse effects were detected after ES1 intake.

Fecal microbiota were similar in both groups but in the ES1 group, 70-80% of the *Bifidobacterium* strains were ES1.
Phase 2 clinical trial in celiac children

- A randomized, double-blind placebo-controlled trial was conducted in 40 CD children aged 2-14 years, using probiotic strain *B. longum* ES1 (Hospital Universitari Sant Joan de Reus and Hospital Sant Joan de Déu de Barcelona)
- For three months the participants received daily $10^9$ cfu of *B. longum* ES1 or placebo
- ES1 administration significantly decreased CD3+ T-lymphocytes and TNF-α concentration in peripheral blood
- Children ingesting ES1 experienced a statistically significant increase in height and also an increase in weight (albeit non-statistically significant)
Reverting dysbiosis

- Microbiota analysis indicated a decrease in *Bacteroides* and *Clostridium* in the ES1 group.
- Children receiving ES1 also had higher bifidobacteria and lactobacillus counts.
Scientific dossier on ES1

Proceliac: on the market

Proceliac

Producto específicamente diseñado para celiacos que contiene leche en polvo desnatada, el probiótico *Bifidobacterium longum ES1* y una serie de nutrientes que refuerzan la dieta del celiaco.

Este producto no sustituye la dieta libre de gluten.

Formato: caja de 14 sobres de 30 g o bote de 420 g
The future of ES1

- Novel products targeting celiacs
- Other inflammatory targets
- Symbiotics
Probiotic against rotavirus


Probiotics as immunity boosters


Biopolis SL
Parc Científic Universitat de València
C/ Catedrático Agustín Escardino Benlloch 9; Edificio B; 469809-Paterna; Valencia
Teléfono: 96-3160299