Review

Nutrafoods (2016) 15:157-160
DOI 10.17470/NF-016-1012-3

Bifidobacterium longum ES1: a probiotic with a strong anti-inflammatory phenotype

Salvador Genovés, Daniel Ramón

Correspondence to:
Daniel Ramón
daniel.ramon@biopolis.es

Keywords:
Probiotic;
Bifidobacterium longum ES1;
Anti-inflammatory properties;
Gut inflammation

Abstract

Bifidobacterium longum ES1 is a probiotic strain with an efficient anti-inflammatory effect which is supported by strong scientific evidence. This strain has been commercialized in several countries as an ingredient for functional foods or nutritional supplements. This article describes the scientific background supporting the phenotype of this probiotic strain.

Coeliac disease, gluten sensitivity and ES1 strain

Coeliac disease (CD) is a chronic enteropathy caused by an uncontrolled immune response to wheat gluten and similar rye and barley proteins in genetically susceptible individuals. It is the most common chronic disease with a prevalence of 0.7–2% in the general population. Non-coeliac gluten sensitivity (GS) is a condition that produces symptoms similar to those seen in CD, but it is not an autoimmune disorder and does not have a genetic component. Currently there are no therapeutic treatments for these conditions. The only alternative is to maintain a meticulous lifelong gluten-free diet (GFD). However, strict adherence to dietary measures is complicated because hidden gluten is found in many so-called 'gluten-free' foods. Consequently, the identification of new treatment alternatives, including specific probiotics, is required.

The *Bifidobacterium longum* ES1 probiotic strain was isolated by scientists at the Institute of Agrochemistry and Food Technology of the National Spanish Research Council (IATA-CSIC) from the faeces of a healthy infant [1]. The strain was deposited with the Spanish National Culture Collection under accession code number CECT 7347 and is protected by a patent that has been licensed exclusively to the Spanish biotech company Biopolis SL. The *B. longum* ES1 strain possesses the general properties of probiotics such as the capacity to inhibit the growth of bacterial pathogens, stability under conditions of gastrointestinal stress (acid pH and high concentration of bile), capacity to adhere to mucin (1–4%), and survival during food preparation and storage, such as refrigeration or lyophilisation [1]. However, the most interesting properties of this *Bifidobacterium* strain are its strong immune-modulatory properties and its capacity to degrade gliadin peptides (Fig. 1). These functional properties have been confirmed in several in vitro and in vivo tests, including human clinical trials.

Biopolis SL, Parc Cientific de la Universitat de València, C/ Catedrático Agustín Escardino 9, Edificio B, 46980-Paterna, Valencia, Spain

Received: April 05, 2016 / Accepted: April 21, 2016

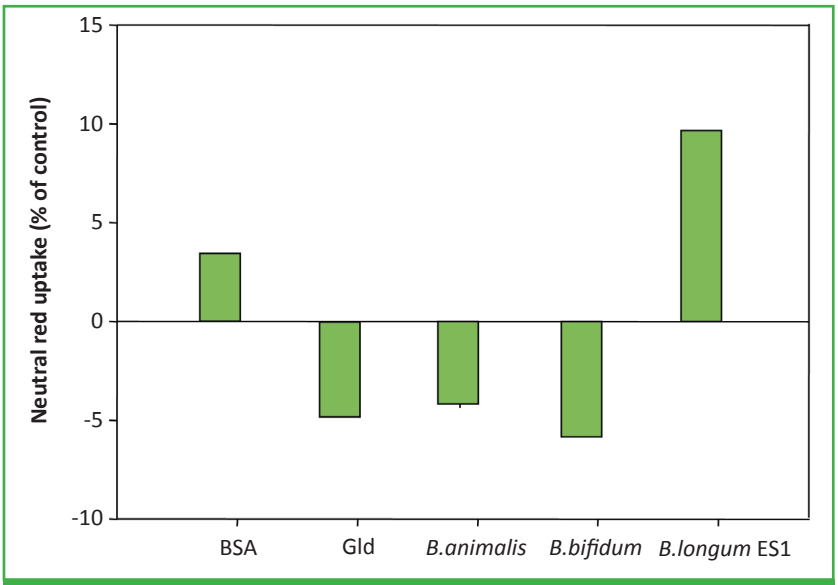

Figure 1 - Neutral red uptake percentages in cell cultures exposed to dialyzable fraction from digests of gliadins (Gld), inoculated or not with three different *Bifidobacterium species*. Negative control of toxicity is a digest of bovine serum albumin (BSA). The strain ES1 is the only one able to protect the cells.

The anti-inflammatory effect of ES1 in mammalian cell cultures

In a preliminary set of experiments on anti-inflammatory activity, the molecular pathways affected by the ES1 strain were elucidated using human cell culture systems. Co-incubation of human peripheral blood mononuclear cells (PBMCs) with the faeces of CD patients provokes a pro-inflammatory cytokine response, significantly increasing TNF-α production and CDC4 expression and decreasing levels of the anti-inflammatory cytokine IL-10. Using this model of inflammation, it has been possible to demonstrate that the ES1 strain was able to revert the pro-inflammatory response of the treated cells by significantly lowering levels of IFN-γ and TNF-α and increasing the production of IL-10. Lower expression of CD4 and CD86 was also detected. This response seemed to be mediated by the NF-κβ pathway [2].

The ES1 anti-inflammatory effect was also examined in an alternative model of inflammation using human colon carcinoma Caco-2 cells exposed to a commercial gliadin extract subjected to in vitro gastrointestinal digestion in the presence or absence of the probiotic strain. mRNA expression of the genes encoding NF-κβ, TNF-α and the chemokine CXCR3 receptor was quantified by RT-PCR and the production of the pro-inflammatory markers IL-1β, NF-κβ and TNF-α assessed by ELISA. Transcriptional analysis demonstrated down-regulation in mRNA expression of the three genes when the ES1 strain was added to the incubation media. Consistent with these results, the *Bifidobacterium* strain was able to reduce the levels of the three pro-inflammatory markers [3]. In a subsequent experiment, the proteomes of the Caco-2 cells exposed to both conditions were determined by 2DE and MALDI-TOF. In the case of the Caco-2 cells exposed to the gliadin-digested extract in the absence of the ES1 strain, a set of differentially expressed proteins involved in cell cytoskeleton disorganization, inflammation and apoptosis was detected. In contrast, gliadin-digested extract in the presence of the probiotic strain influenced the production of proteins involved in calcium homeostasis and cell survival and function [4]. Moreover, co-incubation of gliadins with the ES1 probiotic strain reduces their concentration and the presence of toxic peptides, indicating that this microorganism exerts strong specific proteolytic activity against gliadin and also has the capacity to bind to the toxic peptides derived from the digestion of gliadin.

A more sophisticated system of in vitro human cell inflammation was studied using monocyte-derived dendritic cells (MDDC). Co-incubation of MDDC with Caco-2 cells and enterobacteria isolated from CD patients provoked marked alterations in MDDC morphology, inducing podosome dissolution and dendrites, and activating MDDC adhesion and spreading. Moreover, inflammatory cytokines such as IFN-γ, IL-12 and TNF-α were also produced. In this system the co-incubation of *B. longum* ES1 strain yielded minor MDDC morphological changes and activated adhesion and spreading. Also the production of all measured pro-inflammatory markers was lowered, including the expression of CD40 and CD86. In contrast, the level of the anti-inflammatory cytokine IL-10 was increased [5].

ES1 animal trials

All these in vitro results were confirmed in an animal model using newborn rats subjected to gliadin-induced inflammation in the presence or absence of IFN-γ. The animals were fed placebo or the ES1 strain. Histopathological analysis of the jejunum revealed that the group of animals receiving placebo, whether or not they were sensitized with IFN-γ, showed high cellular infiltration, and reduced villi width and enterocyte height. These changes were partially restored in the groups of animals receiving the ES1 strain in their diet. Moreover, the placebo group sensitised with IFN-γ also had increased expression of the genes encoding NF-κβ and TNF-α and an increase in CD4+, CD4+/Foxp3+ and CD8+ T cell populations. In contrast, the group receiving the ES1 strain showed a reduction in TNF-α gene expression and an increase in the expression of the IL-10 encoding gene (Fig. 2). Also in this group there was a reduction in CD4+ and CD4+/Foxp3+ cell populations, indicating the strong anti-inflammatory response of the ES1 strain [6]. Finally, the jejunum proteome of the treated and non-treated animals was compared. Feeding gliadins caused a broad spectrum of changes in the jejunal proteome related to inflammation, intracellular ionic homeostasis, lipid turnover, cell motility and redox regulation. However, administration of the ES1 strain partially counteracted these changes [7].

It is important to note that gliadin consumption causes intestinal inflammation and mucosal damage commonly associated with the malabsorption of nutrients and ferropenic anaemia. In order to evaluate the role of the ES1 strain in alterations in hepatic Fe deposition and haemoglobin concentration, a study using adult Wistar rats fed gliadins and sensitized or not with IFN-γ was carried out.

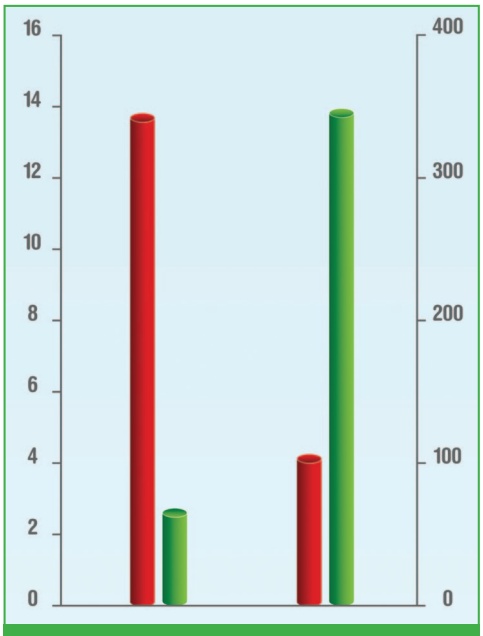

Figure 2 - Newborn rat subjected to gliadin-induced inflammation: production of TNF-α (red bars, ng/g tissue) and IL-10 (green bars, pg/g tissue) in the placebo (left) and ES1 (right) groups

Gliadin feeding increased hepatic Fe deposition and also decreased the expression of the Trf2 gene encoding the liver transferrin receptor. These effects were reversed after administration of the *B. longum* ES1 strain, indicating a putative beneficial effect of the strain in ferropenic anaemia [8].

Food safety assessment of the ES1 probiotic

The safety of the ES1 strain was evaluated following the joint FAO/WHO 'Guidelines for the evaluation of probiotics in food' [9]. The production of some undesirable metabolites such as aminogenic amines, isomers of lactic acid or bile salts was determined. Resistance to antimicrobials was also examined following the EFSA breakpoints [10]. The values for all these traits were very similar to those previously reported in other commercial *Bifidobacterium* probiotic strains. Moreover, an acute ingestion study of the ES1 strain was conducted at the Institut Pasteur de Montevideo (Uruguay) using immune-competent and immune-supressed BALB/c mouse models. No mortality, morbidity or translocation to any organ was detected in any group. Finally, whole genome sequencing of the ES1 strain was carried out using pyrosequencing technology. No relevant genes related to pathogenicity or virulence were detected [11]. The final safety evaluation step was a double-blind, randomized, placebo-controlled intervention trial with a cross-over design carried out in 12 adults to evaluate intake safety during 2 weeks of ES1 consumption in humans and the survival of the strain after gastrointestinal transit. No adverse effects were reported by any of the participants. Additionally, RAPD analyses of colonies isolated from the stool of volunteers indicated that the ES1 strain represented 70–80% of the total bifidobacteria depending on the in-

dividual, demonstrating its ability to survive gastrointestinal transit. These results all demonstrated the food safety status of the *B. longum* ES1 strain.

Human clinical trial

A double-blind, randomised, placebo-controlled intervention trial was carried out in order to evaluate the effects of *B. longum* ES1 ingestion over 3 months by 33 children with newly diagnosed CD and following a GFD. The results indicated a statistically significant height percentile increase in the group receiving the probiotic ES1 strain. Also, reductions in serum TNF-α and CD3+ and HLA-DR+ T lymphocytes were detected.

The results showed that the imbalance in the gut microbiota of coeliac patients following a GFD was counteracted by the parallel administration of ES1. In the placebo group the GFD resulted in significant increases in the ratio of harmless to potentially harmful bacteria (*Bacteroides*+Prevotella+*Escherichia coli*/*Bifidobacterium*+*Lactobacillus*).

In the group receiving the ES1 strain a decrease in *Bacteroides fragilis* was detected [12]. This decrease paralleled a decrease in faecal secretory IgA (sIgA) concentration, the most likely host secretion affecting the localisation, growth and composition of the gut microbiota.

Conclusions

B. longum ES1 is a probiotic strain with an anti-inflammatory phenotype supported by strong scientific evidence. This strain has potential to counteract the Th-1 biased response by inducing high production of the regulatory cytokine IL-10 and low production of the Th1 cytokine IFN-γ. Also its capacity to reduce sIgA and the parallel recovery of gut microbiome dysbiosis in subjects following a GFD are relevant. In conclusion, ES1 is an excellent probiotic for individuals experiencing gluten sensitivity and bowel inflammatory disease. The strain has been commercialized in several food products and nutritional supplements in Canada, the European Union and the USA.

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