BIFIDOBACTERIUM BIFIDUM ROSELL-71

The *Bifidobacterium* genus currently comprises 48 recognized species and is constituted of gram-positive, non-motile, often branched anaerobic bacteria. Bifidobacteria are typical gut inhabitants and represent the predominant bacterial group in the normal intestinal flora of healthy breast-fed newborns.

STRAIN PROPERTIES

1. IDENTIFICATION

Bifidobacterium bifidum is a normal inhabitant of the human colon. A substantial proportion of the Bifidobacterial population in the intestine of infants belong to the *Bifidobacterium bifidum* taxon. *B. bifidum* is now recognized as a highly interesting bacterial species able to benefit human health in the prevention and treatment of gastrointestinal dysfunctions and by interacting with the host immune system[1]. *B. bifidum* Rosell-71 is from human origin and has been selected for its profilic and probiotic qualities.

Strain Identification

Name: *Bifidobacterium bifidum* Rosell-71
Origin: Human
Molecules produced: L(+) Lactic acid, Acetate
Strain deposit: I-3426, CNCM *(Collection Nationale de Cultures de Microorganismes)*, Institut Pasteur, France.
Cell morphology: Irregular rods, non-sporulating, non-motile, isolated or in short chains, gram-positive, heterofermentative, strict anaerobe. Grows well in commercially available media for lactic acid bacteria (RCM, Reinforced Clostridial Medium) at 37°C (98°F) under anaerobic conditions and forms smooth beige colonies.

Phenotypic Identification

Carbohydrate fermentation pattern: API 50 CH
Biochemical characterization: API Zym

Genotypic Identification

16S rDNA sequence confirms the species designation and the specific identity of our strain. The complete *Bifidobacterium bifidum* Rosell-71 genome has been sequenced and annotated[2].

2. SAFETY

- In the QPS (Qualified Presumption of Safety) list published by the EFSA (European Food Safety Authority) (EFSA 2013).
- In the monograph for Live Microorganisms (probiotics) issued by Health Canada – Natural Health Products Directorate (NHPD) (NHPD 2014).
- In the Australian TGA (Therapeutic Goods Administration) list of “Substances that may be used in listed medicines in Australia” (TGA 2011).
- In the International Dairy Federation (IDF) and European Food and Feed Cultures Association (EFFCA) list of microorganisms (Bourdichon *et al.* 2012).
- Available Lallemand safety files in Common Technical Document format.

Antibiotic resistance

Screening for Minimal Inhibitory Concentration (MIC) has been completed with the recommended methods[3]. Microbiological breakpoints were based on the "Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance" by the EFSA Panel on Additives and Products or Substances used in Animal Feed (2012).
In addition, Lallemand Health Solutions has also customized a specific microarray for an optimal screening. This microarray is regularly updated, and more than 350 genes known to be associated with resistance to antibiotics are currently included.
Based on the current testing methods, *B. bifidum* Rosell-71 doesn't possess antibiotic resistance known to be transferable.

3. TECHNICAL FEATURES

Our team of fermentation specialists optimizes growth parameters, cryo-protection conditions and environmental controls to offer optimal probiotic survival.

- Rosell-71 is offered as standardized strain at 50 billion CFU/g.
- Rosell-71 is protected by Bio-Support™ technology.

STRAIN DOCUMENTATION

1. GASTROINTESTINAL SURVIVAL

Resistance to gastric acidity and bile

Microorganisms should remain alive, after passing through the stomach and the upper gastrointestinal (GI) tract, until they reach their target site of action - the lower small intestine.

In acidic conditions, *B. bifidum* Rosell-71 shows a survival rate of 91% (pH 4) and 13% (pH 3) after 2 hours[2].
A dynamic model of the human upper GI tract, the *In Vitro* Digestive System (IViDiS) model, was used to examine the survival of probiotic strains. It has been demonstrated that *B. bifidum* Rosell-71 survives the passage through the stomach and duodenum[2].

2. STUDIES

GASTROINTESTINAL HEALTH

Adhesion capacity:

- The *B. bifidum* species has been shown to adhere to human enterocytes. This property may be mediated through a surface protein also recognized for modulating the adhesion of *B. bifidum* to mucin as well as inducing bacterial aggregation. All these key colonization factors drive the establishment of *B. bifidum* cells in the human gut[1].

Barrier maintenance:

- *Muc3* is a mucin gene in the rat intestine with human homologue that exists as secreted glycoproteins. An oral administration of *B. bifidum* Rosell-71 in rats has shown an increased expression of *Muc3* mucin in the small intestine. This experiment demonstrates the property of *B. bifidum* Rosell-71 to enhance intestinal epithelial cells protective functions in rats[4].

Competition with intestinal pathogens:

- The effect of *B. bifidum* Rosell-71 on the adherence of Shiga toxin-producing *E. coli* O157:H7, also known as enterohemorrhagic *E. coli* (EHEC), has been determined. *E. coli* O157:H7 causes hemorrhagic colitis and hemolytic uremic syndrome. An *in vitro* study has demonstrated that *B. bifidum* Rosell-71 partially inhibits *E. coli* O157:H7 binding to intestinal epithelial cells in a dose-dependent manner, and showed higher inhibitory properties in competition assays than in exclusion assays (Fig.1). However, it is unable to displace *E. coli* O157:H7 bound to intestinal epithelial cells. These results suggest that *B. bifidum* Rosell-71 competes with pathogens for adhesion to epithelial cells[5].

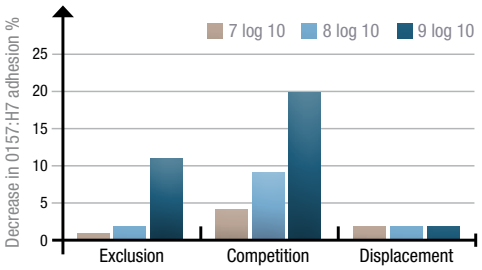

Figure 1: Effect of Bifidobacterium bifidum Rosell-71 on adhesion of E. coli 0157:H7

- *Exclusion: B. bifidum Rosell-71 was first added to intestinal epithelial cells, then E. coli.*
- *Competition: B. bifidum Rosell-71 and E. coli were added simultaneously to intestinal epithelial cells.*
- *Displacement: E. coli was first added to intestinal epithelial cells, then B. bifidum Rosell-71.*

Level of circulating cholesterol:

- *B. bifidum* Rosell-71 has bile salt hydrolase (BSH) activity which allows the deconjugation of bile salts, therefore decreasing the level of circulating cholesterol[2].

IMMUNE HEALTH

Downregulation of inflammatory response:

- To understand the immune modulatory properties of *B. bifidum* Rosell-71, a specific custom microarray has been designed to study the immune response of intestinal cells. The experiment demonstrated an attenuating impact of *B. bifidum* Rosell-71 on the pro-inflammatory response of intestinal epithelial cells HT-29 when compared to the poly(I:C)-only challenge. Poly (I:C) is a synthetic double-stranded RNA ligand, accepted as a challenge model investigating the responses of the innate immune system. This experiment performed on *B. bifidum* Rosell-71 + poly (I:C) co-challenge demonstrated as well an impact at reducing the number of expressed genes connected to pro-inflammatory responses[2].
  Using the same model of infection, it was previously shown that Probiokid® (a blend of *B. bifidum* Rosell-71, *B. infantis* Rosell-33 and *L. helveticus* Rosell-52) was able to attenuate the immune response (T_H1-pro-inflammatory) of intestinal epithelial cells co-challenged with poly(I:C)[6].

Th-1 and Th-2 models of infection:

- In a study carried out on *B. bifidum* Rosell-71 and parasitic-origin inflammation (*Nippostrongylus brasiliensis* - Th-2 model) in the rat, it was shown that *B. bifidum* Rosell-71 has specific and single cytokinergic profiles. As preventive medication administered for 10 days before induction of the ignition, *B. bifidum* Rosell-71 has a very significant effect by reducing secretion of pro-inflammatory-type cytokines such as TNF-αand by modulating the secretion of regulating-type IL-10 cytokines (Fig. 2 and 3)[7].

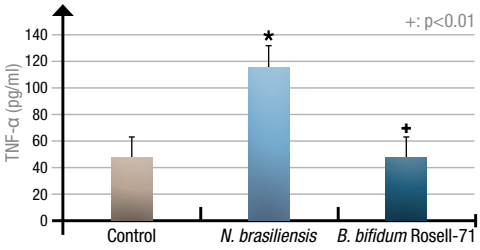

Figure 2: Effect of B. bifidum Rosell-71 on TNF-α production during parasitic origin inflammation in the rat.
**Significant difference vs. control (not infected)*
+ Significant difference vs. N. brasiliensis (infected, not treated)

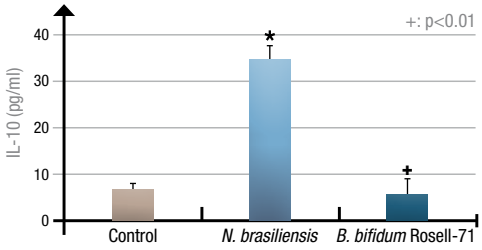

Figure 3: Effect of B. bifidum Rosell-71 on IL-10 production during parasitic origin inflammation in the rat.
**Significant difference vs. control (not infected)*
+ Significant difference vs. N. brasiliensis (infected, not treated)

- An *in vivo* study (n=23) has demonstrated that ProbioKid® synergistically modulates the level of circulating inflammatory immune factors in Th-1 and Th-2 models of infection (*E. coli* and *N. brasiliensis* respectively). Indeed, levels of the pro-inflammatory modulators (IL-1α, IL-1β, IL-6, IFN-γ and TNF-β) was significantly lower in ProbioKid® group than in the control group ($p<0.01$). Furthermore, levels of the anti-inflammatory modulators (IL-4 and IL-10) was also significantly increased in the ProbioKid® group compared with the control group ($p=0.004$)[7].

WINTER INFECTIONS IN CHILDREN

- Effect of daily supplementation with ProbioKid® (*B. bifidum* Rosell-71, *B. infantis* Rosell-33 and *L. helveticus* Rosell-52) has been investigated in a randomized, double-blind, placebo-controlled study. This study was conducted in 135 healthy, school-aged children, who suffered from at least three episodes of ear-nose-throat (ENT), bronchopulmonary or gastric disorder during the course of the previous winter. It has been shown a significant reduction of the risk of any infectious event by 25% when compared to placebo (Fig.4), accompanied by a significant reduction of school absenteeism (Fig.5)[8].

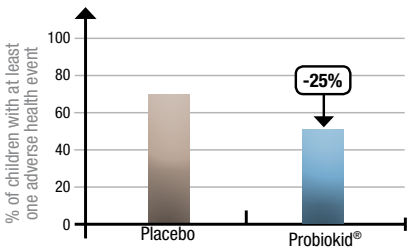

Figure 4: Effect of ProbioKid® on the relative risk of infectious disease in children. p=0.044

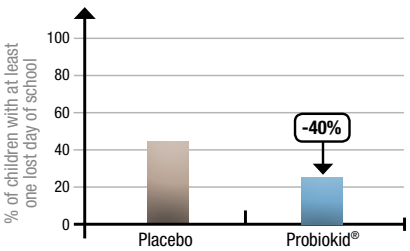

Figure 5: Effect of ProbioKid® on the number of children who lost at least one day of school because of an adverse health event. p=0.043

COLD AND FLU IN ACADEMICALLY STRESSED STUDENTS

- The effect of *B. bifidum* Rosell-71 on the percentage of healthy days in academically stressed students has been studied in a randomized, double-blind, placebo-controlled trial conducted on 581 participants. The model of acute psychological stress used in the study is associated with increased incidence of cold/flu. It has been shown an increase in the proportion of healthy days ($p<0.05$) equivalent to a reduction by 45% of the likelihood that a participant would report a cold/flu during a day while on the probiotic compared to those on the placebo (Fig.6). Proportion of participants reporting more than one day of cold/flu during the 6-week intervention period was also significantly lower with *B. bifidum* Rosell-71 than with placebo ($p<0.05$) (Fig.7). This translates into fewer and shorter episodes of cold/flu with an average reduction of symptoms of approximately a half day. The use of statistical predictive models also highlighted the impact of the level of stress and the gender on cold/flu incidence. The probiotic effect was all the more important in these susceptible groups[9].

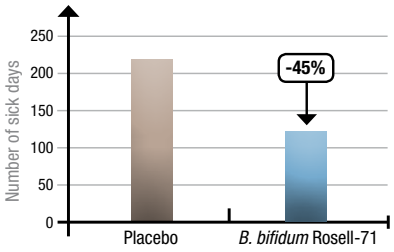

Figure 6: Effect of B. bifidum Rosell-71 on the number of sick days in academically stressed students. p<0.05

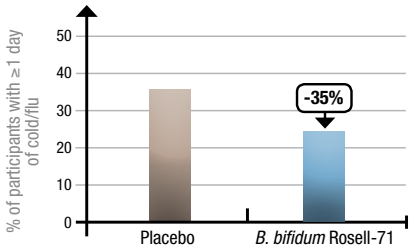

Figure 7: Effect of B. bifidum Rosell-71 on the proportion of academically stressed students reporting more than 1 day of cold/flu. p<0.05

1- Turroni F. *et al.* (2014) *Bifidobacterium bifidum* as an example of a specialized human gut commensal. Front Microbiol. 21;5:437

2- Lallemand Internal Report

3- ACE-ART (CT-2004-506214)

4- Dykstra N.S *et al.* (2011) Pulse probiotic administration induces repeated small intestinal *Muc3* expression in rats. Pediatr. Res. 69(3): 206-11

5- Kostrzynska M. *et al.* (2002) The effect of probiotic organisms on the adhesion of *Escherichia coli* O157:H7 to intestinal epithelial cells. Presented at the 4th Food Network meeting, Lacombe, Alberta, Canada

6- Macpherson C. *et al.* (2014) Multi-strain probiotic modulation of intestinal epithelial cells' immune response to a dsRNA ligand, polyinosinic:polycytidylic acid. Applied and Environmental Microbiology. 80(5):1692-700

7- Cazzola M. *et al.* (2010a) Immunomodulatory impact of a symbiotic in TH1 and TH2 models of infection. Ther Adv respir Dir. 4(5): 259-270

8- Cazzola M. *et al.* (2010b) Efficacy of a synbiotic supplementation in the prevention of common winter diseases in children: a randomized, double-blind, placebo-controlled pilot study. Ther Adv Respir Dis. 4(5), 271-278

9- Langkamp-Henken B. *et al.* (2015) *Bifidobacterium bifidum* R0071 results in a greater proportion of healthy days and a lower percentage of academically stressed students reporting a day of cold/flu: a randomised, double-blind, placebo-controlled study. British Journal of Nutrition. doi: 10.1017/S0007114514003997.

For more information, please contact: **healthsolutions@lallemand.com** or **www.lallemand-health-solutions.com**