

LACTOBACILLUS HELVETICUS

ROSELL-52

- The *Lactobacillus* genus currently consists of over 125 species and encompasses a wide variety of organisms. These organisms are strictly fermentative, aerotolerant or anaerobic and have complex nutritional requirements.

STRAIN PROPERTIES

1. IDENTIFICATION

Lactobacillus helveticus is a lactic acid bacterium widely used in the manufacture of fermented dairy products. In addition to the technological importance, some strains of *L. helveticus* also show health-promoting properties⁽¹⁾. The bacterium's specific name is an adjective derived from "Helvetia", the Latin name for the region occupied by the ancient Helvetii (and for modern Switzerland). *L. helveticus* Rosell-52 is from a dairy origin and has been selected for its prolific and probiotic qualities.

Strain Identification

Name: *Lactobacillus helveticus* Rosell-52

Origin: Dairy

Molecules produced: L(+) Lactic acid, D(+) Lactic acid, traces of Acetic acid, and Folate. *L. helveticus* Rosell-52 shows an elevated proteolytic activity which maximizes the probability of releasing bioactive peptides⁽¹⁾.

Strain deposit: I-1722, CNCM (*Collection Nationale de Cultures de Microorganismes*), Institut Pasteur, France.

Cell morphology: Rods, non-sporulating, non-motile, gram-positive, obligately homofermentative, facultative anaerobe; tends to form pairs or short chains. Grows well in commercially available media for lactic acid bacteria (MRS, Mann Rogosa and Sharp) at 37°C (98°F) and forms small white colonies.

Phenotypic Identification

Carbohydrate fermentation pattern: API 50 CH

Biochemical characterization: API Zym

Genotypic Identification

Multi-Locus Sequence Typing (MLST) confirms the species designation and the specific identity of our strain⁽²⁾. *L. helveticus* Rosell-52 was formerly identified as *Lactobacillus acidophilus* and reclassified as *Lactobacillus helveticus* in 2006⁽³⁾. The complete genome of *L. helveticus* Rosell-52 has been sequenced and annotated⁽⁴⁾.

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 2012)

- In the Australian TGA (Therapeutic Goods Administration) list of “Substances that may be used in Listed medicines in Australia” (TGA 2011)
- In the Brazilian ANVISA (Agência Nacional de Vigilância Sanitária) list of “Bioactive substances and probiotics” (ANVISA 2014)
- 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⁽⁶⁾. 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, *L. helveticus* Rosell-52 doesn't possess any 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-52 is available as “non-dairy” strain.
- Rosell-52 is offered as standardized strain at 150 billion CFU/g
- Rosell-52 is protected by Bio-Support™ technology and available with Probiocap® technology.

STRAIN DOCUMENTATION

1. GASTROINTESTINAL SURVIVAL

Resistance to gastric acidity and bile

Probiotic strains 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, *L. helveticus* Rosell-52 shows a survival rate of 53% (pH4) and 47% (pH3) 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. *L. helveticus* Rosell-52, delivered in a capsule form, remains stable under normal stomach and duodenal conditions⁽⁶⁾.

Survival in faecal samples after transit

A trial done on healthy individuals who received a dietary supplement containing *L. helveticus* Rosell-52 demonstrates survival of the strain through the human intestinal tract. No alteration of the microbiota was observed in healthy subjects⁽⁷⁾.

2. STUDIES



GASTROINTESTINAL HEALTH

Adhesion capacity:

- *L. helveticus* Rosell-52 is able to bind to intestinal epithelial cell lines (T84 and Caco-2) which may be important for competition with pathogens, stimulation of mucus production and modulation of the host immune system (Fig. 1)^(8,9).

Barrier maintenance:

- In challenge tests with *E. coli*, *L. helveticus* Rosell-52 has been shown to protect the integrity of the epithelium by limiting the loss of trans-epithelial electrical resistance (TEER), a measure of barrier integrity⁽⁸⁾.
- Surface-layer proteins (Slps), crystalline envelope of the outer bacteria cell surface, allows *L. helveticus* Rosell-52 to have a strong adhesion capacity to intestinal epithelial cells and to occupy the attachment sites of pathogenic bacteria such as *E. coli*, thereby avoiding pathogen adhesion and translocation⁽¹⁰⁾.
- By decreasing *E. coli* adhesion capacity, *L. helveticus* Rosell-52 blocks cytoskeletal rearrangements by *E. coli* and thus maintains tight junctions of intestinal epithelial cells^(8,9).

Inhibition of intestinal pathogens growth and adhesion:

- *L. helveticus* Rosell-52 is able to inhibit the adhesion of both enteropathogenic (EPEC) *E. coli* O127:H6 and enterohemorrhagic (EHEC) *E. coli* O157:H7, the cause of protracted diarrhea and hemorrhagic colitis, to intestinal epithelial cells in a concentration-dependent manner *in vitro*⁽⁸⁾.
- *L. helveticus* Rosell-52 is able to inhibit *Citrobacter rodentium* growth (a close relative of *E. coli*) in a time-dependent manner *in vitro*⁽¹¹⁾.
- Another *in vitro* study has demonstrated that *L. helveticus* Rosell-52 inhibits *Campylobacter jejuni* invasion (a common bacterial causes of enterocolitis in humans) (Fig. 2)⁽¹²⁾.
- *L. helveticus* Rosell-52 has been shown to inhibit the growth and decrease the viability of diarrhoeagenic *Salmonella typhimurium*⁽⁹⁾.
- *L. helveticus* Rosell-52 was also able to partially inhibit adherence of *Helicobacter pylori* to human epithelial cells in a dose-dependant manner in an *in vitro* cell model (Fig. 3)⁽¹³⁾.

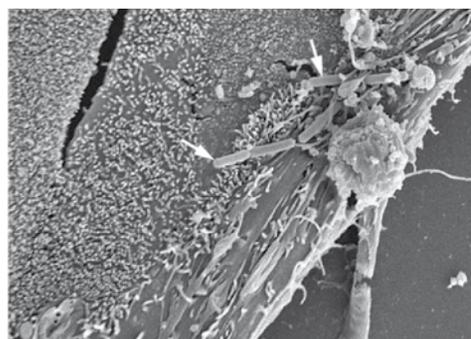


Figure 1: Adhesion of Rosell-52 to T84 epithelial cells. Courtesy of Dr. Phil Sherman, Hospital for Sick Children, Toronto, Canada.

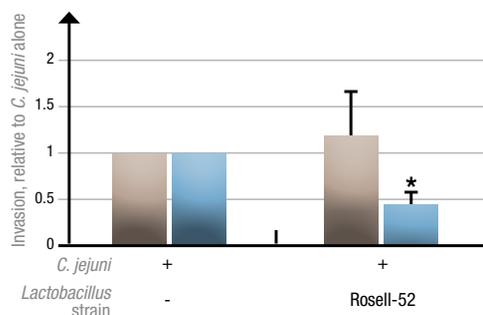


Figure 2: Strain-specific inhibition of *Campylobacter jejuni* invasion of intestinal epithelial cells ($p < 0.0005$)

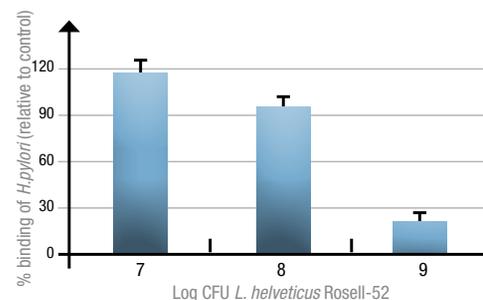


Figure 3: Competition of *H. pylori* adherence to human epithelial cells by *L. helveticus* Rosell-52.

In a review performed on a combination of *L. helveticus* Rosell-52 with *L. rhamnosus* Rosell-11 (Lacidofil®), it was showed that the probiotic blend could have a beneficial effect on antibiotic-associated diarrhea (AAD) and acute gastroenteritis, irritable bowel syndrome (IBS), *Helicobacter pylori* eradication and lactose intolerance⁽¹⁴⁾.

ANTIBIOTIC-ASSOCIATED DIARRHEA (ADD): Several randomized trials conducted on the blend of *L. helveticus* Rosell-52 and *L. rhamnosus* Rosell-11 have demonstrated its efficacy to prevent antibiotic-associated diarrheas when used during antibiotherapy, especially in children who are more sensitive to ADD^(15,16).

IRRITABLE BOWEL SYNDROME (IBS): A open study (50 patients) has shown that the administration of *L. helveticus* Rosell-52 and *L. rhamnosus* Rosell-11 improved clinical symptoms in IBS patients with longstanding symptoms. It has been demonstrated an improvement of frequency and consistency of stools for 84% of the patients and a reduction of flatulence for 62% of the patients⁽¹⁷⁾.

HELICOBACTER PYLORI ERADICATION: Two clinical trials (397 patients in total) have demonstrated that the addition of *L. helveticus* Rosell-52 (one of the two strains of Lacidofil®) to usual eradication therapy improves eradication efficacy of *H. pylori* and reduces complications after antibiotic therapy^(18,19).

LACTOSE INTOLERANCE: Tested in patients suffering from lactose intolerance, the combination *L. helveticus* Rosell-52 and *L. rhamnosus* Rosell-11 has been shown to improve the quality of life of lactose intolerant patients by reducing abdominal pain, number of stools, and improving lactose adsorption⁽²⁰⁾.



IMMUNE HEALTH

Modulation of the immune response:

- *L. helveticus* Rosell-52 increases circulating antibodies IgM (produced when a body is challenged by antigens) and IgG (secondary antibody response as a maturation of antibody response) *in vitro*. Consequently, it increases the pool of antibodies able to react quickly to infection (Fig.4)⁽²¹⁾.
- The production of the pro-inflammatory cytokine IL-8 by the HT-29 cell line (gastric epithelial cells stimulated by pathogen presence or inflammation), whose function is to stimulate trans-endothelial migration of neutrophils to a site of infection, is significantly down-regulated by *L. helveticus* Rosell-52 *in vitro*⁽²²⁾.
- A *Nippostrongylus brasiliensis* parasite infection in rats leads to a strong increase in both Th-2 (IL-4, IL-5, IL-6, IL-10) and Th-1 (TNF- α , IFN- γ) cytokine levels. Giving *L. helveticus* Rosell-52 treatment significantly decreases the *N.brasiliensis* effect on intestinal mucosa cytokine levels such as IL-10 and the values obtained are similar to uninfected controls (Fig.5). It is suggested that *L. helveticus* Rosell-52 exerts an immunomodulatory effect which can positively influence functional parameters in either Th-1 and Th-2 oriented physiopathological conditions⁽²³⁾.

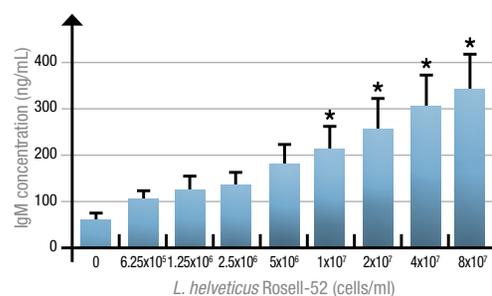


Figure 4: Influence of *L. helveticus* Rosell-52 (cells/mL) on the IgM concentration (ng/L). *p<0,01 (compared to control)

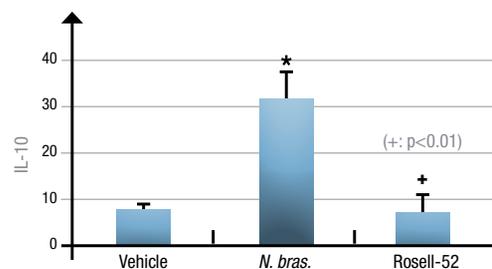


Figure 5: Serum concentration of IL-10 in rats pretreated with *L. helveticus* Rosell-52 or vehicle, to a challenge of *N. brasiliensis*.

WINTER INFECTIONS IN CHILDREN: A preparation containing *Lactobacillus helveticus* Rosell-52, *Bifidobacterium infantis* Rosell-33 and *Bifidobacterium bifidum* Rosell-71 (Probiokid®) has been evaluated in 135 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. This study showed a significant reduction of the risk of any infectious event by 25% when compared to placebo, accompanied by a significant reduction of school absenteeism⁽²⁴⁾.



WOMEN'S HEALTH

- **Adhesion capacity:** Quantitative analysis of adhesion shows that *L. helveticus* Rosell-52 displayed concentration-dependent adhesion in cervical HeLa cells (Fig.6)⁽⁹⁾.
- **Protection against urogenital pathogens:** An *in vitro* study demonstrated the antagonist activities of *L. helveticus* Rosell-52 against vaginosis-associated (*Gardnerella vaginalis* and *Prevotella bivia*) and uropathogenic (*Escherichia coli*) pathogens. *G. vaginalis* and *P. bivia* are associated with bacterial dysbiosis whereas *E. coli* may cause urinary tract infections (Fig.7)⁽⁹⁾.

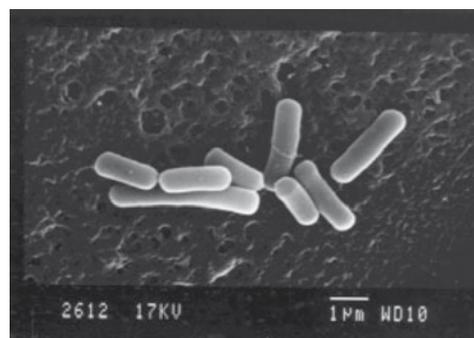


Figure 6: Adhesion of Rosell-52 to human cervical HeLa cells. Courtesy of Dr. F. Atassi, Institut National de la Santé et de la Recherche Médicale, Paris, France.

DECREASED INCIDENCE OF VAGINAL DYSBIOSIS:

- A randomised controlled study has been performed on 96 women receiving prophylactic antibiotic therapy after Cesarean section delivery. It has been shown that 89.3% of patients receiving a supplementation of *L. helveticus* Rosell-52 (in combination with *L. rhamnosus* Rosell-11) with antibiotic therapy were considered eubiotic (i.e. having a balanced microbiome) following therapy, while none of the patients of the control group were eubiotic⁽²⁵⁾.
- In another study involving 103 pregnant women, *L. helveticus* Rosell-52 (in combination with *L. rhamnosus* Rosell-11) was given before and after cesarean operation. It was shown a reduced opportunistic microflora (*Candida albicans*, *Staphylococcus aureus* and *E. coli*) with a preventive supplementation of the probiotic combination, and a decreased colonization of amniotic fluid and gastrointestinal tract of new born⁽²⁶⁾.

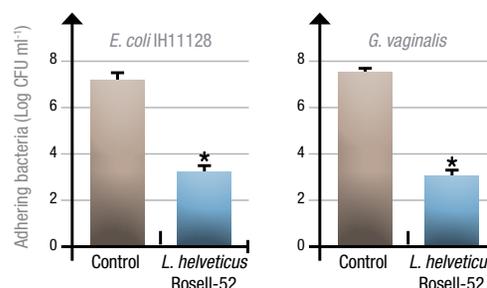


Figure 7: *In vitro* antibacterial activity of *L. helveticus* Rosell-52 ($p < 0.05$)



PHYSICAL AND EMOTIONAL EQUILIBRIUM

Today, it appears that the microbiota plays a role in the bidirectional dialogue between the brain and the gut. Thus, probiotics capable to interact with the digestive microbiota can have an effect on psychological troubles such as stress and anxiety.

- In a preclinical study, it has been shown that oral administration of Probio'Stick®, a combination of *L. helveticus* Rosell-52 and *B. longum* Rosell-175 has a significant anxiolytic-like effect in a rat model (n=36) comparable to Diazepam, an anxiolytic sedative used as a positive control⁽²⁷⁾.
- In a rat model of post-myocardia infarction depression, a condition associated with increased mortality in human, probiotics had a preventive effect against depressive behaviour and decreased the tendency of brain cells to enter into apoptosis (also named programmed cell death, a form of cellular suicide) in areas of brain associated with mood and behaviour (limbic and hippocampic areas). This mechanism could involve pro-inflammatory cytokines and lead to the restoration of the integrity of the intestinal barrier^(28,29).

IMPROVEMENT OF PHYSICAL AND PSYCHOLOGICAL SYMPTOMS OF STRESS:

- A clinical trial (n=75) has shown that a daily administration of *L. helveticus* Rosell-52 associated with *B. longum* Rosell-175 allows a significant reduction of physiological symptoms associated with chronic stress, in particular abdominal pain and nausea⁽³⁰⁾.
- A clinical study conducted on 55 subjects, has revealed that this blend allows a reduction of anxiety and depression signs in human volunteers after 30 days of treatment, using psychological tests (HADS, Hospital Anxiety and Depression Scale and HSCL-90, The Hopkins Symptom Checklist-90) (Fig.8) and a biomarker of stress (cortisol)⁽³¹⁾.

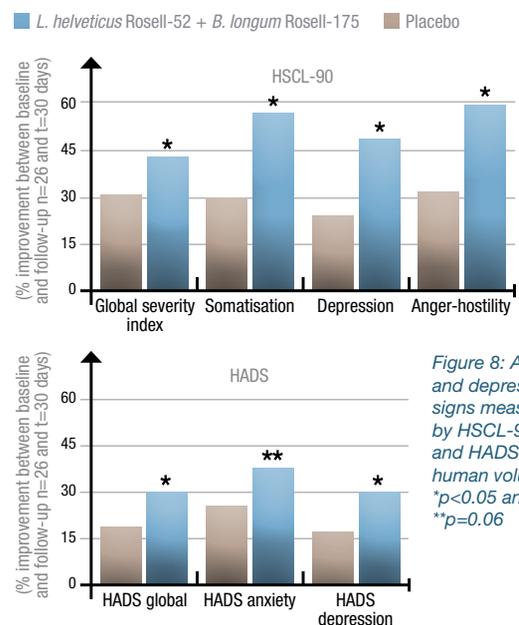


Figure 8: Anxiety and depression signs measured by HSCL-90 and HADS on human volunteers *p<0.05 and **p=0.06



ATOPIC DERMATITIS

IMPROVEMENT OF ATOPIC DERMATITIS IN CHILDREN:

- A clinical trial, conducted on 58 children, has shown that Lacidofil® (*L. helveticus* Rosell-52 and *L. rhamnosus* Rosell-11) is able to significantly reduce clinical signs of atopic dermatitis in children (measured with SCORAD, SCORing Atopic Dermatitis - clinical tool), increasing their quality of life⁽³²⁾.
- **Immunomodulation properties:** In the same study, a significant increase of the antibody IgG4 was observed after *L. helveticus* Rosell-52 and *L. rhamnosus* Rosell-11 treatment, signifying a better tolerance to cow milk. Moreover, it has been demonstrated a decreased activation of T-cells, indicating a reduced immune response⁽³²⁾.

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

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