

ProBactilardii®

DuoLife CLINICAL FORMULA PROBACTILARDII® is a comprehensive food supplement, consisting of 2 complementary capsules. The *Synbiotic* capsule contains a prebiotic (in the form of acacia fibre) and the proprietary BACTILARDII® probiotic formula, based on 4 bacterial strains and 1 yeast strain. BACTILARDII® is a formula created exclusively for DuoLife by a world expert in the field of probiotics – the Canadian company Lallemand. The *Postbiotic* capsule – contains a postbiotic in a formula based on butyric acid. This food supplement features not only an innovative composition, but also a patented formula and patented direct packaging, ensuring maximum protection of microbial strains contained inside the capsule.



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The preparation is composed of natural ingredients of the highest quality. The pro-health effect of the microbial strains contained in the dietary supplement as well as acacia fibre and butyric acid has been documented by numerous clinical studies (over 100 clinical trials).

PROBIOTICS are products containing selected live strains of microorganisms that have a beneficial effect on the human body by improving the balance of the intestinal microbiota¹.

PREBIOTICS are products that are selectively used by beneficial microorganisms of the microflora (mainly intestinal, but not only) in humans, bringing health benefits to the human body¹.

POSTBIOTICS are defined as the combination of all bioactive ingredients produced by bacteria, e.g., during fermentation, which are beneficial when introduced into the human body¹.

When?

The intestinal microflora of a healthy human includes approximately 100 trillion (!) beneficial microorganisms (bacteria, fungi and even viruses), by number exceeding 10 times the total number of cells in the human body. As many as 500–1000 useful species can live in the human intestines, most of them in the large intestine – up to 1 trillion per gram of the intestinal content, accounting for 80% of the dry weight of faeces². The number of genes in the microbiome is approx. 150 times the number of human genes³. The composition of the microbiota depends on many factors, however bacteria of the genus *Bacteroides*, *Bifidobacterium* and *Lactobacillus* are almost always dominant. In addition to genetic determinants, the composition of intestinal microflora can be affected mainly by age, diet, geographical region, health conditions, and medicines used.

The pro-health effects of microorganisms living in the digestive tract are documented by thousands of clinical studies and scientific reports. A number of metabolic and immunological reactions necessary for the proper

functioning of the human body occur only due to the presence of beneficial microflora. Microbiome provides good gastrointestinal function, good intestinal peristaltic, optimum availability of group B vitamins and vitamin K for the human body. It promotes nutrient absorption, supports intestinal barrier functions and immune system performance. It protects intestinal epithelial cells to help protect the body from toxins and free radicals. Moreover, the positive effect of the intestinal microflora on the maintenance of normal brain functions has been proven^{3,4}.

The composition and number of the microbiome may be affected by a sudden change in diet, caused, for example, by a change of place of residence, travel to tropical or exotic countries with different food. Excessive stress, lifestyle changes, fatigue or disease can also lead to adverse changes in the microbiome. One of the most common causes of microflora disorders is the use of antibiotics, and it may take up to several weeks to recover from an antibiotic treatment³. In such situations, supplementation based on beneficial microorganisms can significantly support the function of the gastrointestinal tract. The use of probiotic supplements is also suitable for healthy people who wish to support the condition of the gut and the whole body daily.

DuoLife CLINICAL FORMULA PROBACTILARDII® is intended for use as support for optimal body functions in the case of:

- ▶ people who want to support the functioning of their digestive tract on a daily basis;
- ▶ people with gastrointestinal issues related to disorders of the natural intestinal microflora, including constipation or diarrhea;
- ▶ people with digestive problems related to excessive stress;
- ▶ people during and after antibiotic treatment (antibacterial and/or antifungal);
- ▶ people planning trips, especially to exotic and tropical countries, with a different menu, to be used before and during the trip;
- ▶ people who want to support the functions of their immune system, including those with reduced immunity due to excessive stress;
- ▶ people who want to support optimal functions of their central nervous system, including optimal mood;
- ▶ people with lactose intolerance;
- ▶ people who are elderly, weakened, or malnourished.

How?

DuoLife CLINICAL FORMULA PROBACTILARDII® supports:

- ▶ proper functions of the digestive tract in healthy people;
- ▶ proper functions of the digestive tract during antibiotic therapy;
- ▶ digestive tract functions during diarrhea and bacterial, viral and fungal infections;
- ▶ functions of the digestive tract in people living under excessive stress;
- ▶ optimal functioning of the immune system;
- ▶ proper functioning of the intestinal microflora;
- ▶ optimal condition of the large intestine;
- ▶ proper digestive processes;
- ▶ optimal biosynthesis of B vitamins and vitamin K in the large intestine;
- ▶ functioning of the central nervous system – especially optimal mood and emotional balance;
- ▶ normal lactose metabolism;
- ▶ antioxidative processes;
- ▶ normal blood cholesterol level;
- ▶ removing toxins from the body;
- ▶ convalescence after bacterial, viral and fungal infections of the gastrointestinal tract.

Composition of DuoLife CLINICAL FORMULA PROBACTILARDII®:

- ▶ **The first Synbiotic (prebiotic and probiotic) capsule** contains:
 - 1) as a **PROBIOTIC**: freeze-dried live bacterial cultures of ***Bifidobacterium bifidum* Rosell® R0071**, ***Bifidobacterium longum* Rosell® R0175**, ***Lactobacillus helveticus* Rosell® R0052** and ***Lactobacillus rhamnosus* Rosell® R0011** and the yeast strain of ***Saccharomyces boulardii* CNCM I-1079**, which together form the probiotic blend of BACTILARDII® reserved for DuoLife
 - 2) as a **PREBIOTIC: acacia fiber**

- **The second Postbiotic capsule** contains:
3) as a **POSTBIOTIC: butyric acid** in the form of glycerin tributyrate (tributylin)



Ingredients:

The Synbiotic capsule (prebiotic-probiotic): proprietary probiotic BACTILARDII® formula containing a total of 9×10^9 CFU (9 billion colony-forming units) of freeze-dried live bacteria and live yeast cultures, including 8×10^9 CFU of bacterial cultures (*Bifidobacterium bifidum* Rosell® R0071, *Lactobacillus helveticus* Rosell® R0052, *Lactobacillus rhamnosus* Rosell® R0011 and *Bifidobacterium longum* Rosell® R0175) and 1×10^9 yeast cultures (*Saccharomyces boulardii* CNCM I-1079), acacia fiber, potato starch – carrier, vitamin C (L-ascorbic acid) of natural origin, 1.5 mg/caps. – antioxidant, shell component: hydroxypropyl methylcellulose (HPMC).

The Postbiotic capsule: granular glycerin tributyrate in sunflower oil 380 mg/capsule, including butyric acid 91 mg/capsule, shell ingredients: hydroxypropyl methylcellulose (HPMC), copper complexes of chlorophylls and chlorophyllins – colourant.

The amount of microorganisms is guaranteed until the end of the product's shelf life, provided that the storage conditions indicated on the label are followed.

Directions for use:

Adults and children over 12 years of age: 1 capsule from each container a day, wash down with cool water. The combined use includes 2 capsules taken together – a probiotic with a prebiotic in the first capsule and a postbiotic in the second. If necessary, the intake of each capsule can be increased up to 2 times a day. Do not exceed the recommended maximum daily dose.

Children from 7 to 12 years of age: The use of the product (both capsules) is allowed after prior consultation with a doctor.

Children from 3 to 7 years of age and pregnant and lactating women: Using probiotic and prebiotic capsules should be consulted with a doctor. The use of postbiotic capsules is not recommended.

The capsules are only intended for children who can swallow a capsule. For children who cannot swallow a capsule, you can pour the content out of the capsule and give it with a spoon or additionally dissolve it in a small quantity of water and consume immediately after preparation.

The product is not intended for children under 3 years of age.

In the case of antibiotic therapy, it is recommended to consume the preparation while taking the antibiotic, preferably 1 hour after taking the drug. It is also recommended to continue supplementation for 7–10 consecutive days after the end of antibiotic therapy.

In the case of trips abroad, especially to exotic countries and areas with a tropical climate, the preparation should be used 5 days before departure, throughout the stay and 5 days after returning.

The product should not be used as a substitute for a varied diet. Balanced diet and a healthy lifestyle are the basis for proper functioning of human body.

Only a doctor can decide about possible use of higher doses. Should you have any doubts concerning the use of the formulation, consult a doctor or a pharmacist.



It is advantageous to combine **DuoLife CLINICAL FORMULA PROBACTILARDII®** with: DuoLife Medical Formula BorelissPro®, ProImmuno®, ProSelect®, ProMigren®, ProRelaxin® and ProDeacid®.

What makes DuoLife CLINICAL FORMULA PROBACTILARDII® different?

► **Innovative composition: a blend of prebiotic, probiotic and postbiotic in one product:**

a) PREBIOTIC: acacia fiber included in the soluble fiber fraction, stimulating the development of natural bacterial microflora.

b) PROBIOTIC: proprietary microbial blend of **BACTILARDII®** containing: 8 billion bacterial strains in **proportions reserved for DuoLife** and 1 billion yeast strains. The total amount of microorganisms is **9 billion CFU = 9×10^9 CFU** (CFU = *colony-forming unit*) of freeze-dried live bacteria and live yeast cultures **GUARANTEED** in the formula **AT THE END OF THE PRODUCT'S SHELF LIFE**. The BACTILARDII® formula with acacia fiber was created **exclusively for DuoLife** by a world-class specialist in the field of probiotic formulas – the Canadian company **Lallemand**. Lallemand provides bacterial strains, e.g. for the Lacidofil preparation, registered as a drug in Poland and many other countries.

The stability and survival of microorganisms in the BACTILARDII® formula during 24-month storage at 20–25°C has been **tested and laboratory-confirmed** by a reputable laboratory in the United States.

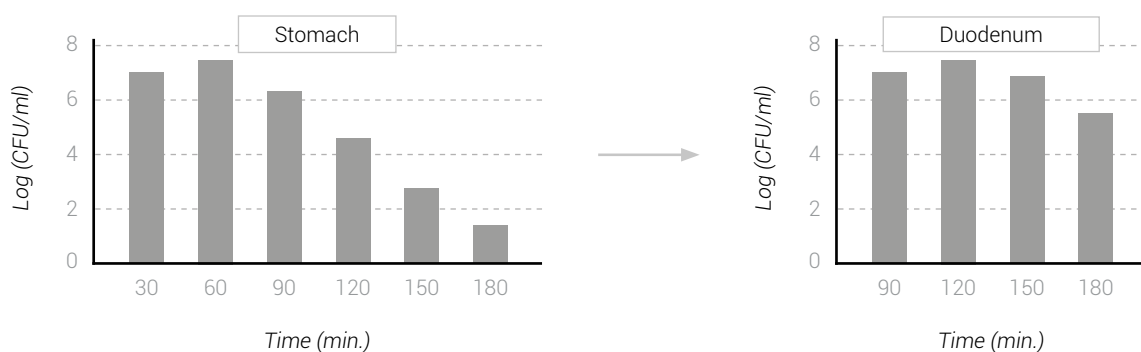
The BACTILARDII® formula includes the bacterial strains ***Bifidobacterium bifidum* Rosell® R0071**, ***Bifidobacterium longum* Rosell® R0175**, ***Lactobacillus helveticus* Rosell® R0052** and ***Lactobacillus rhamnosus* Rosell® R0011** and the yeast strain ***Saccharomyces boulardii* CNCM I-1079**, in **DuoLife-exclusive proportions** and also in **full portions used in many clinical trials**.

c) POSTBIOTIC: lactic acid formula considered to be a product of metabolism healthy for the intestines, produced by the beneficial intestinal microflora.

- **Natural ingredients**, including 4 bacterial strains and 1 yeast strain in the form of LIVE freeze-dried microorganisms.
- **The performance** of individual microbial strains has been **documented by NUMEROUS clinical studies, including at least 30 clinical studies for the specific Lallemand bacteria strains used in the product (R0011, R0052, R0175 i R0071)**.
- **Synergy between all components.**
- **Proprietary innovative formula** – probiotic strains are protected against the adverse effects of external conditions during production and storage, as well as against the action of gastric acid and bile using the proprietary **BIO-SUPPORT™ technology** developed by Lallemand. The stability of the strains due to the formula used within the gastrointestinal tract has been proven in preclinical *in vitro* tests⁵ and in clinical studies⁶.

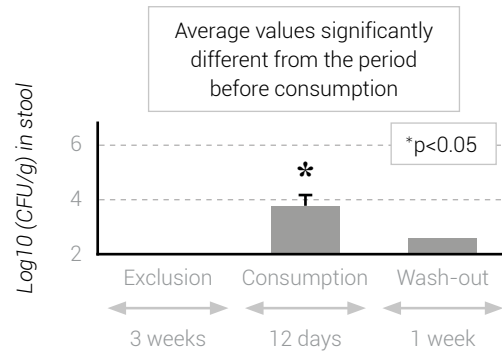
Resistance during passage through the digestive tract:

- Experimental studies performed for the R0052 and R0011 strains, using an *in vitro* model (IViDiS, *in vitro Digestive System*), showing good stability of the strains in the gastric and duodenal environments⁵.



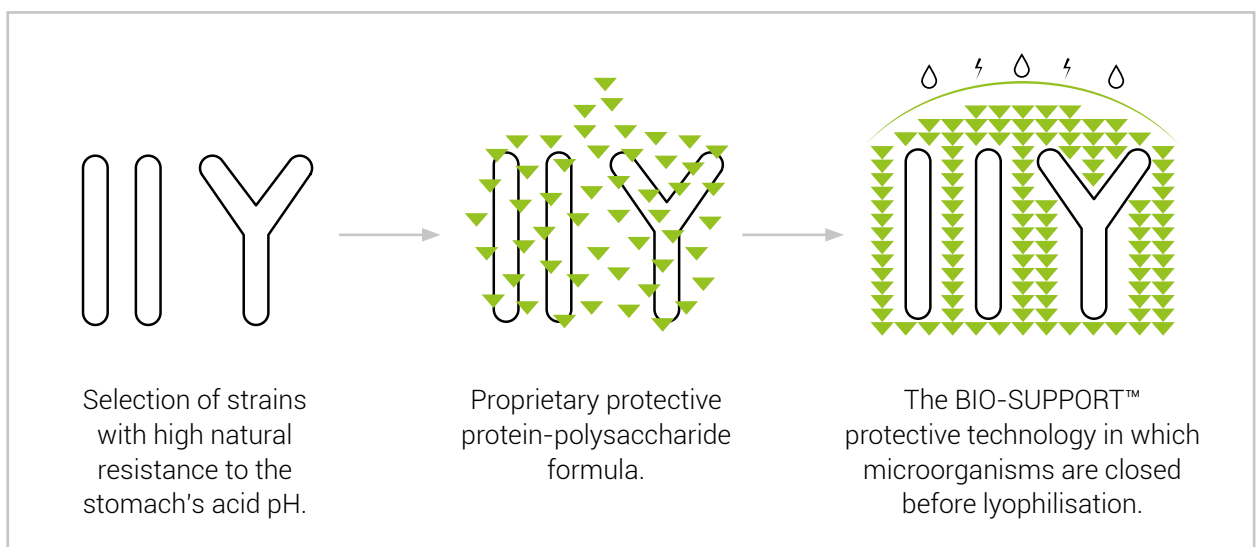
Conclusions: The survival of strains R0052 and R0011 is optimal up to 90 minutes of passage through the stomach and another 90 minutes through the duodenum, which allows concluding about the stability of the strains throughout the passage through the upper part of the gastrointestinal tract under physiological conditions.

- Clinical studies in healthy volunteers taking the dietary supplement with the R0011 strain showed good survival of bacteria throughout the human gastrointestinal tract⁶.



Conclusions: The number of R0011 bacilli in the faeces of the subjects before consuming the supplement (3 weeks, *exclusion*) and during consumption (12 days, *consumption*) and during the one-week wash-out period.

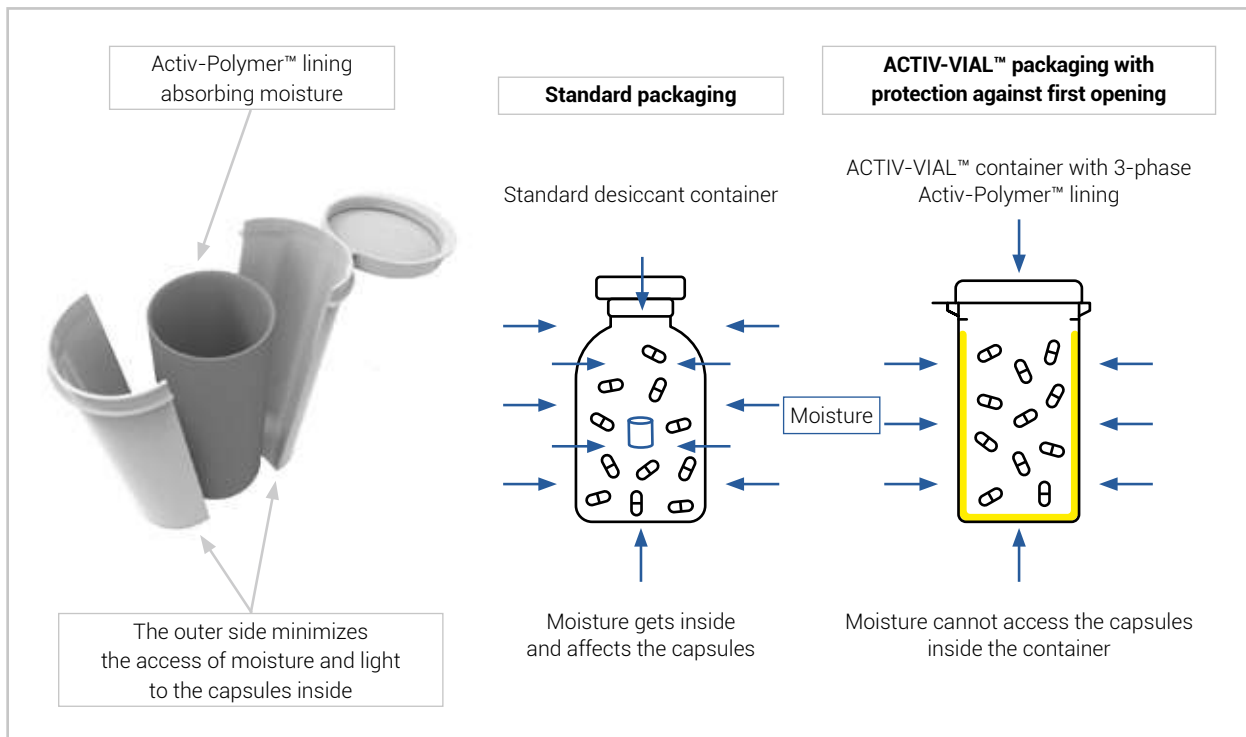
BIO-SUPPORT™ technology operation scheme – ensuring protection of all Lallemand strains.



- ▶ **Additional benefits of the formula in the form of microgranules contained in HPMC capsules:** the formula of butyric acid was created in the form of microgranules enclosed in natural hydroxypropyl methylcellulose capsules, which provide additional protection of the ingredients against the effects of adverse environmental conditions and gastric acid. The BACTILARDII® formula has also been enclosed in HPMC capsules. HPMC capsules have a delayed release time and their properties were tested *in vivo* using scintigraphic methods at the Bio-Images Research Laboratory in Glasgow, Scotland. The low moisture content inside the capsules additionally helps protect the ingredients against losing their health-promoting properties.
- ▶ **Innovative ingredients, unique on the market: two separate capsules in two separate direct packages in one product: one capsule with a probiotic and prebiotic together (*Synbiotic*), and the other capsule with a postbiotic (*Postbiotic*)** – the recommended use is both capsules together 1–2 times a day. The breakdown of ingredients into two capsules enables the technological combination of various raw materials in one preparation, while not disturbing their optimal synergistic effect and giving 100% guarantee of optimal survival of probiotic strains.
- ▶ **Patented, innovative ACTIV-VIAL™ direct packaging certified for drugs** from the American company of CSP Technologies – a white, opaque container **of a pharmaceutical standard** for probiotics, made of the highest quality material with an **Activ-Polymer™** lining, guaranteeing **no** access of moisture, oxygen and light to the capsules contained in the package, **at a level better than the traditional blister**. An analogous packaging adapted to the requirements of the raw material was used for capsules containing postbiotics. Once opened, the Activ-Polymer™ liner also ensures complete stability of the capsules throughout the entire period of use. The packaging is equipped with **a system protecting against the first opening**, which

guarantees that the product is brand new and has not been previously tampered with by unauthorised persons. **The packaging is also free of bisphenol A (BPA)**, a compound with controversial health effects⁷.

DuoLife CLINICAL FORMULA PROBACTILARDII® contains 2 separate ACTIV-VIAL™ containers – one with probiotic and prebiotic and the other with postbiotic in one outer collective packaging.



- ▶ **The product DOES NOT CONTAIN artificial fillers and is GMO-free** – the raw materials used to develop the supplement DO NOT come from genetically modified plants; natural substance that protects the strains against the harmful effects of atmospheric oxygen **is vitamin C of natural origin – L-ascorbic acid** (described on the label as an antioxidant, pursuant to the EU Regulation (EC) 1831/2003).
- ▶ **The product is GLUTEN-FREE** – can be used by people with gluten intolerance.
- ▶ **The product is suitable for vegetarians.**
- ▶ **Concentrated formula** – thus offering convenient use of the dietary supplement – usually both capsules taken together once a day.
- ▶ **Convenient storage – room temperature 20-25°C** with laboratory-confirmed stability of the strains in these conditions.

What are the valuable properties of the strains contained in the preparation *Bifidobacterium longum* Rosell® R0175 and *Lactobacillus helveticus* Rosell® R0052?

Bifidobacterium and *Lactobacillus* bacteria are among the most abundant in healthy intestinal flora. Among them the genus of *Bifidobacterium longum* and *Lactobacillus helveticus* are probiotic strains with widely documented pro-health effects. They promote optimal colon function, regulate intestinal peristalsis, helping to reduce chronic constipation, including in the elderly⁸. They also help to restore normal intestinal functions more quickly in people with diarrhoea of different origins – bacterial, viral or the so-called travellers' diarrhoea. In addition, they support the immunological functions of the body, promoting the fight against pathogenic microorganisms, support the normal metabolism of lactose, and contribute to the optimal absorption of minerals, vitamins and other nutrients from the intestines. They also help to maintain the proper level of cholesterol in the blood and protect the cells of the digestive tract against the harmful effects of oxidative stress⁹⁻¹².

Importantly, however, the two specific strains used in the DuoLife CLINICAL FORMULA PROBACTILARDII® dietary supplement: *Bifidobacterium longum* Rosell® R0175 and *Lactobacillus helveticus* Rosell® R0052 **have additional, clinically documented synergistic pro-health effects**¹³⁻¹⁵. The combination of the R0175 and R0052 bacterial strains has a beneficial **effect on psychological functions in healthy people**, helping to maintain

an optimal mood and **promoting protection against the adverse effects of stress on the functioning of the gastrointestinal tract and the central nervous system** (Figure 1A and 1B). The R0175 and R0052 strains, through the so-called gut-brain axis, can play a role in the proper functioning of the central nervous system and help to protect against stress, anxiety and mood disorders. They can also help to reduce the intestinal ailments caused by excessive emotions. The results of the clinical trials conducted are published and publicly available¹³⁻¹⁵.

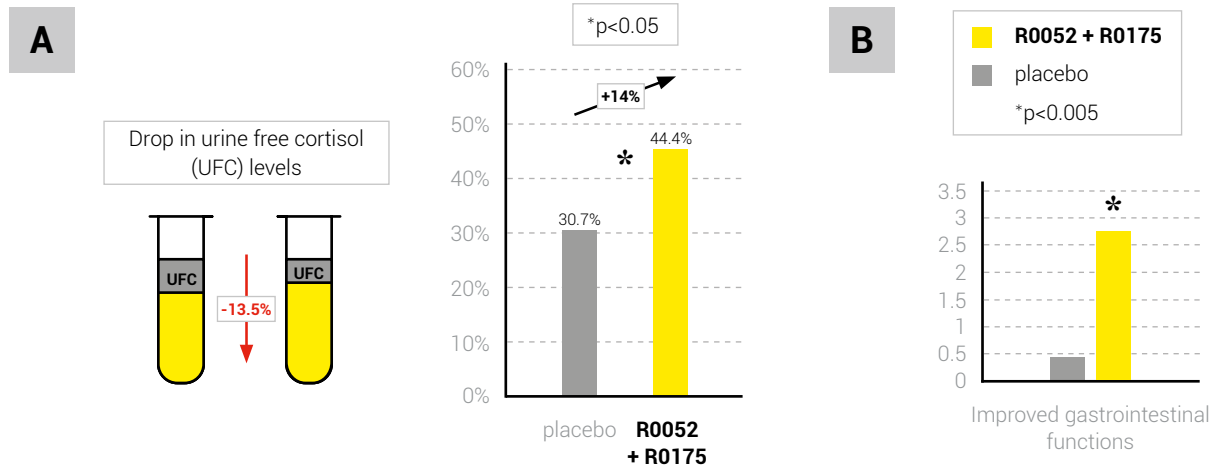


Figure 1. The synergistic effect of the R0052 and R0175 strains has been documented in clinical trials conducted in 2011 (A) and 2008 (B). Figure A: Improvement of psychological functions (global mood score; HSCL-90 Global Severity Index) compared to placebo, combined with a decrease in urinary stress biomarker (cortisol) with 30 days of use for both strains (3×10^9 CFU in total) in 55 healthy volunteers. Figure B: Average improvement in gut function in 75 healthy volunteers exposed to occasional stress during 21 days of using a mixture of R0052 and R0175 strains. Based on ¹⁴⁻¹⁶. Placebo = reference group, not receiving probiotics.

All clinical trials have also demonstrated a high safety profile of the tested bacterial strains in humans¹³⁻¹⁵.

The amounts and proportions of bacterial strain colonies R0175 and R0052 used in clinical trials correspond to the amounts contained in the DuoLife CLINICAL FORMULA PROBACTILARDII® preparation.

Preclinical trials were also carried out for both strains (7 studies in total), which demonstrated the strains' barrier effect and their adhesion to the intestinal epithelium, which determines the probiotic pro-health effect¹⁶ (Figure 2).

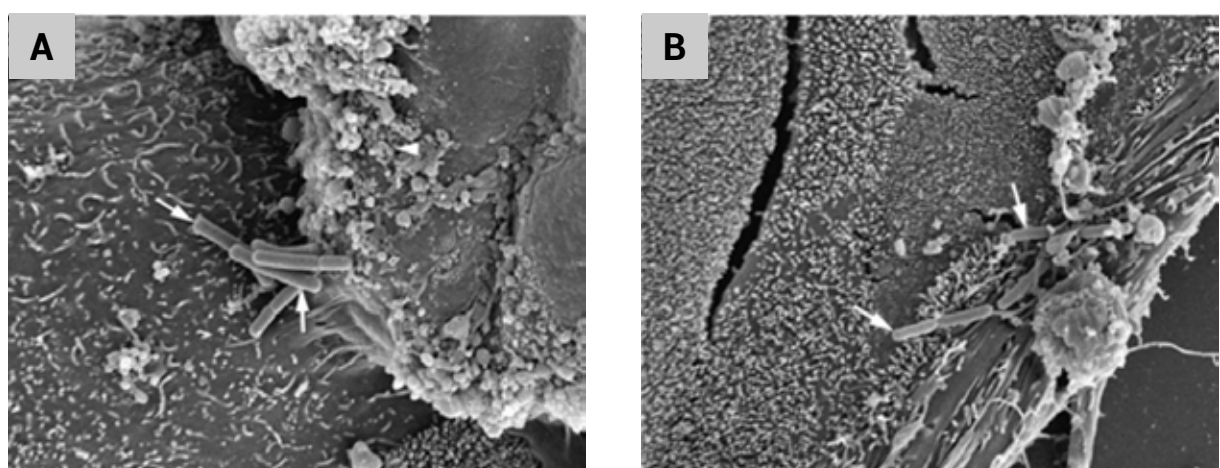


Figure 2. Adhesion of the bacilli of *Lactobacillus rhamnosus* Rosell® R0011 (A) and *Lactobacillus helveticus* Rosell® R0052 (B) to the intestinal epithelial cells determining their probiotic effect. Bacteria are indicated by white arrows. Image from an electron microscope, based on ¹⁶.

What are the results of clinical trials with the *Bifidobacterium bifidum* Rosell® R0071 strain?

Bifidobacterium bifidum Rosell® R0071 **helps to maintain the optimal functions of the intestines and the immune system, and by influencing the gut-brain axis, it supports the proper functioning of these systems in stressed people.** The results of clinical trials from 2015¹⁷ confirm the pro-health properties of the strain, which may significantly contribute to the maintenance of optimal functions of the immune system in stressed people, who often have decreased immunity associated with excessive emotions. The strain was used in clinical trials for 6 weeks in a portion comparable to that included in the DuoLife CLINICAL FORMULA PROBACTILARDII® food supplement.

Lactobacillus rhamnosus Rosell® R0011 also has a well-documented pro-health clinical effect that is synergistic with other strains contained in the DuoLife CLINICAL FORMULA PROBACTILARDII® food supplement.

The R0011 strain is particularly valuable for optimal intestinal function and optimal immunity. **In combination with the R0052 strain, it has been tested in 26! clinical trials**, half of which were conducted in children^{18–23}. According to the results of these studies, R0011 promotes the digestion of lactose in people with intolerance to this sugar, helps in faster return to normal gastrointestinal functions in diarrhoea of various origins, supports intestinal peristalsis, which also allows it to contribute to reducing constipation. The mechanism of probiotic activity and the effect on the health of the gastrointestinal tract and the immune system have also been investigated in many preclinical studies^{16, 24–26} (Figure 2).

All trials with specific Rosell strains (R0052, R0175, R0071 and R0011) used in DuoLife CLINICAL FORMULA PROBACTILARDII® were conducted in accordance with **the gold standard of clinical trials as randomized, double-blind, placebo-controlled trials.**

Why is *Saccharomyces boulardii* yeast considered an excellent probiotic?

The beneficial effects of the *Saccharomyces boulardii* yeast on the human body have been proven **in dozens of clinical trials in adults and children**, as well as in numerous preclinical studies^{27–29}. The *Saccharomyces boulardii* yeast may promote gut function in people with traveller's diarrhoea as well as bacterial and viral diarrhoea. It also has a positive effect on the optimal performance of the gastrointestinal tract and immune system in healthy people. These yeasts are resistant to most antibiotics, hence **they are perfect as protective probiotics** for administration **during antibiotic therapy**; they can also be safely used in people with gastrointestinal mycoses caused by *Candida* spp. They have a clinically proven very high safety profile for use in both children and adults^{27–29}.

The acacia fibre used in the food supplement has prebiotic properties and is a medium for beneficial intestine bacteria.

Acacia fibre belongs to the soluble fibre fraction; **it is a prebiotic. It stimulates the development of natural intestinal microflora**, promotes optimal functions of the digestive tract, supports intestinal peristalsis, regulating bowel movements and preventing flatulence and chronic constipation³⁰. It also helps to maintain optimal blood cholesterol levels³¹. The combination of a prebiotic with a probiotic in one capsule of DuoLife CLINICAL FORMULA PROBACTILARDII® enhances the effect of both components in a synergistic manner.

Butyric acid is a natural compound with clinically proven properties that is highly valuable for humans. How does it work?

Butyric acid is a product of fermentation of undigested carbohydrates (fibre) by beneficial bacteria in the intestinal microflora. **It is classified as a postbiotic** because **it has a beneficial effect in supporting the functions of the**

digestive and immune systems. It is naturally biosynthesized in the large intestine and its supplementation can support the effects of butyric acid synthesized in the gut. In the DuoLife CLINICAL FORMULA PROBACTILARDII® preparation, butyric acid is present in the form of glycerin tributyrinate (tributyryn), a very bioavailable form, thanks to which the compound can be pro-health even after being absorbed into the blood³². In addition, the microcapsule form used in the preparation allows butyric acid to reach the large intestine and act locally there³³. Butyric acid promotes the optimal condition of the intestines, supports the intestinal barrier functions, intestinal peristalsis, nourishes intestinal epithelial cells, can support the body's fight against local inflammation in the intestine. Moreover, it promotes the proper liquid absorption and helps maintain the proper level of blood cholesterol³⁴⁻³⁶. Moreover, it has been shown that butyric acid, once absorbed into the bloodstream, helps maintain optimal blood pressure³⁷ (Figure 3).

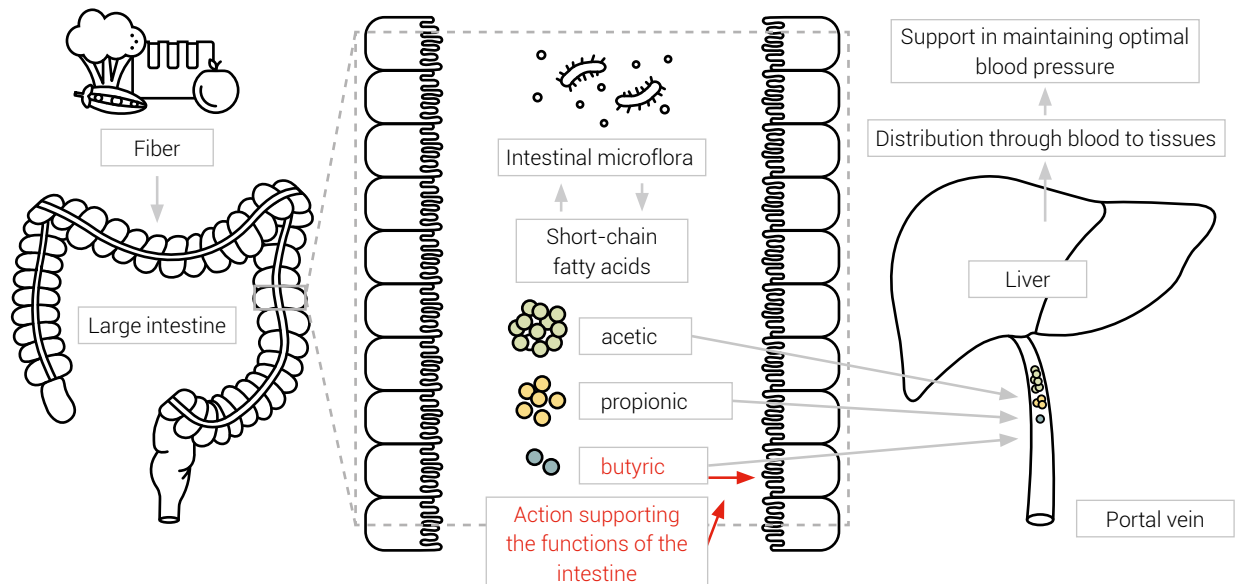


Figure 3. The pro-health role of butyric acid. Together with other short-chain fatty acids, butyric acid nourishes intestinal epithelial cells, helping to support the gut's barrier function and immune system. After being absorbed into the blood, butyric acid may help maintain normal blood pressure. Based on ³⁷.

i The references for DuoLife Clinical Formula ProBactilardii® can be found on a separate binder sheet.

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