Probiotic support for the immune system and targeted strategies to manage health outcomes
The human gut normally hosts 100 trillion bacteria of about 500 species

<table>
<thead>
<tr>
<th>Bacterial type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Commensal</strong></td>
<td>Resident bacteria that neither effect nor benefit from their human host, e.g., most <em>Escherichia coli</em> strains</td>
</tr>
<tr>
<td><strong>Mutualistic</strong></td>
<td>Resident bacteria that can benefit from their host and provide benefit to the host, e.g., <em>Lactobacillus spp.</em></td>
</tr>
<tr>
<td><strong>Pathogenic</strong></td>
<td>Resident bacteria that harm the host, e.g., <em>Clostridium perfringens</em></td>
</tr>
</tbody>
</table>

A healthy microbiota has many benefits

### Microbiota and related concepts defined

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbiota</strong></td>
<td>Microorganisms in the gut, skin, and mouth of the body</td>
</tr>
<tr>
<td><strong>Microbiome</strong></td>
<td>The collective genes of the human microbiota</td>
</tr>
<tr>
<td><strong>Genome</strong></td>
<td>The genes of an organism, such as a human</td>
</tr>
</tbody>
</table>

The genetic information in each of our human bodies includes a genome and a microbiome. Of this total genetic material, more than 99 percent is microbial.
Microbiome: understanding how microbial genes affect human health

Contemporary medical research initiatives promise to help us harness the potential of our resident microbes to achieve better health.
The gut and its microbiota

Definitions and basic concepts
The gut and its key functions

Intact and functional gut

Food digestion for absorption of macro- and micronutrients

Immune functions, with 70% of the immune system in the gut

Gut digestion and absorption

- Chemical digestion of proteins and fats
- Chemical digestion of carbohydrates, fats, polypeptides, nucleic acids
- Absorption of peptides, amino acids, glucose, fructose, fats, water, minerals, and vitamins
- Absorption of ions, water, minerals, vitamins

Liver
Gallbladder
Stomach
Pancreas
Small intestine
Large intestine

Type and quantity of bacteria and fungi vary through the gut

- **Stomach**: $10^1 / \text{g}^*$
  *Lactobacillus, Helicobacter*

- **Duodenum**: $10^3 / \text{g}^*$
  *Bacilli, Streptococcaceae*

- **Ileum**: $10^7 / \text{g}^*$
  *Actinobacteria*

- **Colon**: $10^{12} / \text{g}^*$
  *Bacteroidetes*

*Number of organisms per gram of homogenized tissue or fluid

The gut immune system

The impact of probiotics on the immune system
Mechanisms by which probiotics exert their effects

- Competition for dietary ingredients as growth substrates
- Reduction of inflammation
- Production of growth substrates for other bacteria
- Improved barrier function
- Competitive exclusion for binding sites
- Stimulation of innate immune response

Probiotic Bacteria May Modulate Innate Immune Responses via Many Different Mechanisms

The new “rules” for supplementation and growth of gut microbes

• Not all probiotics are the same; the genus, species, and strain determine their effect.
• Target the probiotic to the indication.
• Support growth of healthy microbiota with prebiotics, and thus help limit growth of potential pathogens.
• More gut bacteria is not necessarily better than less.
Not all probiotics are the same

- *Lactobacillus salivarius* is a probiotic widely used for treatment of intestinal inflammation.
- But not all strains are equally effective. A recent study tested 33 different strains of *L. salivarius*.
- Of these, strain *UCC118* and others protected barrier function, while 13 afforded little or no barrier protection.

Disruption of tight junctions in the intestinal epithelium underlies inflammatory bowel disease and dysbiosis. Probiotic treatment can improve barrier integrity.

Use the right probiotic for each indication

<table>
<thead>
<tr>
<th>Probiotic genus, species, strain</th>
<th>Reported benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus salivarius UCC118</em>(^{1,2})</td>
<td>Protection against infection by food-borne <em>Listeria</em>; support of intestinal epithelium integrity by maintenance of tight junctions</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus GG</em>(^{3,4})</td>
<td>Prevention and reduction of atopic dermatitis in children; reduction of episodic diarrhea in children</td>
</tr>
<tr>
<td><em>Lactobacillus reuteri RC-14 and Lactobacillus rhamnosus GR-1</em>(^{5})</td>
<td>Prevention or treatment of bacterial vaginosis</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus NCFM and Bifidobacterium lactis Bi-07</em>(^{6})</td>
<td>Reduction of cold and influenza symptoms in children</td>
</tr>
</tbody>
</table>

The credibility of health claims for specific probiotic species and strains must be established through science-based clinical studies.

Use the right probiotic for each indication

<table>
<thead>
<tr>
<th>Probiotic genus, species, strain</th>
<th>Reported benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus plantarum</em> HEAL9 and <em>L. paracasei</em> 8700:2&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td>Reduction of common cold infections or duration of symptoms in adults</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> 299V&lt;sup&gt;3,4&lt;/sup&gt;</td>
<td>Support of gut barrier function; improvement of IBS symptoms</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em> NCFM, <em>L. paracasei</em> Lpc-37, <em>B. lactis</em> Bi-07, and <em>B. lactis</em> Bi-04&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Reduction of antibiotic-associated diarrhea</td>
</tr>
</tbody>
</table>

Although most probiotics used today are regarded as safe for healthy individuals, it is always important to consider the risk-benefit ratio before prescribing a probiotic for each individual patient.

Clinical application of probiotics for immune support
The immune response to an infection occurs in multiple stages
Different types of white blood cells are responsible for each stage
Probiotic Selection Criteria and Clinical Data

1. Selection of an efficient strain-combination based on the immune responses in a human trial
2. Two (2) randomized clinical trials supporting the product efficacy
How does daily intake of a probiotic impact white blood cells?

59 subjects in a blinded, randomized, placebo controlled study received probiotic product, 1x daily, oral application. $10^{10}$ cfu

- 6 groups received probiotic
- 1 group received placebo
- Blood draw and screening for INNATE IMMUNITY (Phagocytic Cells and NK T cells) and ADAPTIVE IMMUNITY (T cells)

2 weeks or 5 weeks (L. plantarum 299v only)

L. paracasei 8700:2 increased phagocytosis of E. coli by White Blood Cells

http://www.biofortified.org/2010/03/glowing-phagocytosis/

L. paracasei 8700:2 increased the NKT cell population

A natural killer (NK) cell recognizes MHC I on a healthy cell and does not kill it.

An infected cell that does not present MHC I is killed.


http://cnx.org/content/m44821/latest/?collection=col11448/1.9
L. plantarum tended to increase the proportion of CD8 T cells expressing markers of activation and markers of memory.
The immune response to an infection occurs in multiple stages. Different types of white blood cells are responsible for each stage.
Immune Response to Viral Infections

L. Paracasei 8700:2 and L. plantarum HEAL9 were selected & combined for the optimal effects on different parts of the immune response.
Randomised, double-blind and placebo-controlled study using new probiotic lactobacilli for strengthening the body immune defence against viral infections

Anna Berggren · Irini Lazou Ahren · Niklas Larson · Gunilla O¨nning

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Abstract Background. The aim of this study was to investigate whether consumption of Lactobacillus plantarum HEAL 9 (DSM 15312) and Lactobacillus paracasei 87002 (DSM 13434) could affect naturally acquired common cold infections in healthy subjects.

Methods A randomised, parallel, double-blind placebo-controlled study was performed to investigate whether intake of this probiotic mixture could reduce the risk of common cold episodes, number of days with common cold symptoms, frequency and severity of symptoms, and cellular immune response in common cold infections. A total of 272 subjects were supplemented daily with either 10^8 cfu (colony forming units) of probiotics (N = 135) or control (N = 137) for a 12-week period.

Results The incidence of acquiring one or more common cold episode was reduced from 67% in the control group to 55% in the probiotic group (p < 0.05). Also, the number of days with common cold symptoms were significantly (p < 0.05) reduced from 1.6 days in the control group to 6.2 days, in the probiotic group, during the 12-week period. The total symptom score was reduced during the study period from a mean of 44.4 for the control group to 33.6 for the probiotic group. The reduction in pharyngeal symptoms was significant (p < 0.05). In addition, the proliferation of B lymphocytes was significantly counteracted in the probiotic group (p < 0.05) in comparison with the control group.

Conclusion In conclusion, intake of the probiotic strains Lactobacillus plantarum HEAL 9 (DSM 15312) and Lactobacillus paracasei 87002 (DSM 13434) reduces the risk of acquiring common cold infections.

Keywords Probiotic · Immune defense · Common cold · Lactobacillus plantarum · Lactobacillus paracasei · DSM 15312 · DSM 13434

Introduction The occurrence of common cold is very frequent in the community, and on average, children have 6–8 and adults 2–4 colds per year [1]. By tradition, foods or dietary supplements with high levels of vitamin C or Echinacea have been consumed to try to reduce the incidence or severity of common cold. It is known that the symptoms associated with common cold are a result of the immune response by the host towards the infection. Therefore, compounds with the right type of anti-inflammatory activity are supposed to be effective antivirals. Examples of compounds with anti-inflammatory activity are probiotics. The current definition by The World Health Organization of probiotics is “live microorganisms which when administered in adequate amounts confer a health benefit on the host” [2]. However, the effect on the immune system is highly strain-specific, and evaluation of effects on the immune system must be done using the strains per se.

Different probiotic strains have been shown to affect the immune system and also the incidence of common cold. In young children, intake of probiotics reduced missed school days [3] and absence of day care [4] because of illness. In the study by Haataja et al. [4] conducted on 571 children aged 1–6, intake of Lactobacillus GG during a 7-month period resulted in a relative reduction in the number of children suffering from respiratory infections.

RCT (Randomized Control Trial) 1, Berggren

## RCT1, Berggren: Clinical Trial Design

<table>
<thead>
<tr>
<th>Design</th>
<th>Double blind, randomized, placebo controlled with 272 adult test subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centers</td>
<td>Two centers in Sweden, Lund and Uppsala</td>
</tr>
<tr>
<td>Primary endpoint</td>
<td>Reduction of common cold incidence</td>
</tr>
<tr>
<td>Secondary endpoints</td>
<td>Evaluation of number, length and severity of the episodes</td>
</tr>
<tr>
<td></td>
<td>Effect on immune markers (selected cell population at the Uppsala center)</td>
</tr>
<tr>
<td></td>
<td>Fecal increase of lactobacilli (colonization)</td>
</tr>
<tr>
<td>Inclusion criteria</td>
<td>Healthy volunteers</td>
</tr>
<tr>
<td>Age ≥ 18 years</td>
<td></td>
</tr>
<tr>
<td>Medicinal product, dosage, type of application</td>
<td>Probiotic product or placebo, 1x daily, oral application</td>
</tr>
<tr>
<td></td>
<td>Combination of <em>L. plantarum</em> HEAL9 (DSM 15312) and <em>L. paracasei</em> 8700:2 (DSM 13434) at 1x10^9 cfu/day</td>
</tr>
<tr>
<td>Duration of treatment</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Number of visits:</td>
<td>4: -2, 0, 2, 12 weeks</td>
</tr>
</tbody>
</table>

RCT1, Berggren, Results Summary

1. Common cold incidence was reduced (p<0.05).

2. Number of sick days was reduced (p=0.0499).

3. Pharyngeal symptoms were reduced (p=0.027).

4. Evidence of the two probiotic strains in faecal material after 2 and 12 weeks of intake indicates effective survival in the gut (p<0.001).

Randomized, Double Blind and Placebo Controlled Study Using a Combination of Two Probiotic Lactobacilli to Alleviate Symptoms and Frequency of Common Cold

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ABSTRACT

Purpose: The efficacy of ProbiDefendum, a combination of the two lactobacilli Lactobacillus plantarum HEAL6 and Lactobacillus paracasei 87002, on the severity, duration and frequency of cold episodes was studied in a randomized double blind, placebo controlled clinical trial. Methods: A total of 310 subjects with increased risk for common cold infection (at least two episodes of common cold during the last six months) were enrolled and were randomly assigned to receive either active product (A-group) or placebo (B-group) over a period of 11 weeks. Subjects reported the occurrence and severity of cold episodes in a diary, scoring 13 different symptoms of common cold over a period of 7 days after the episode began. Results: In the total study population, cold episodes were reported in 148 cases (47.7%). In the A-group, 54 subjects reported one and 18 subjects reported 2 episodes whereas 63 subjects had none. In the B-group, 53 subjects reported one, 34 subject reported 2 episodes and 79 subjects reported none. Although the number of episodes was similar in both groups, cold episodes in the active group were significantly shorter than in the placebo group. The total sum score of cold symptoms was significantly lower in the active group as compared with the placebo group. The daily intake of the probiotic dietary supplement ProbiDefendum over a period of 12 weeks efficiently alleviated symptoms of common cold and the duration of cold episodes.

Keywords: Probiotics; Lactobacillus plantarum HEAL6; Lactobacillus paracasei 87002; Immune; Common cold; Clinical Study

1. Introduction

Common cold is an upper respiratory tract infection that can be caused by more than 100 different viruses. The most prominent of them is human rhinovirus (HRV), which itself comprises more than 130 serotypes [1]. The virus enters the human body through the mouth or the nose. Symptoms include sore throat, running nose, nasal congestion, sneezing and coughing, sometimes accompanied by headache and muscle aches. Until now, no efficient antiviral drugs are available and treatment options that only aim at alleviating specific symptoms. Episodes of common cold usually resolve within 7 days; but longer episodes are observed in real. Adults experience 2 - 5 cold episodes per year whereas children have 7 - 10 such viral infections [2]. Common cold is a substantial contributor to the number of sick days in schools, day care facilities and the work environment. In a Swedish survey from 2008, the total productivity loss in Sweden due to common cold infections was estimated to be about € 2.7 billion per year, which is not only caused by the number of sick days, but also by low productivity while at work, and absenteeism of care given. Reducing the lost productivity by 1 day per person and year in Sweden alone would potentially save more than € 500 million. The survey also revealed that the productivity loss due to reduced working capacity of subjects at work having a common cold infection or allergic rhinitis and because of absence from work are very similar [3]. Developing efficient treatment options for common cold is therefore of high public health.

Open Access
## RCT2, Busch: Clinical Trial Design

<table>
<thead>
<tr>
<th>Design</th>
<th>Double blind randomized trial of 310 adult patients (155/group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centers</td>
<td>Four centers in Berlin, Germany participated</td>
</tr>
<tr>
<td>Primary endpoint</td>
<td>Reduction of cold symptoms (Total sum score)</td>
</tr>
</tbody>
</table>
| Secondary endpoints | Reduction in the length of the cold periods  
Reduction in the number of cold periods  
Global evaluation of the efficacy and safety by the subjects  
Global evaluation of the safety by the investigators |
| Inclusion criteria | Age $\geq$ 18 years  
Increased liability to common cold (at least 2 episodes within 6 months) |
| Medicinal product, dosage, type of application | Probiotic product, 1x daily, oral application  
Same as RCT1 |
| Duration of treatment | 12 weeks/subject maximum, or until second cold period |
| Number of visits: | 3: 0 (baseline), after 6 weeks, after 12 weeks |

Reduction of individual cold symptoms

<table>
<thead>
<tr>
<th>Area</th>
<th>Symptom</th>
<th>Significant, p&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Condition</td>
<td>Headache</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Pain in a limb</td>
<td>+</td>
</tr>
<tr>
<td>Throat</td>
<td>Sore throat</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Difficulty Swallowing</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hoarseness</td>
<td>+</td>
</tr>
<tr>
<td>Nose</td>
<td>Runny nose</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Congested nose</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Yellow secretion</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Bloody secretion</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sneezing</td>
<td>-</td>
</tr>
<tr>
<td>Bronchial</td>
<td>Cough</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Yellow secretion</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Other secretion</td>
<td>-</td>
</tr>
</tbody>
</table>

Subjects in the probiotics arm reported significantly reduced symptom severity scores in 9 out of 13 symptoms recorded.

Statistically significant reduction ($p < 0.001$) of the total sum score of the common cold symptoms.

Subjects in the probiotics arm reported significantly reduced total symptom scores over the course of a cold episode.

Subjects in the probiotics arm reported significantly reduced cold episode length.
70% of subjects in the probiotics arm reported the intervention to be of good or very good efficacy.

HCPs conducting the study rated the efficacy similarly.

Busch et al – Summary Results

1. The Global Symptom Score was improved by 33% in the active versus the placebo group (highly significant 0.001).

2. The individual score for all (13) measured cold symptoms was lower in the active group compared with the placebo group and 9 of 13 symptoms were significantly lower.

3. The length of the cold episodes was reduced by more than one day from 6.7 to 5.6 (highly significant 0.001).

4. Close to 50% of those given Probi’s product considered it very effective and more than 20% considered it effective. The numbers were considerably lower in the placebo group and less than 1% considered the treatment very effective. Therapy was blind at time of evaluation.
Lactobacillus plantarum HEAL9 and Lactobacillus paracasei 8700:2
KEY TAKEAWAYS

• Rationally selected on the basis of human immune response modification
• Unique combination of two strains (Rask et al.)

• In two state-of-the-art designed clinical studies (randomized, double blind, placebo controlled) with a total of 600 subjects, the following clinical effects were achieved (significant reduction of):
  • the incidence of common cold (Berggren et al.)
  • the number of sick days (Berggren et al. and Busch et al.)
  • common cold symptoms (Busch et al.)
Mechanisms by which probiotics exert their effects

- Competition for dietary ingredients as growth substrates
- Reduction of inflammation
- Production of growth substrates for other bacteria
- Improved barrier function
- Competitive exclusion for binding sites
- Stimulation of innate immune response

Not all probiotics are the same. Genus, species and strain make a difference. Probiotics should be selected on the basis of targeted outcome.

What’s in your toolbox?

  • Need to know the genus, species and strain
  • Papers are not always freely available to the public
• Probiotic Advisor: https://www.probioticadvisor.com/
  • Experienced Reviewers
  • Subscription based service
• Canadian Probiotics Clinical Guide: http://www.probioticchart.ca/
  • Evidenced based review of products on the market in Canada
  • Many of the brand names are sold in the US
  • A US version is also in the works for 2016/17
• International Scientific Association for Pre and Probiotics www.isapp.net
  • Useful links and information about selecting probiotics
• World Gastroenterology Organization
  • Review of evidence base for probiotics and targeted health conditions
• MHICN – Digestive Health Section www.mhicn.com
  • Breaking news and other useful tools
Thank you!

Questions?

Insert references here