Probiotics
Nutrition Masters Course

Noelle Patno, PhD
Learning Objectives

• Review early pioneers of research on the intestinal microbiome and its re-emergence as a focal point of immunology

• Understand how the microbiome relates to health and disease

• Clearly define and select a quality probiotic based on WHO/FAO guidelines

• Identify key aspects of a probiotic strain as described in published literature

• Explore emerging microbiome-related research
Early Pioneers of Microbiome-Related Research
How Long Have Microorganisms Been Studied?

• In 1590, Zacharia Janssen, a spectacle maker, discovered that two lenses magnified better than one—inventing the first microscope\(^1\)
• During the late 17\(^{th}\) century, Antonie van Leeuwenhoek was the first to connect the use of a microscope with microbiology\(^2\)
• Between 1665-1683, the existence of microscopic organisms was discovered by Robert Hooke and Antonie van Leeuwenhoek\(^3\)

Genetics highlights

1866—Gregor Mendel discovers inheritance in pea plant experiments

1953—Discovery of double-helix model of DNA structure

1986—First semi-automated DNA sequencing machine

1988—Quantitative/real-time PCR (qPCR) method developed

2000—First commercially available sequencing method

2001—Human genome unraveled

2004—Next Generation Sequencing (NGS)

Microbiome highlights

1676—Discovery of bacteria by Antonie van Leeuwenhoek

1881—Culture-dependent methods for stool analysis

1885—First human gut microbiome studied

1959—Germ-free mice reared

1996—First human fecal sample sequenced

2006—Study of microbiome in age and country

2007—Start human microbiome project (HMP)

2009—First study of microbiome in health and disease: lean vs. obese

## 1800s: Pioneers of the Immune System

<table>
<thead>
<tr>
<th>Élie Metchnikoff, PhD</th>
<th>Paul Ehrlich, PhD</th>
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</table>
| • Commonly referred to as the father of cellular innate immunity\(^1,3\)  
• Discovered phagocytosis by macrophages and microphages as a critical host-defense mechanism\(^2\) | • Considered to be one of the fathers of humoral adaptive immunity\(^3\)  
• Described the role of antibodies in the immune response to bacterial infection\(^3\) |

Early Use of Probiotics by Metchnikoff

- In the late 1890s, Élie Metchnikoff spent time in remote villages of Eastern Europe where the majority of villagers were centenarians. He noted they drank a fermented yogurt that contained *Lactobacillus bulgaricus*.

- “For suppressing putrefactive colonic bacteria” Metchnikoff recommended daily doses of probiotics in the form of “soured milk (i.e., yogurt) prepared by a group of lactic bacteria, or of pure cultures of the *Bulgarian bacillus* (*Lactobacillus bulgaricus*), but in each case (accompanied by) a certain quantity of milk, sugar, or sucrose.”

• Abigail Salyers, PhD from University of Illinois first drew attention to the overuse of antibiotics (in the early 1990s) and helped raise awareness of the importance of diverse intestinal microbiome (and attention to the damage caused by overuse of antibiotics)

• Her research focused on the ecology of microorganisms in the human body and the transfer of antibiotic-resistance genes, particularly genes among the *Bacteroides* species

Patrice Cani, PhD

- Dr. Cani’s main research involves the investigation of interactions among the gut microbiota, the host, innate immune system and others related to metabolic disease.\(^1,2\)

- His more recent publications discussed *Akkermansia muciniphila*—one of most abundant species in the human intestinal microbiota.\(^3\)

- It is a novel candidate to improve metabolic disorders associated with:\(^3\)
  1. Liver diseases
  2. Obesity
  3. Cardiometabolic disorders

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Relationship Between the Microbiome and Health
Human Microbiome Project (HMP)

## Relevant Definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td><strong>Microbiota</strong></td>
<td>Microorganisms of a particular site or habitat (such as the gut microbiota)</td>
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<tr>
<td><strong>Microbiome</strong></td>
<td>Refers to the entire habitat of a human, including the microorganisms (bacteria, archaea, lower and higher eukaryotes, and viruses) and their genomes (i.e., genes)</td>
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Two Parts of the Human Microbiome Project

5 major body sites characterized

- Nasal passages
- Oral cavity
- Skin
- Gastrointestinal tract
- Urogenital tract

3 different microbiome-associated health conditions

- Type 2 Diabetes
- IBD
- Pregnancy complications and preterm birth

Significance of the Human Microbiome Project

1. Developed a new database system—much more efficient, and organized for searching, storing and accessing data

2. Developed analytical tools to compare patterns in datasets

3. Established a catalog of reference bacterial genomes to compare across multiple body sites
Publications on Microbiome: 2013-2017

The Impact of the Microbiome on Health Begins Early

Initial colonization of the gut in infants affects health later in life\(^1\)

- Factors influencing the development of the infant microbiota include\(^1\)
  - Diet
  - Family environment
  - Caesarean vs. vaginal birth
  - Diseases and therapies

- Alterations in the establishment of a healthy gut microbiota and factors that *decrease* microbial diversity in infants and young children may affect the risk of developing disease\(^2\)

Risk factors influencing the maternal microbiota (Gestational Period)

- Weight gain
- Maternal BMI
- Diet
- Antibiotics

Maternal influence (Postnatal Period)

- Breast milk
  - Microbes
  - HMOs

Metabolic disease
- Obesity
- Diabetes

GI conditions
- IBS
- IBD
- Crohn’s

Immune disease
- Asthma
- Allergy

At birth, infant exposed to maternal microbiota

Successive colonization impacts host metabolism and immune education

Some Factors Affecting the Microbiome

**Early Life**
- Maternal BMI
- Pre-term Pharmacotherapy (e.g., antibiotics, steroids)
- 0-3 months Caesarean vs. Vaginal Birth
- 1-3 years Breastfeeding cessation
- Day care attendance

**Adult Life**
- Cigarette smoke
- Allergens
- Infections
- Air pollution
  - Age
  - Gender
  - BMI
  - Onset of chronic diseases
  - Diet
  - Pharmacotherapy (e.g., antibiotics, steroids)

Adapted from Shukla SD et al. *Clin Transl Immunology*. 2017;6(3):e133.
Different Diets Impact the Microbiome

Western diet

Mediterranean diet

Gluten-free diet

• Different diets affect microbial dysbiosis and diversity\(^1\)

• Dysbiosis, an imbalance in bacterial composition, can be categorized into one or more of the following\(^2\):
  1. Loss of beneficial organisms
  2. Excessive growth of potentially harmful organisms
  3. Loss of overall microbial diversity

• Dysbiosis is associated with diseases\(^2\)

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The Diversity of the Microbiome is Associated with Disease

Increased diversity is linked to lower risk for metabolic or immunological diseases.\(^1\)

Decreased diversity has been associated with human diseases.\(^2,3\)

Diversity Refers to the Different Types of Bacteria and Abundance of Each Type


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Activity of the Microbiota

• Gut bacteria benefit the host in a variety of ways, such as
  - Regulating gut motility
  - Producing vitamins
  - Transforming bile acid and steroids
  - Metabolizing foreign substances
  - Absorbing minerals
  - Activating and destroying toxins

• Bacteria produce metabolites including short-chain fatty acids (SCFAs), such as acetic, propionic, and butyric acids, which are potentially therapeutic

Potential Mechanisms of Bacteria in the Intestine

Mechanisms
1. Enhance epithelial barrier
2. Adhere to intestinal mucosa
3. Inhibit pathogen adhesion
4. Competitively exclude pathogenic microbes
5. Produce anti-microbial peptides, bacteriocins, or metabolites such as lactic acid, short-chain fatty acids such as butyrate
6. Modulate the immune system

What Are Bacteriocins?

- Bacteriocins are peptides produced by bacteria, which can kill or inhibit other bacterial strains\(^1\)
- They affect the changing composition of microbial communities\(^2\)
- For example, in an *in vitro* study, *Lactobacillus salivarius* UCC118 was shown to induce bacteriocin ABP-118 gene expression upon adhesion to human intestinal epithelial cells\(^3\)

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Illustration:
Two Key Mechanisms of Action of Probiotics:

1. Enhance epithelial barrier
2. Produce anti-microbial substances
Prebiotic Definition from the ISAPP

A prebiotic is a substrate that is selectively utilized by host microorganisms conferring a health benefit


Dietary fibers (fibers from cereals, grains, fruits, vegetables, nuts and legumes, are longer than oligosaccharides) are resistant to human digestion and may or may not be fermentable by gut bacteria

Prebiotic Benefits Include *Bifidobacteria* and SCFA Increases

- Prebiotics may modify the intestinal microbiome and also impact human health, such as improve metabolic, immune and gastrointestinal health\(^1\)
- While earlier studies focused on FOS and inulin, emerging prebiotics have been identified:
  - IMO\(^s\) (Isomaltooligosaccharides) are well-tolerated prebiotic soluble fibers that produce high levels of *Bifidobacteria* without causing as much gas as inulin (*in vitro*)\(^2\)
  - In human breast milk, 2′-*fucosyllactose* (2′FL) is one of the most abundant human milk oligosaccharides (HMOs). HMOs, including 2′FL, support the selective growth of certain *Bifidobacteria*\(^3\)
  - Both IMOs\(^2,4\) and 2′FL\(^3\) support the production of SCFA (short chain fatty acids)

Several Factors Influence the Density, Diversity, and Activity of Gut Bacteria


- **External influences**
  - Diet
  - Prebiotics
  - Probiotics
  - Antibiotic usage
  - Illness
  - Lifestyle
  - Environmental factors

- **Internal host properties**
  - Age
  - Genetics
  - Stress
  - Physiologic processes
  - Digestive tract physiology

Homeostasis

Density, diversity, and activity of the gut bacteria

Dysbiosis

Diseases

Selecting a Quality Probiotic
Probiotic Definition

**ISAPP definition:**
“Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.”\(^1\)

**FAO/WHO definition:**
“Live microorganisms which when administered in adequate amounts confer a health benefit on the host.”\(^1,2\)

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**ISAPP:** International Scientific Association for Probiotics and Prebiotics  
**FAO:** Food and Agriculture Organization of the United Nations  
**WHO:** World Health Organization

Guidelines for Choosing Quality Probiotics

- Strain-specificity
- Proper units
- Potency research
Genus, Species, & Strain

Bacteria are categorized by genus, species, and strain. Each probiotic bacteria MUST be identified at this level; each strain carries unique health benefits.

*Lactobacillus acidophilus NCFM®*
CRN/IPA Guidelines for Probiotic Viability

- Look for appropriate units on label: colony-forming units (CFUs)
- Ensure amount of CFUs listed is guaranteed up until expiration date, not just at time of manufacture
- Main concern should be the available CFUs at time of consumption

Do greater CFU quantities = greater health benefits?

• Some products MAY work better at higher CFUs than others. This may be due to weak activity of the strain or lack of strain-specific selection

• Effective CFUs (dose selection) should be based upon a strain’s (or combination of strains) well-documented clinical health benefits for that specific patient population

• Doses can range from 100 million to over a trillion CFUs per day

Probiotics: 2018 ISAPP Dispelling Myths

Is more better?
The best dose is one that has been tested in humans and resulted in positive health outcomes. Doses can range from 100 million to over a trillion CFU per day.

Are greater number of strains better?
It depends on the literature. Some studies show benefits of a single-strain probiotic while others show specific combinations of probiotic strains have a positive outcome. More CFU does not guarantee a more beneficial product.

Do probiotics have to alter my microbiota to be effective?
No. As probiotics and their produced substances pass through the gut they interact with immune cells, dietary components in the gut and other microbes.

Evidence is Lacking for the Health Benefits of Fermented Foods

• Fermented foods may provide general health benefits but **they are not characterized by strain type or amount of bacteria.** Lack of characterization makes them difficult to study.

• A recent meta-analysis of kombucha concludes that it does not have a defined health benefit¹

• However, well-defined strains of bacteria have been studied at specific amounts for certain health conditions

Example of Specific Strains Studied at Specific Doses

Article title: Probiotics reduce symptoms of antibiotic use in a hospital setting: A randomized dose response study.

“equal amounts of *Lactobacillus acidophilus NCFM®, Lactobacillus paracasei Lpc-37, Bifidobacterium lactis Bi-07, and Bifidobacterium lactis Bl-04*...capsules contained the probiotic combination at either a low-dose: [4.17 billion colony forming units (CFUs)] or high-dose: 17.00 billion CFUs] or placebo.”

Example of Specific Strains Studied in Specific Population

Article title: Probiotic effects on cold and influenza-like symptom incidence and duration in children.

“...326 eligible children (3-5 years of age) were assigned randomly to receive placebo (N104), Lactobacillus acidophilus NCFM (N110), or L. acidophilus in combination with Bifidobacterium animalis subsp lactis Bi-07 (N112). Children were treated twice daily for 6 months.”

“CONCLUSION: Daily dietary probiotic supplementation for 6 months was a safe, effective way to reduce fever, rhinorrhea, and cough incidence and duration and antibiotic prescription incidence, as well as the number of missed school days attributable to illness, for children 3 to 5 years of age.”

Population-Specific, Dose and Strain-Specific Study Example

- Randomized, placebo-controlled trial to assess illness reduction
- Population: Healthy, physically active adults (241 males, 224 females, 18-60 years old, who exercise at least 30 min 3x/week)
- Dose: Lactobacillus acidophilus NCFM and Bifidobacterium animalis subsp. lactis Bi-07 (NCFM & Bi-07) $5 \times 10^9$ CFU each per day
- Duration: 150 days (5 months)
- Results:
  - Delayed the onset of upper respiratory tract illness events
  - NCFM + Bi-07 group had a significantly higher level of physical activity load (Duration x intensity) (on average, 4 exercise sessions/week)
    - Exercise duration and intensity was significantly increased
- Possible that NCFM and Bi-07 helps reduce illness’ negative effects on physical activity

Stability Testing

- Stability testing should be conducted under the same temperature conditions as the recommended storage conditions on the finished product label.
- Label should reflect label claim through end of shelf life (up until expiration date).
- As few publications on strain storage conditions exist, stability testing should be conducted to provide data to support proper storage conditions.

Storage Conditions

• The storage conditions are determined by the results of the stability tests and/or published literature on the strain

• Each strain may require different storage conditions (refrigeration or room temperature)

• Manufacturers should provide instructions for storage and handling based on individual formulations and packaging and conditions throughout product’s shelf life

Probiotic Yeast

• *Saccharomyces boulardii* is a very well studied, commonly used probiotic yeast that was found in a meta-analysis to be effective in children and adults in reducing the risk of antibiotic-associated diarrhea\(^1\)

• It has been studied to provide symptom relief for various gastrointestinal conditions (IBS, Crohn’s)\(^1,2\)

• Quantification of yeast is not consistent; some manufacturers list by weight (milligrams) and others list by CFU\(^3\)

Identifying Marks of a Quality Probiotic

1. Strain specified
2. CFU (Colony Forming Unit) labeled
3. Quantity is guaranteed through expiration
4. Storage conditions are listed
Summary:
Characteristics of a Quality Probiotic

1. Strain specified
2. CFU (Colony Forming Unit) labeled
3. Quantity is guaranteed through expiration
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## Evidence-Based, Strain-Specific Probiotic Health Benefits

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<tr>
<td><strong>Lactobacillus acidophilus NCFM and Bifidobacterium lactis Bi-07</strong></td>
<td>Gastrointestinal, immune health, and recurring intestinal distress support; different doses are suggested for children and adults&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Lactobacillus plantarum 299v</strong></td>
<td>Helps support occasional irritation and bowel discomfort; promotes integrity of the gastrointestinal barrier&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Bifidobacterium lactis B420</strong></td>
<td>Supports body weight maintenance&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14</strong></td>
<td>May help maintain healthy vaginal microflora and support urogenital health&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Lactobacillus paracasei 8700:2 and Lactobacillus plantarum HEAL9</strong></td>
<td>Helps support healthy nasal, sinus, and respiratory function&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Bifidobacterium lactis (Bi-07), Lactobacillus acidophilus NCFM, Bifidobacterium lactis (Bi-04) and Lactobacillus paracasei (Lpc-37)</strong></td>
<td>Provides relief for abdominal discomfort associated with loose stools and occasional diarrhea&lt;sup&gt;7&lt;/sup&gt;</td>
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<tr>
<td><strong>Lactobacillus acidophilus NCFM, Bifidobacterium lactis Bi-07 and Fructooligosaccharides (FOS)</strong></td>
<td>Supports immune health and digestive support. Fructooligosaccharides is a prebiotic shown to promote the growth of beneficial bacteria.(^1)</td>
</tr>
<tr>
<td><strong>Bifidobacterium lactis HN019, Lactobacillus rhamnosus HN001, and Saccharomyces boulardii</strong></td>
<td>Provides support for immune health and occasional loose stools. May be ideal for patients who travel often.(^3)</td>
</tr>
<tr>
<td><strong>Bifidobacterium animalis ssp lactis (BB-12) and Lactobacillus rhamnosus GG</strong></td>
<td>Strains are shown to support healthy microbial balance in infants and young children.(^4)</td>
</tr>
<tr>
<td><strong>Saccharomyces boulardii, Bifidobacterium lactis Bi-07, Lactobacillus plantarum Lp-115, Lactobacillus salivarius Ls-33, Lactobacillus NCFM, Streptococcus thermophilus St-21, and Bifidobacterium lactis BI-04</strong></td>
<td>Combination of strains provide multidimensional support for both the upper and lower GI tract for digestive and immune health.(^2)</td>
</tr>
<tr>
<td><strong>Lactobacillus salivarius UCC118</strong></td>
<td>May influence tight junctions between intestinal cells and may beneficially influence immune cell signaling processes.(^5,6)</td>
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Lactobacillus salivarius UCC118
Effect of *L. salivarius* UCC118 on Tight Junction Proteins

**Study Design**

- Human Intestinal Epithelial Cell Line: CaCo2
- Oxidative stress: Hydrogen Peroxide (H$_2$O$_2$) exposure
- Outcome: Localization of tight junction proteins
- Hypothesis: UCC118 will prevent the cellular internalization of tight junction proteins associated with oxidative stress

Pretreatment with UCC118 prevents disruption of intestinal epithelial cell tight junctions, in a validated in vitro model of human intestinal epithelial cell oxidative stress.

Image selected from Figure 5 in Miyauchi E et al. Am J Physiol Gastrointest Liver Physiol. 2012;303(9):G1029-1041. License 4359481306032 from APS
Conclusions

• UCC118 prevented the internalization of tight junction proteins after oxidative stress
• Not all strains of *Lactobacillus salivarius* have this capacity
• UCC118 protects tight junction functionality in intestinal epithelial cells

Adapted from Miyauchi E et al. *Am J Physiol Gastrointest Liver Physiol.* 2012;303(9):G1029-1041.
In vivo effectiveness of bacteriocin produced by L. salivarius UCC118

• To demonstrate the in vivo effectiveness of the bacteriocin produced by L. salivarius UCC118, researchers at University College Cork conducted an infectious challenge study in an animal model:
  o Pathogen: Listeria (EDGe expressing)
  o Animal model: Mice
  o Treatment Groups:
    – Control
    – UCC118
    – UCC118 – bacteriocin-deficient
  o Detection method: Listeria in liver, whole body imaging

• These types of pathogen challenge studies are not typically conducted in humans for ethical reasons.

UCC118 prevents *Listeria* infection, via a bacteriocin dependent mechanism in mice

Mouse (Control) + Listeria Infection

Mouse treated with *Lactobacillus salivarius* UCC118 + Listeria Infection

Mouse treated with *Lactobacillus salivarius* UCC118, bacteriocin deficient + Listeria Infection

Conclusions

• *L. salivarius* UCC118 prevented *Listeria* infection in an animal model\(^1\)
• The effect was dependent upon the production of bacteriocin by UCC118\(^1\)

• In addition, *L. salivarius* UCC118 has been shown to reduce certain *Firmicutes* genus members in mice and pig microbiota and *Spirochetes* levels in the mice and pig microbiota\(^2\)
  o This effect was also dependent upon the production of bacteriocin\(^2\)

Emerging Data on *L. salivarius* UCC118

- **Intestinal colonization**
  - Detection in feces\(^1,2\)
  - Detection at surface of ileum and adhesion to colon (*in vivo*)\(^3\)

- **Efficacy**
  - Pilot scale study in patients with Crohn’s disease\(^4\)
  - Pilot quality improvement study in patients with SIBO\(^5\)

**L. salivarius** UCC118 in an open label study in patients with Crohn’s disease

- 21 consecutive patients with **mildly active** Crohn’s disease needing a therapeutic change
- Patients were already taking a stable dose of oral 5-ASA but were not on steroids, had a CDAI of 150-320
- Trial involved probiotic therapy (1x10^{10} organisms in yogurt/day for 6 weeks) instead of **steroid therapy**
- Efficacy was quantified in terms of a change in CDAI and steroid avoidance
- Compliance was confirmed by faecal isolation of the probiotic in all subjects

What is CDAI? Crohn’s Disease Activity Index is a composite measure of the symptoms of Crohn’s.
A score < 150 = remission
150-450 = active disease
> 450 = severe disease

CDAI scores ranged from 175-250 at study enrollment

For the 19 patients who completed the trial without steroids,
- Mean CDAI at week 0 was 208
- Mean CDAI at 3 weeks was 167
- Mean CDAI decreased to 146.6 at 6 weeks (p=0.0049, significant)

At 2 months, 11 of the subjects remained steroid free (did not need steroids to manage their Crohn’s)

In the same clinical study, UCC118 improved TNF-\(\alpha\) response in patients with Crohn’s disease

While there was no statistically significant change in TNF-\(\alpha\) levels (106.14 pg/ml at week 0, 14.617 pg/ml at week 6; \(p>0.05\)), those patients with a high baseline TNF-\(\alpha\) level had significantly reduced levels at week 6.

## Limitation and Strengths of the Study

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>• Decrease in disease score and TNF-α levels without steroid therapy is notable</td>
<td>• Open label</td>
</tr>
<tr>
<td>• Crohn’s flare rarely clears up spontaneously without aggressive treatment</td>
<td>• No control group</td>
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UCC118 Improved Symptoms in Patients with Small Intestinal Bacterial Overgrowth (SIBO)

Quality Improvement Trial, Cleveland Clinic Center for Gut Rehabilitation and Transplantation, Outpatient Clinic

- **L. salivarius UCC118** was swapped out for the usual probiotic and given in addition to standard of care
  - 29 patients with SIBO were given *L. salivarius* UCC118 (10⁸ CFU/day) daily for 90 days
  - Patients reported symptoms at 30, 60 and 90 days
  - Patients’ comments regarding changes to their SIBO symptoms were recorded

Patients in the trial reported an improvement in their symptoms

Select comments from patients regarding their experience taking UCC118 for SIBO symptoms

“Before my diarrhea was so severe I couldn’t make it out of the house. Now I can make it to the grocery store.”

“I have not had diarrhea since midway through the trial.”

“The foul odor of my stool has really gone away since taking the probiotic.”

“I can tell my gas symptoms have worsened since I stopped taking the probiotic.”

“I was able to get off antibiotics for 3 months.”

“My symptoms disappeared after 30 days and I was able to resume my daily activities.”

“I have been symptom-free of SIBO for 2 weeks, I am amazed, astounded, and ecstatic!”

“My diarrhea is no longer present and my stool is more consistent.”

“My SIBO symptoms improved in first 1-2 weeks and have been the same since.”

“I was symptom-free for 18 hours after starting the probiotic—that’s the longest relief I’ve had in years.”

## Limitation and Strengths of the Study

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<td>• Replacement of standard of care probiotic with UCC118 was notable</td>
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<td>• Investigators reported decreased antibiotic usage and decreased time to antibiotics in addition to patient reported improvement of symptoms</td>
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A randomized controlled human study shows the safety of oral supplementation of UCC118 in pregnant patient populations\(^1\)

In vitro experiments show that UCC118 treatment of human mesenteric lymph node (MLN) cells may lead to IL-10 production.\(^2\) IL-10 is a cytokine that plays an important role for the proper functioning of the immune system.\(^3\)

Bifidobacterium animalis ssp lactis 420
B420 probiotic strain—
Mechanisms of action

All of the actions below are supported by pre-clinical and/or clinical data with B420

- Increased SCFA production, which may increase production of satiety hormones
- Reduced food intake
- Decreased bacterial and LPS translocation

Summary of mechanisms of action

*Bifidobacterium animalis* ssp *lactis* 420 has three key potential mechanisms of action that may explain its effects of reducing energy intake in the clinical study

1. Animal studies demonstrate that B420 may increase levels of the anorectic (appetite-reducing) gut peptide GLP-1

2. The clinical study shows that B420 increases total intestinal short-chain fatty acid concentration, which may promote the production of GLP-1

3. *In vitro* and animal studies demonstrate that B420 may improve gut barrier function, which may reduce LPS and bacterial translocation from the gut, which may lower LPS signaling in adipose tissue, reducing the signals associated with weight gain

B420 clinical data\(^1\) —

**Body fat**

*Bifidobacterium animalis* ssp. *lactis* 420 helps control body fat

*In a 6-month clinical study, of overweight individuals, those taking *Bifidobacterium animalis* ssp. *lactis* 420 showed reduced body fat mass compared to placebo group


* Indicates placebo vs. B420 groups significantly different at 6 months (p<0.05; per protocol post-hoc analysis)
B420 clinical data[^1] — Body weight

- *Bifidobacterium animalis* ssp. *lactis* 420 helps control body weight and body weight regulation
- Preliminary evidence shows that *Bifidobacterium animalis* ssp. *lactis* 420 may help contribute to long-term weight maintenance


* Indicates placebo vs. B420 groups significantly different at 6 months (p<0.05; per protocol post-hoc analysis)
Emerging Microbiome-Related Research
Fecal Microbial Transplants

• Fecal Microbial Transplants (FMT) transfers feces
  o Earliest record of usage in 4th century China as “yellow soup” for diarrhea and severe food poisoning treatment
  o Chinese and others used it more and in 18th century, it spread to Europe
  o Currently the only known FDA approved FMT treatment is for recurrent infection with Clostridium difficile, which often flourishes after severe antibiotic use and infection during colitis

• FMT is being explored for the treatment of
  o IBD
  o Insulin resistance and Type 2 Diabetes
  o Dyslipidemia
  o Atherosclerosis
  o Hepatic steatosis

The Gut Microbiome May Impact Multiple Areas of Health

Emerging Research: Soil-Based Probiotics (Spore-Forming Bacteria)

• The genus *Bacillus* strains are gaining interest for their enhanced tolerance and survival under the harsh environment of the gastrointestinal tract

• Not all *Bacillus* strains are soil inhabitants; they can be isolated from other sources (air, water, vegetables, human/animal gut)

• Recent research suggests that spore-forming bacteria do carry probiotic attributes

• *Bacillus coagulans* strains (GBI-30, 6086, MTCC 5856, and others) have been the most researched in recent years

• Research is very new and clinical data is very limited

Multiple Pathways for Communication between the Microbiome and the Brain

- Vagal stimulation
- Neurotransmitters
- Hormones
- Metabolites
- Immune signaling
- MAMPs
- Metabolites

CNS

Vagus nerve

Circulatory system

Immune system

MAMP = Microbe-associated molecular patterns
Communication between the brain and gut, including gut microbes, impacts health

Adapted from Grenham S et al. *Front Physiol.* 2011;2:94.
Key Messages

• Different factors such as diet, drugs, lifestyle and environment can affect the gut microbiome and may result in dysbiosis and loss of diversity

• Microbial dysbiosis and lower diversity are associated with metabolic and gastrointestinal diseases

• Recommending clinically-studied strains of probiotics (that fit proper criteria/guidelines from ISAPP, CRN and IPA for dose (CFU), strain-specification, and quantity guaranteed at expiration) with specific indications may help support general health and alleviate certain digestive, respiratory, metabolic and urogenital symptoms

• Continued research of the human microbiome is necessary in healthy and diseased populations to investigate potential diagnostic and therapeutic solutions