A New Understanding of Probiotics and The Human Microbiome

The latest research on nature's true probiotics

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Natural Medicine & Digestive Health Expert
President of MicroBiome Labs
NEW Current Understanding – The Source

The Human Microbiome Project (HMP) – NIH + 5 years

Why the HMP project?
- Human flora has never been completely characterised. Limitations of microbiology with obligate anaerobes
- Metagenomics — This has allowed for the characterization and study of the total GI microbial population as it relates to health, wellness and disease
- Launched by the NIH 5 years ago — 200 researchers, 80 research institutes

Facts from the Human Microbiome Project (HMP)
- We are more bacteria than human! — 10 trillion human cells vs. 100 trillion bacteria cells
- There are over 1,000 different species of commensal organisms in the GI out of 35,000 possible
- Bacterial genes outnumber human genes more than 150 to 1. (25,000 human genes vs. 3.3 million microbial genes)
- No two individuals have the exact same composition — not even twins. Phylum level conserved — Species level vast difference
- The intestinal microbiota is not homogeneous
Spatial and temporal aspects of intestinal microbiota composition.

A  Proximal GI tract
- Stomach: $10^3$ Lactobacillus, Veillonella, Helicobacter
- Duodenum: $10^3$
- Jejunum: $10^4$ Bacilli, Streptococcaceae, Actinobacteria, Actinomycesae, Corynebacteriaceae
- Ileum: $10^7$
- Colon: $10^{10}$ Lachnospiraceae, Bacteroidales

B  Epithelium
- Epithelial surface
- Mucus layer
- Intestinal lumen
- Feces
- Clostridium
- Lactobacillus
- Enterococcus
- Bacteroides
- Bifidobacterium
- Streptococcus
- Enterobacteriaceae
- Enterococcus
- Clostridium
- Lactobacillus
- Ruminococcus

C  Birth
- 1 year
- Death
- Increasing numbers
- Composition evolves continuously
- Factors:
  - Maternal colonization
  - Diet
  - Environmental exposures
  - Antimicrobial therapies

Sekirov I et al. Physiol Rev 2010;90:859-904

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838 Days to adult-like microbiota

ANY DIVERSION FROM THIS NATURAL PROCESS LEADS TO DISEASE
WHAT IS A HEALTHY MICROBIOME?

A DIVERSE MICROBIOME

Diversity Gives Strength:
1) Can do more functions
2) More functional redundancies
3) Diverse communities are more resistant to invasion

What Effects The Microbiome?
1) Age
2) Diet
3) Antibiotic Use
4) Physiology
5) Genetics - loosely
WHAT ARE STRATEGIES FOR PROBIOTIC SUPPLEMENTATION?

CURRENT STRATEGY – RESEEDING THE GUT
The Current Solutions in Dietary Supplements

Supplement Facts

Available in 14ct, 30ct & 60ct.

Serving Size: 1 Capsule

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount per Serving</th>
<th>% Daily Value</th>
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<tbody>
<tr>
<td>Ultimate Flora Critical Care Blend</td>
<td>255 mg</td>
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<tr>
<td>Bifidobacterium lactis</td>
<td></td>
<td></td>
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<tr>
<td>Bifidobacterium breve</td>
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<tr>
<td>Bifidobacterium longum</td>
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<td>Total Bifido Probiotic Cultures</td>
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<tr>
<td>Lactobacillus acidophilus</td>
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<tr>
<td>Lactobacillus casei</td>
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<td>Lactobacillus plantarum</td>
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<td>Lactobacillus salivarius</td>
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<td>Lactobacillus thermosus</td>
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<tr>
<td>Lactobacillus bulgaricus</td>
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<tr>
<td>Total Lacto Probiotic Cultures</td>
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<tr>
<td>Total Bifido/Lacto Cultures</td>
<td>50 billion†</td>
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Daily Value (DV) not established

Other Ingredients: Vegetable capsule (gelatin and shell), cellulose.
Directions: For best results, take one capsule daily with a light meal.

WARNING: Consult your physician before using this or any product. If you are pregnant, nursing, trying to conceive, taking medication or have a medical condition.

Supplement Facts

Serving Size: 3 Capsules (1.800 mg) Amount Per Serving % Daily Value

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<tr>
<th>Nutrient</th>
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<th>% Daily Value</th>
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<tr>
<td>Calories</td>
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<tr>
<td>Total Fat</td>
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<tr>
<td>Total Carbohydrate</td>
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<td>&lt;1%*</td>
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<tr>
<td>Vitamin C (Total Tocopherols)</td>
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<tr>
<td>Vitamin E (as Tocotrienol)</td>
<td>7.51 IU</td>
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<td>Brazilian green propolis (non-bacteroides diocecoo)</td>
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<tr>
<td>P-aminobenzoic acid</td>
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<td>Kaeferinide</td>
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<td>Aspartic acid (as L-aminoacid)</td>
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<tr>
<td>Flosssed oil (as L-aminoacid)</td>
<td>320 mg</td>
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</tr>
<tr>
<td>Proprorietory lacto acid bacteria blend*</td>
<td>21.6 million CFU</td>
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| ** Bifidobacterium sp. Blasi, Bifidobacterium sp. Infantis, Bifidobacteria Longum, Enterococcus Faecalis 7518, Lactobacillus acidophilus, Lactobacillus enter, Lactobacillus Bulgaricus, Lactobacillus acidophilus, Lactobacillus plantarum, Entrobacoccus thermophilus.

*Percent Daily Values based on a 2,000 calorie diet. **Daily value not established.
†Other ingredients: Capsule = Tapioca, prepressed from Veratin 2600 root, glyceerin spuit, soybean oil, and caragamin (red aga).
‡Other Ingredients: Vegetable capsule, gelatin spuit, soybean oil, and caragamin (red aga).
THE RESEEDING PLAN

ISSUES

SURVIVAL
FITNESS
NATURAL/STRATEGY
QUALITY
SURVIVAL
The Food Standards Agency (FSA) study w/ Reading University (UK)
Dr. G.R. Gibson, Dr. G. Rouzaud, Dr. J. Brostoff and Dr. N. Rayment

Purpose:
1) Evaluate the probiotic effect of commercial products in the human gut. Any affect on gut flora.
2) 35 strains from commercial products were studied. Primarily *lactobacillus sp.* and *bifidobacterium sp.*
3) Evaluate the survivability of common probiotics through the GiT.

**STEP 1:** Survival through gastric juices in the stomach. 20 minutes at pH 1-3
- pH 1 = None survived with any viability. Unable to recovery
- pH 2-3 = 18 of the 35 showed around 50% survival

**STEP 2:** Survivors through the stomach were tested for survival in the upper small intestines via bile acid tolerance
- Of the 18 tested, only 6 showed viability in the presence of bile acid salts and would have a chance to make it to the large intestines.

**STEP 3:** The 6 strains were then chosen for survival testing in lower intestines
- Of the 6 strains, 4 strains showed viability in lower intestinal conditions

**Only 4 of 35 strains would survive to enter the large intestine and the survivors would have less than 50% survival**
Survival of Probiotics in Simulated Gastric Fluid

Study Design
- 4 Products tested: 1 leading USA retail brand supplement; 1 leading yogurt and 1 leading Greek yogurt vs. MegaSporeBiotic™
- USP Simulated Gastric Solution – pH 1.3 for 2 hour then bile salts.
- Data is based on percent survival of bacterial population.
- Test products (other than MegaSpore) were sourced directly by the lab from store shelves.

Results
- Both yogurts had starting counts – regular yogurt had higher counts – but both yogurts saw over 99.99% of “probiotic” bacteria die off in the stomach.
- The leading USA supplement brand had nearly a 4 fold overage but over 99.9% died off in the stomach.
- MegaSporeBiotic showed 100% survival with no loss at all.
ISSUES - THE RESEEDING PLAN

FITNESS:
- Aerobic bacteria seeding an Anaerobic Environment.
- Evade immune destruction.
- Do they fit in your gut? – binding sites.

Larsen, N et al 2007: *The adhesion abilities of 11 strains of Lactobacillus in a intestinal cell line model.* Included popular strains such as: L. rhamnosus GG, L. reuteri ATCC 55730, L. johnsonii NCC 533 and L. reuteri DSM 12246.

RESULTS: The best adhesion was 38% by L. reuteri, second best was 24% and the rest went down from there.

IS IT NATURAL:
- Does it happen in nature?
- The issue of Finite Overload – goes against expanded diversity.
Some strains used may not have studies to show their benefits.
Mislabeling of probiotic strains has been found to be an issue

Several documented cases of mislabeled probiotics – a panel of EU experts in microbial characterization found the following:
1) Products were incorrectly labeled with the wrong strains
2) Products contained markedly reduced numbers than claimed
3) Found presence of strains not labeled – in some cases they were pathogenic.

“Quality control of probiotics is lacking, study suggests.” (Nutraingredients-USA, 20-Nov-2015)

A new study by scientists at the University of California has found that contents of many bifidobacterial probiotic products differ from the ingredients listed.

"After testing 16 probiotic products available in local Californian stores and also online, they found only one of the products exactly matched the bifidobacterial species claims on the label. Some products had pill to pill and lot to lot variation."
BUT WHY DO SOME “PROBIOTICS” WORK?


**Multicenter, randomized, controlled trial of heat-killed Lactobacillus acidophilus LB in patients with chronic diarrhea.**


“At the end of the treatment, the clinical symptoms were markedly improved in the Lacteol group, indicating that *L. acidophilus LB* is more effective than living lactobacilli in the treatment of chronic diarrhea.”


**Live and heat-killed Lactobacillus rhamnosus GG: effects on proinflammatory and anti-inflammatory cytokines/chemokines in gastrostomy-fed infant rats.**

Li N, Russell WM, Douglas-escobar M, Hauser N, Lopez M, Neu J.

“In conclusion, both live and heat-killed LGG provided by the enteral route decrease LPS-induced proinflammatory mediators and increase anti-inflammatory mediators.”

**Effects of the Consumption of Heat-killed Enterococcus faecalis EC-12 Preparation on Microbiota and Metabolic Activity of the Faeces in Healthy Adults**

Atsushi Terada, Wakoto Bukawa, Tatsuhiko Kan and Tomotari Mitsuoka

Microbial Ecology in Health and Disease 2004; 16: 188/194

Faecal values on all end-points seemed to improve by the administration of heat killed EC-12
WHAT ARE STRATEGIES FOR PROBIOTIC SUPPLEMENTATION?

CURRENT STRATEGY – RESEEDING THE GUT

REVISED STRATEGY – BACK TO BASICS
THE IMPORTANCE OF ENVIRONMENTAL BACTERIA

- **Distinct Distal Gut Microbiome Diversity and Composition in Healthy Children from Bangladesh and the United States**
  Audrie Lin, et al 2013

  “The distal gut of Bangladeshi children harbored significantly greater bacterial diversity than that of U.S. children, including novel lineages from several bacterial phyla.”

- **Human gut microbiota community structures in urban and rural populations in Russia**
  Alexander V, et al 2013

  “the original microbial community structures occurred in hosts from urban populations 2.6-fold less frequently than in the rural hosts, which implies that the rural population’s microbiota community was the healthy original”

- **Comparison of fecal microflora of elderly persons in rural and urban areas of Japan.**
  Benno Y, et al 1989

  “found significant rural-urban disparities in microbiota composition. Rural populations had much higher bifidobacteria levels...”
THE IMPORTANCE OF ENVIRONMENTAL BACTERIA

STUDIES ON THE HADZA TRIBE OF TANZANIA

- Some of the last hunter-gatherer people on earth.
- They live an ancient, ancestral life.
- Their environment hasn’t changed for 1000’s of years.
- Massive exposure to ancestral microbial community.
- Vastly different microbiota compared to westernized populations.
- Virtually no common digestive diseases such as Crohn’s, UC, Colon Cancer, Reflux, etc.
What kind of probiotic features are required?
REQUIRED FEATURES OF NATURE’S PROBIOTIC

• Naturally survive the harsh gastric environment

• Be of a strain that is found in the Microbiota
  It has to have a binding site and belong in the gut.

• Must have evolutionary significance

• Must be a facultative anaerobe

• Must have a bi-phasic life cycle

• Must have clinical demonstration of safety and efficacy
So what is nature’s design for supplemental probiotics?
Bacterial Spores

- In particular, *Bacillus* spores as they are the most widely studied and most widely used probiotics outside of the supplement market.

- *Bacillus* spores were the first commercial probiotics. They were also the first prescription probiotics starting in 1958:
  - Enterogermina® (Sanofi-Aventis, Italy)
  - Bacti-Subtil® (Aventis Pharma, France)

- Used extensively in agriculture and aquaculture
  - AlCare®, BioGrow®, BioPlus®2B, NeoFerm BS10, LiquaLife®, etc.

- Most widely used and well studied strains in humans are:
  - *Bacillus Subtilis*
  - *Bacillus Licheniformis*
  - *Bacillus Coagulans*
  - *Bacillus Clausii*
  - Introducing – Patented Bacillus Indicus HU36™
Key Features of Bacterial Spores

- They form robust endospore and can withstand harsh temps, desiccation, low pH, gastric barriers, antibiotics, UV radiation, solvents, enzymes and even high pressures.

- They are found all over the environment (soil, vegetation, dust, rocks, aqua-environments, digestive systems of insects, marine life, mammals, etc.

- Spores remain dormant for over 50 million years.

- Found all over the ancient environment: ice-core studies.

- They colonize very effectively in the Human GIT and have been found to colonize very effectively in the GIT of several different animals.

- Are found as part of the normal human commensal flora.

- LONG history of use in industries where efficacy is closely measured (pharma, agricultural).

- Extremely safe.

- Their use as probiotics is supported by evolution – true commensal organism.
The Amazing Endospore

Figure 2

Spore structure and locations of components of the spore germination apparatus. The sizes of the various spore layers are not drawn to
Colonization

- Numerous studies and a long history of use have confirmed survivability of spores through the GIT.
- Studies have shown that spores are well adapted to germinate in the small intestines, grow and proliferate and then can re-sporulate in the lower GI.
- This ability to re-sporulate is clearly an evolutionary adaptation as several functions of bacillus spores require re-sporulation in the lower GI (exp: GALT development).
- Bacillus spores are found in the GI of insects, animals and humans, indicates a universal function as a commensal probiotic.
- Studies now indicate that the environment is simply a vector to transfer the bacteria from host to host, the spores are better suited for life in the GI, but designed to be passed via the environment.
• Microbiota Shift and Balance

Spores have the ability to shift the microbiota and favor the growth of good bacteria.

They do this by:

▪ Producing a significant number of antibiotics that control bacterial growth.
  (Example: Coagulin, Subtilisin, Amicoumacin, Surfactin, Iturins A, and Bacilysin)

▪ Competitive exclusion (CE) of pathogenic organisms.
  (They compete for space and nutrients.)

▪ Increasing the growth of important GIT commensals, such as lactobacillus.
  (They produce compounds that feed the commensal)

▪ A recent Gut Model study demonstrated almost a 30% shift in microbiota population with a continuous administration of bacillus spores.
• **Immunomodulation**

The intestines possess the largest amount of lymphoid tissue in the human body

**GALT** – Gut Associated Lymphoid Tissue

**Peyer’s Patch** – found in the ileum of the small intestines.

**Mesenteric glands** – sites of amplification

Immune sampling occurs in the lumen – an immune response is generated in the mucosa and then amplified in the mesenteric glands.
• **Immunomodulation**

- Bacillus spores help **Develop the GALT**.
  (It does this with the cooperation of Bacteriodes Fragilis sp.)
- Bacillus spores ** Increases circulating T and B Lymphocytes.**
  (By becoming active in the small intestines and stimulating the Peyer’s patch.)
- Bacillus spores **Shift the body from Th2 inflammatory to Th1 adaptive.**
  (via stimulation of TLR 2/4, CCR-5, IL-12, IFN-g and TNF-a)
- Bacillus spores **Improve Pattern recognition to curb Autoimmune and Allergic immune response.**
  (via Toll-like receptors(TLR) stimulation in Peyers Patch)
- Bacillus spores shift from **Innate immune response to Adaptive immune response.**
  (via Dendritic activation)
• Digestive Aid
  - *Bacillus* probiotics has been shown to **produce key enzymes** that help the digestion of food products and alleviate bloating, cramping and discomfort.
  - **Changing Dysbiosis** - Dysbiosis is also responsible for incomplete digestion of consumed foods. Studies have shown that *Bacillus* probiotics help prevent the growth of harmful bacteria and promotes the growth of beneficial bacteria – thus alleviating dysbiosis. Better flora, better digestion.
  - **Direct digestion** of resistant starches, proteins, fats, plant matter and non-starch polysaccharides
  - **Reduces Inflammation** in the GI which helps digestion and assimilation of nutrients.
  - **Detox** - Bacillus has been shown to neutralize genotoxic compounds encountered in the GIT.
    - Examples: Vomitoxin, also known as deoxynivalenol (DON), found in wheat, corn and other grains is neutralized by *bacillus* in the GIT.
    - Example: Zearalenone (ZEA) is naturally produced by the fungus found on cattle, milk and even plants – impacts fertility and a number of other conditions. Bacillus has been shown to neutralize this toxin in the GIT.
• Key Nutrient Production
  • Production of vitamins – Menaquinones, full array of b-vitamins, etc.

  • Bacillus also digests resistant starches and non-starch polysaccharides (NSP; major components of dietary fiber) to short-chain fatty acids (SCFA) - acetate, propionate, and butyrate. Increased SCFA production by nearly 40% over normal flora.
    • SCFA stimulate colonic blood flow and fluid and electrolyte uptake.
    • Helps heal the colon and studied extensively as an anti-cancer agent.
    • Increases fat metabolism.
    • Major energy source for colonocytes.
    • Supports the growth of good commensal organisms (lactobacillus and bifidobacter).
    • Lowers cholesterol and triglycerides.
    • Reduces gut and systemic inflammation.
    • Decreases Intestinal permeability – helps leaky gut.

  • NEW Bacillus Indicus HU36 – produces high levels of carotenoids that are of the highest bioavailability.
APPLICATIONS of Bacillus Spores

- Immune modulation: Allergies, Asthma, Rheumatoid Arthritis, immune dysfunctions.
- Immune stimulation of peripheral T-lymphocytes and B-lymphocytes: Fights colds, flus, sinus infections, ear infections, gut infections, etc.
- Improved urogenital health and flora: Decrease in frequency of urinary tract infections
- Control of gut microbial growth: Reduction in side effects related to antibiotic therapy, Treatment for small intestinal bacterial overgrowth (SIBO)
- Reduced gut inflammation: Use for Crohn’s disease, IBS, ulcerative colitis, obesity, etc.
- Detoxification of the intestinal tract: Dermatitis, eczema, psoriasis, fatigue, fibromyalgia, etc.
- Improved digestion - improved nutrient absorption: Fatigue, malaise, athletes, osteoporosis, CVD
- Reduction of cholesterol: CVD, peripheral artery disease, high BP, arrhythmias, etc.
- Production and absorption of critical nutrients i.e. carotenoids, quinols, vitamins and enzymes: Reduction of oxidative stress – overall health improvement
1. Must have a position in the microbiota – its important for a probiotic to be able to colonize well to afford its beneficial function. Without a spot for it, the probiotic will not be able to compete.

2. Must have sufficient numbers to colonize and cause stimulation for benefit.

3. Supplemented Probiotics, by evolution, should naturally occur in the environment (where they gain exposure to the host) and be stable both in the environment and the GIT.
Function of Spores in MegaSporeBiotic

- Colonization
- Microbiota Shift
- Nutrient Production
- Digestion
- Immune Modulation
Developed under advisement of spore expert Dr. Simon Cutting, Ph.D. (Developed by a Practitioner for Practitioner only use.)

• Utilises verified, branded and registered strains – the only way to ensure proper characterization.
• Contains 5 efficacious bacillus spore probiotics in a consortium – each strain is backed up by a history of use and by published science.
• All strains are produced in a cGMP facility under drug manufacturing guidelines.
• Guaranteed prescription grade and licensed from Royal Holloway London University.
• Delivers over 4 billion live probiotic cells daily – a dose that not only matches but exceeds all prescription products of the same strains. The single most powerful spore probiotic formulation.
• Blended with a pre-biotic to enhance colonization.
• Guaranteed 100% spore format for maximum survivability and precise dosing
• THE FIRST probiotic formulation to deliver powerful, highly-bioavailable carotenoids via in vivo production. Bacillus Indicus is a patented, carotenoid rich strain.
• As far as we know, MegaSpore is the only probiotic that contains strains shown to increase SCFA production by 40%.
Why these 5 strains?

**Bacillus Subtilis HU58™**
- Widely used, safe and highly effective.
- Produces over 12 affective antibiotics
- Makes nattokinase and vitamin K2
- Highly important for immune development - GALT

**Bacillus Indicus HU36™**
- Potent immune stimulation
- Produces high levels of carotenoids – lycopene, astaxanthin, beta-carotene, lutin.
- Produces quinols and vitamins
- The most effective antioxidants on the market.

**Bacillus Clausii**
- Most widely used probiotic drug in the world.
- Potent immune stimulator
- Antibiotic resistant for use during antibiotic treatment

**Bacillus Licheniformis**
- Produces antibiotic bacitracin.
- Produces protease for digestion and helps protein digestion.
- Produces whole spectrum of B vitamins and Folic acid

**Bacillus Coagulans**
- Very well studied and long history of use.
- Very effective against IBS, Crohn’s
- Produces L+ optical form of lactic acid
- Potent immune stimulation

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  - Very effective against IBS, Crohn’s
  - Produces L+ optical form of lactic acid
  - Potent immune stimulation
- **Gut permeability study**: Double-blind, placebo controlled human trial using post-prandial endotoxemia. Pilot trial showed complete gut remodeling in 30 days. Study to be completed in 6 weeks.
- **SIBO Study using ROME III Criteria**: Double-blind, placebo controlled, metacentric study on IBS symptoms and safety blood markers. Study is beginning.
- **Hepatic Encephalopathy Study**: Double-blind, placebo controlled study on lowering digestive and blood ammonia levels in liver failure patients. Study completed, manuscript is under preparation. Data shows that even in the presence of rifaximin, Megaspore’s HU58 strain was able to lower blood ammonia levels in liver failure patients with no adverse events.
- **Mucosal Secretory IgA study**: Double-blind, placebo controlled study on increasing the expression of mucosal secretory IgA.
- **In Development with the National Institutes of Health**: Double-blind, placebo controlled study on HIV Enteropathy in HIV+ subjects.
• Special offers open to Practitioners who have tuned in and open an online account at www.megasporebiotic.co.uk

• **Buy 12** bottles of Megasporbiotic & get **2 extra Free**  
  (This offer is open until 5th May 2016)

• Use discount code **MEGA10** for **10%** off all new orders for the next 3 months through end of July 2016.

• **BANT** Members get **12.5%** off all orders.

• **Questions?** email sales@tmcventures.com
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Apply for an account online at
www.megasporebiotic.co.uk
0845 528 1296   (+44) 845 528 1296
Email: orders@tmcventures.com

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