

MICROBIOME RESTORATION POST ANTIBIOTIC THERAPY

THE USE OF A NOVEL PROBIOTIC COMBINATION TO BLUNT THE RESPONSE OF ANTIBIOTIC THERAPY ON THE CORE GUT MICROBIAL ECOLOGY

BY SARAH G. ELLIS, ND, MS





LEARNING OBJECTIVES

- Discuss antibiotic use in the United States
- Review how antibiotics impact overall health and microbiome health
- Introduce RestorFlora, ingredients, dosing, and supporting research
- Review *Saccharomyces Boulardii*, *Bacillus Subtilis*, *Bacillus Coagulans*, and *Bacillus Clausii*



ANTIBIOTIC USE IN THE USA

- 47 million antibiotic courses are prescribed for infections that don't require antibiotics
- Over time, bacteria can mutate their genes, making antibiotics less effective and infections more deadly
- Antibiotic resistance develops as bacteria and fungi develop the ability to defeat the drugs designed to kill them
- 50,000 antibiotic-resistant pathogens related deaths per year in US and Europe

COMMON CAUSES OF ACUTE ANTIBIOTIC EXPOSURE

Otitis media

Upper Respiratory Infections

Strep throat

UTIs

GI infections

COMMON CAUSES OF LONG-TERM ANTIBIOTIC EXPOSURE

Systemic infection

**Prosthetic joint/valve
infection**

Organ transplantation

Splenectomy

Recurrent UTIs

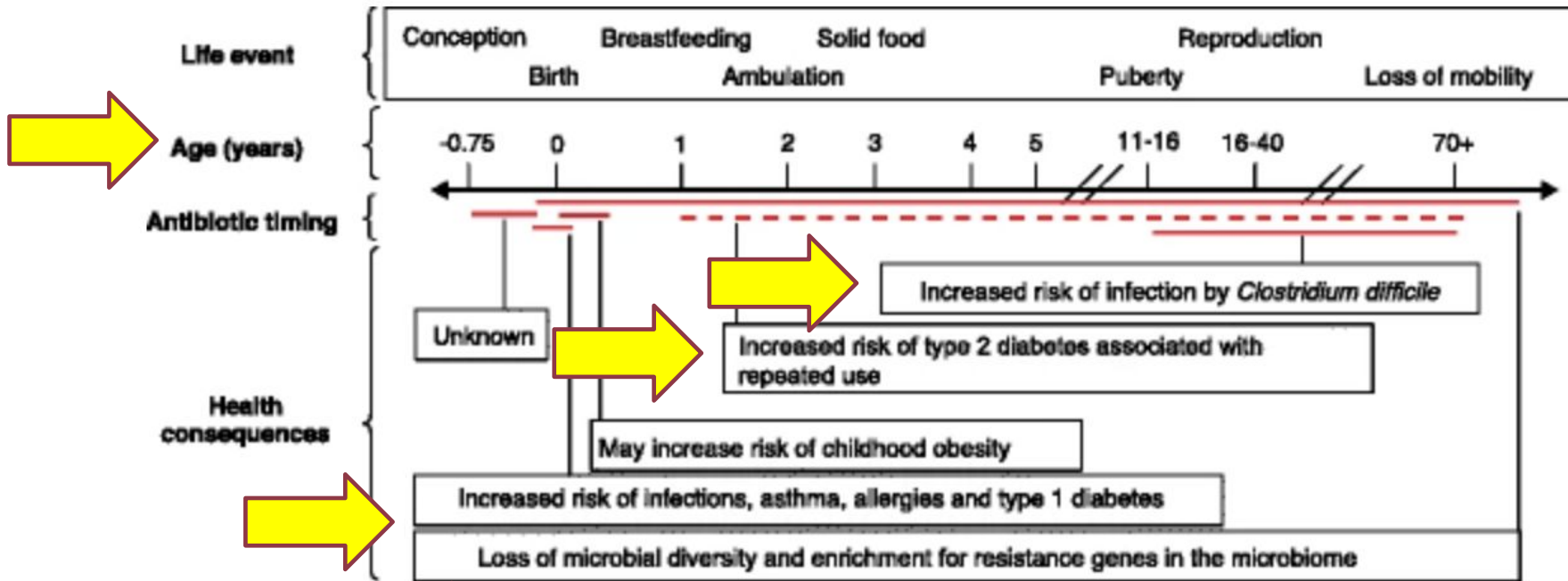
HEALTH IMPACT OF ANTIBIOTIC USE

- Broad Spectrum leads to a systemic effect, rather than localized
- Disrupt the gut ecology and can lead to dysbiosis
 - Amoxicillin can cause changes that last 30 days to 2 months
 - Cipro & Cefprozil have longer impacts, sometimes permanent
- Antibiotic associated diarrhea
- Overuse can cause metabolic, immunological, and developmental disorders



HEALTH IMPACTS OF ANTIBIOTIC USE

- Damages alpha diversity
- Increases susceptibility to pathogens
- Causes damage to intestinal barrier
- Increases susceptibility for antibiotic resistance
- Alter gene expression
- Less mitochondria per cell
- Inappropriate immune activity



HEALTH IMPACT OF ANTIBIOTIC USE

Red lines indicate that a single dose of antibiotics within the time period has been linked to a health consequence, whereas a dotted red line indicates that multiple doses of antibiotics within the time period are required to observe a link.

EARLY ANTIBIOTIC EXPOSURE:

In 5 out of 9 studies, antibiotics (including penicillins and macrolides) were associated with reduced bifidobacteria.

Amoxicillin exposure was associated with complete disappearance of *Bifidobacterium adolescentis*.

4 studies showed decrease in *Lactobacillus* for up to 12 months following exposure to penicillin and up to 24 months following macrolide use.

3-fold increase in *Clostridium* within 6 months of exposure to macrolides.

Reduced *Clostridium* clusters IV and XIVa which are inducers of T regulatory immune cells and play a role in regulating or suppressing other cells in the immune system.

Akkermansia mucinophila completely disappeared with azithromycin use.



Pre-antibiotics

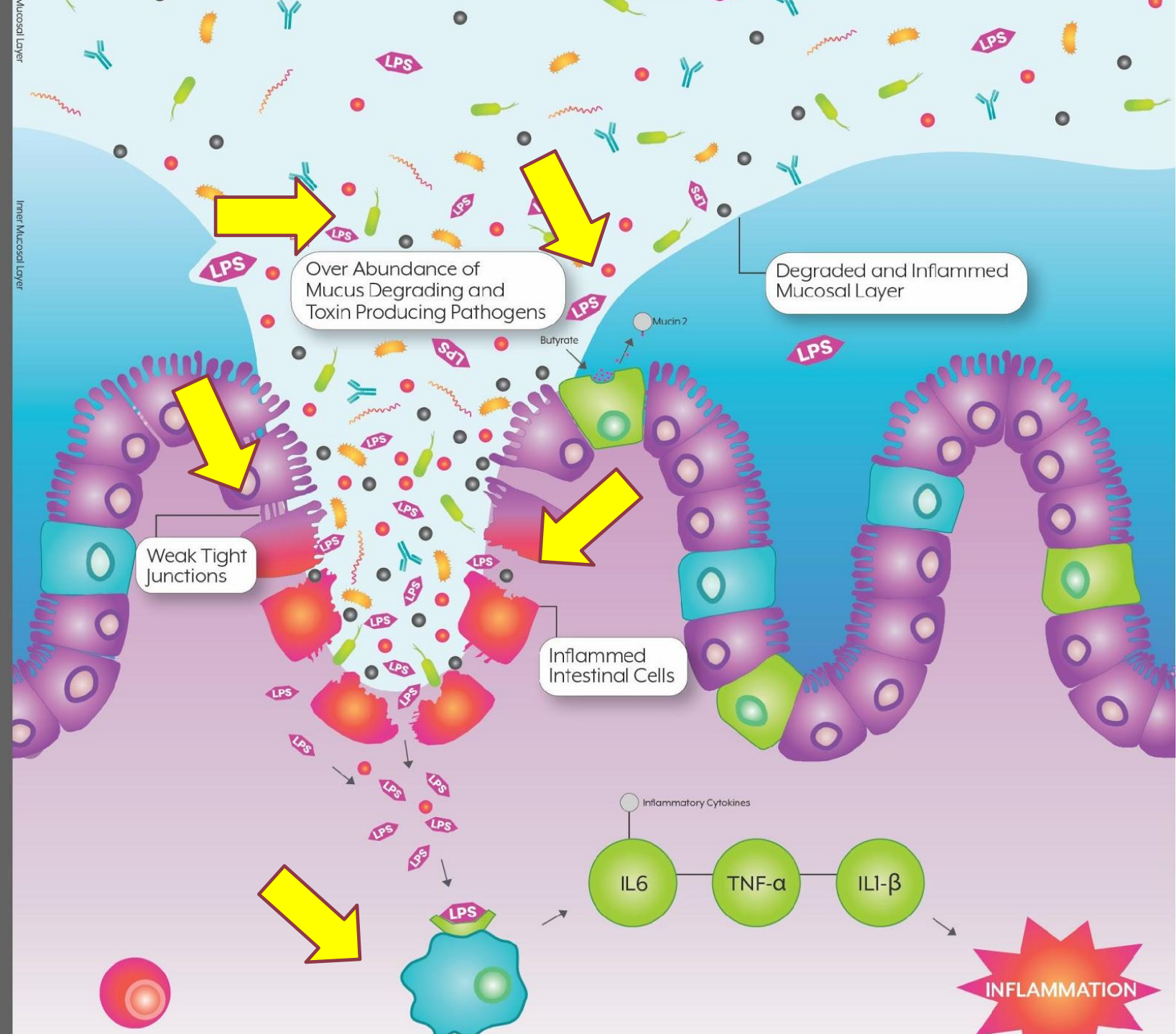
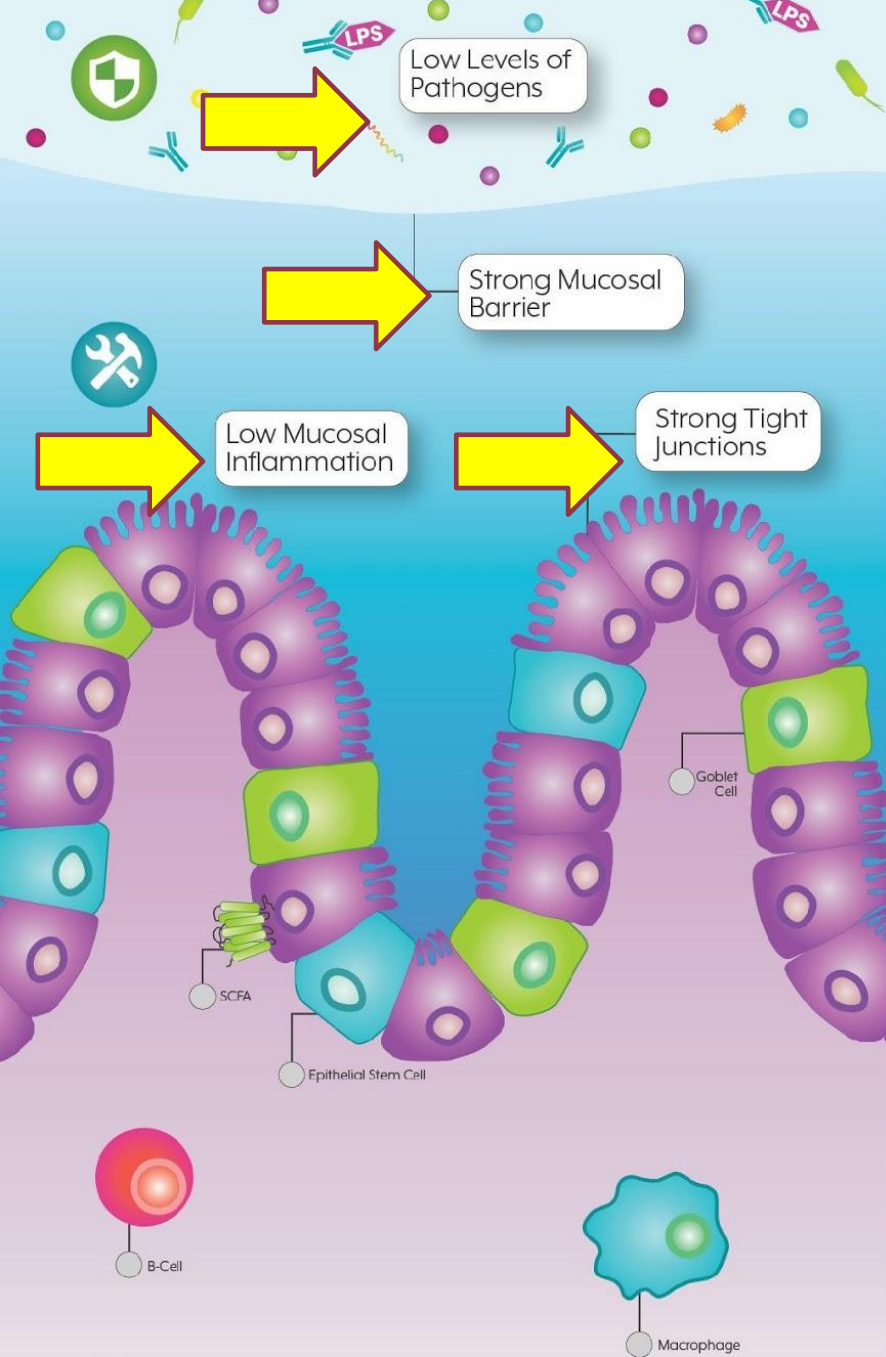


During antibiotics



Post-antibiotics

ANTIBIOTICS AND THE MICROBIOME





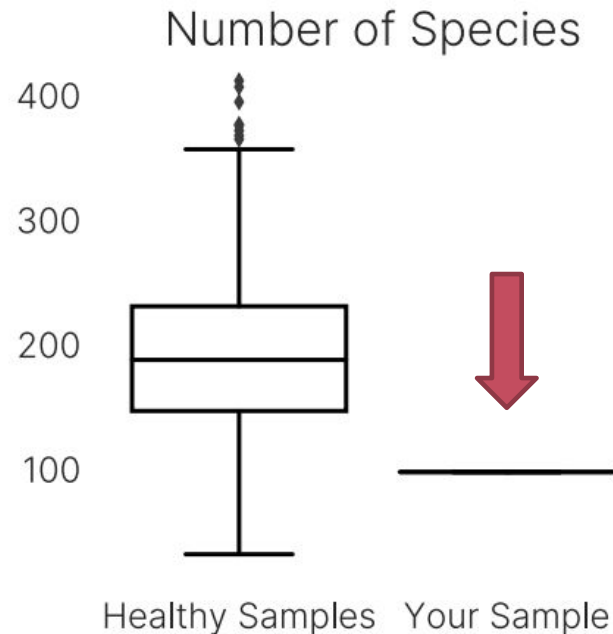
Prescription Antibiotics
DYSBIOSIS RISK FACTORS

- Natural Antimicrobials
- Pesticides
- Infections
- Excessive Alcohol Intake
- Smoking
- Stress
- Lack of Sleep
- Intense Exercise
- Standard American Diet/Saturated Fats

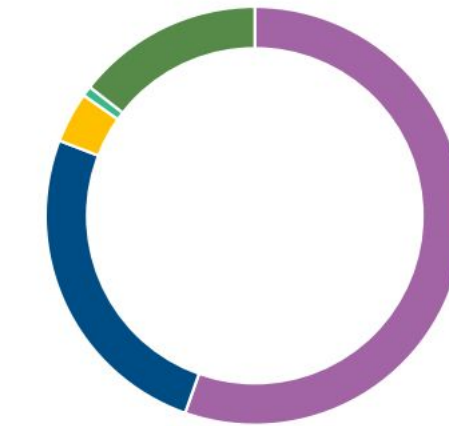
DYSBIOSIS RISK FACTORS ALTER THE CORE MICROBIOME

Alpha Diversity

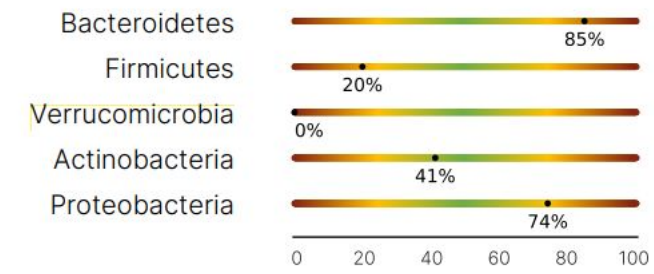
Number of species in the gut microbiome: **98**
Your Alpha Diversity was found to be: **6.18**



Phylum Level Your Sample



- Bacteroidetes 55.40%
- Firmicutes 25.45%
- Proteobacteria 3.92%
- Actinobacteria 0.78%
- Others 14.45%



BACTERIAL CONTRIBUTIONS

Commensals	Opportunistic/Pathogenic
Short-Chain Fatty Acids	Ammonia
Vitamins	Hydrogen Sulfide
Antioxidants	Methane
Neurotransmitters	Toxins and virulence factors
Optimize the gut pH	Lipopolysaccharide (LPS)



MICROBIOME IMPACT

- Metabolic dysfunction
- Autoimmunity
- Skin conditions
- Nervous system disorders
- Hormone balance
- Liver health and function
- Anxiety and depression

GUT DYSBIOSIS



Cardiovascular Disease
Atherosclerosis



Parkinson Disease
Alzheimers Disease
Multiple Sclerosis
Depression
Anxiety
Pain
Stress



Hypothyroidism



Obesity
Type 2 Diabetes



Inflammatory Bowel Disease
Irritable Bowel Syndrome
Ulcerative Colitis



Sarcopenia
Rheumatoid Arthritis
Cachexia
Frailty

THE STANDARD CURE

- Re-seeding with probiotics
 - Taking probiotics and assuming that once they reach the gut microbiome they will colonize and thrive





PROBIOTIC CHALLENGES

- Cannot survive gastric barrier
- Cannot withstand antibiotic therapy
- Single strain, single effects
- Re-seeding doesn't mean colonizing
- Not designed to survive in an oxygenated environment during manufacturing

THE SOLUTION





RESTORFLORA™

- Blend of Bacillus Subtilis HU58, Bacillus Clausii,, and Saccharomyces Boulardii
- Effective GI protection during antibiotic therapy
- Beneficial in treating antibiotic associated diarrhea (AAD), food born illness, candida, H pylori, and E. coli overgrowth



RESTOREFLORA™

- Probiotic Blend- 2 Billion CFU
- B subtilis HU58
- B clausii SC109
- *Saccharomyces cerevisiae var boulardii* CNCM-I-1079- 5 Billion CFU |

A UNIQUE, DUAL-ACTION FORMULA

of probiotic yeast, *Saccharomyces boulardii*, with two widely used probiotic strains that together offer the ultimate digestive support.**

SUGGESTED USE: Ages 5+

Take 1 capsule daily with a meal or as directed by your healthcare practitioner. Children under 5 years of age, please consult with your healthcare practitioner.

CAUTION: If you are pregnant or nursing, taking any medication, or have a medical condition, consult your doctor before using any dietary supplement. Do not use if safety seal is broken or damaged. Keep out of reach of children.

****These statements have not been evaluated by the Food and Drug Administration (FDA). This product is not intended to diagnose, treat, cure, or prevent any disease.**



RESTOR FLORA™

Dual-Action
Formula For
Digestive
Support**

Dietary Supplement

50
Capsules

GASTROINTESTINAL HEALTH

SUPPLEMENT FACTS

Serving Size 1 Capsule
Servings Per Container 50

Amount Per Serving	% Daily Value
Proprietary Probiotic Blend	360 mg †
<i>Saccharomyces cerevisiae</i> 5 billion CFU † <i>var boulardii</i> (CNCM-I-1079)	
<i>Bacillus clausii</i> (SC-109)	1 billion CFU †
<i>Bacillus subtilis</i> , HU58™	1 billion CFU †

† Daily values not established.

OTHER INGREDIENTS: Cellulose, vegetable capsule (cellulose and water).

MANUFACTURED FOR:

Microbiome Labs (904) 940-2208
1332 Waukegan Rd | Glenview, IL 60025



US010523



SUGGESTED USE

- **Ages 5+** First 7 days, take 2 capsules at the same time per day after a meal or as directed by your physician while taking antibiotics. The following 7 days, take 1 capsule per day after a meal or as directed by your physician for 7 days.
- Capsules can be opened, and contents sprinkled on soft foods, water, juice or other non-carbonated beverages for easy consumption.

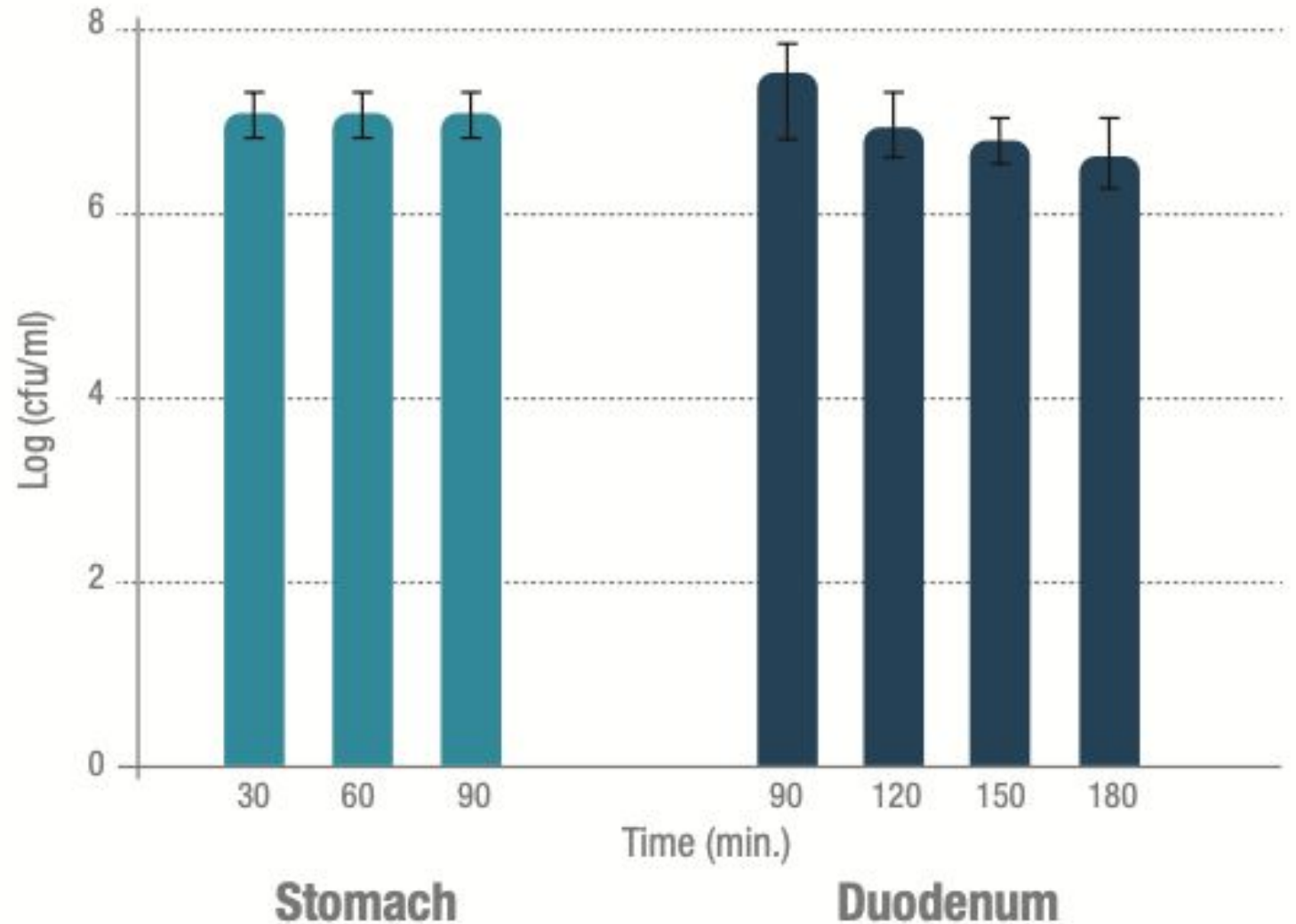


SACCHAROMYCES BOULARDII PROPERTIES

- Commensal yeast probiotic
- Used commercially for over 30 years
- Resistant to gastric acidity
- Naturally resistant to antibiotics
- Does not colonize
- Reaches therapeutic concentrations within 3 days and is fully cleared from GI tract in 3-5 days

SURVIVAL

- Survival of *S. Boulardii* CNCM-I-1079
- Survives in stomach
- Survives in Duodenum
- Can be administered with or without food





S. BOULARDII COMMON AREAS OF RESEARCH

- Antibiotic associated diarrhea
- Occasional diarrhea and traveler's diarrhea
- Helicobacter pylori
- Clostridium difficile
- Infant and pediatric gut health
- Irritable Bowel Syndrome



ANTIBIOTIC ASSOCIATED DIARRHEA (AAD)

- 5-25% of patients experience diarrhea after a round of antibiotics
- Bacteria that metabolize high molecular weight carbohydrates can be diminished
- High molecular weight carbohydrates accumulate in the colon and cause water to be pulled into the intestinal tract
- Higher concentrations of unabsorbed bile acids also cause an influx of secretions that are linked to diarrhea

S. BOULARDII REVIEW PAPER

- Properties
- Clinical efficacy
- Mechanisms of action



Efficacy and safety of the probiotic *Saccharomyces boulardii* for the prevention and therapy of gastrointestinal disorders

Theodoros Kelesidis and Charalabos Pothoulakis

Abstract: Several clinical trials and experimental studies strongly suggest a place for *Saccharomyces boulardii* as a biotherapeutic agent for the prevention and treatment of several gastrointestinal diseases. *S. boulardii* mediates responses resembling the protective effects of the normal healthy gut flora. The multiple mechanisms of action of *S. boulardii* and its properties may explain its efficacy and beneficial effects in acute and chronic gastrointestinal diseases that have been confirmed by clinical trials. Caution should be taken in patients with risk factors for adverse events. This review discusses the evidence for efficacy and safety of *S. boulardii* as a probiotic for the prevention and therapy of gastrointestinal disorders in humans.

Keywords: efficacy, gastrointestinal disorders, probiotic, *Saccharomyces boulardii*, safety

Introduction

There is increasing evidence that the gastrointestinal microflora is a major regulator of the immune system, not only in the gut, but also in other organs [Gareau *et al.* 2010]. The nonpathogenic yeast *Saccharomyces boulardii* has been prescribed in the

marketed as a dietary supplement [McFarland, 2010]. Several mechanisms of action have been identified directed against the host as well as pathogenic microorganisms and include regulation of intestinal microbial homeostasis, interference with the ability of pathogens to colonize

Ther Adv Gastroenterol

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S. BOULARDII MECHANISMS OF ACTION: LUMINAL

- **Antimicrobial Activity:** prevents growth of pathogenic organisms and reduces translocation of pathogens across the intestinal barrier
- **Anti-toxin Effects:** neutralizes and breaks down pathogenic toxins and virulence factors
- **Cross-talk with Normal Microbiota:** helps re-establish normal microbiota more rapidly

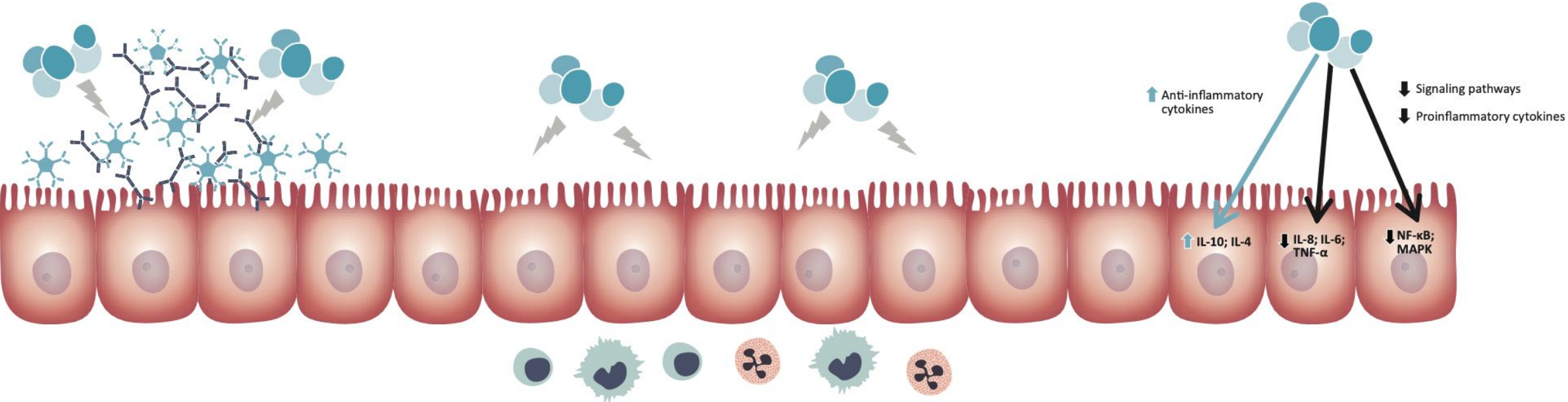
S. BOULARDII MECHANISMS OF ACTION: TROPHIC ACTION

- Reduces mucosal inflammation
- Enhances enzyme secretion that helps prevent diarrhea
- Restores normal levels of SCFAs
- Stabilizes GI barrier function and strengthens tight junctions

S. BOULARDII MECHANISMS OF ACTION: IMMUNE REGULATION



- Innate Immunity:
 - Monocytes and granulocytes
 - Increases Kupffer cells in liver
- Adaptive Immunity:
 - Enhances SIgA
 - Stimulates Treg cells
- Reduces pro-inflammatory cytokines
 - Increases secretion of IL-10

S. BOULARDII MECHANISMS OF ACTION: IMMUNE REGULATION





Increase of immunoglobulin levels



-  Significant increase of IgA levels in children with acute diarrhea⁽¹⁾
- Significant increase of IgA levels in mice administrated with *C. difficile* toxin A⁽²⁾
-  Increase of IgM levels⁽³⁾




Increase of red and white blood cells



-  Increase of leucocytes levels^(2,4) (white blood cells) including macrophages, lymphocytes, neutrophils
-  Increase of erythrocytes levels⁽⁴⁾ (red blood cells – not shown)

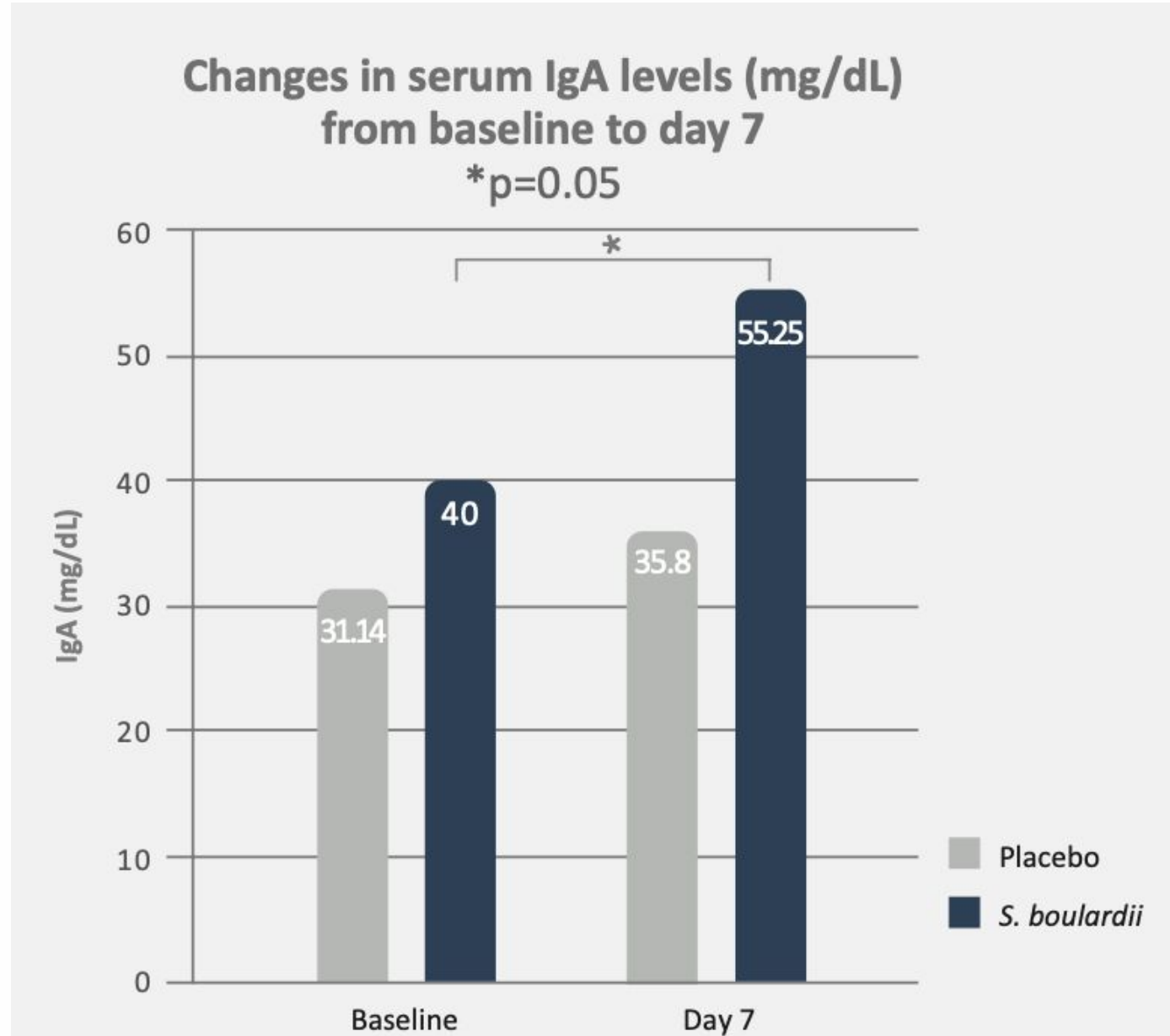
In vitro and *in vivo* demonstrated involvement in immune response



-  Enhancement of anti-inflammatory cytokines production (IL-4 or IL-10)⁽⁵⁾
-  Reduction of proinflammatory cytokines production (IL-8, IL-6, TNF-α)⁽⁵⁾
-  Inhibition of host's signaling pathways (NF-κB or MAPK)⁽⁵⁾

S. BOULARDII & SIGA LEVELS IN CHILDREN

- Immune response enhancement in children with acute gastroenteritis
- 27 children aged from 6 months to 10 years with acute diarrhea
- Twice daily administration of *S. boulardii* 250mg or placebo for 7 days. Results after 7 days compared to baseline with *S. boulardii*:
- Significant increase of IgA levels.
- Significant decrease of C-reactive protein levels.
- Significant increase of CD8 lymphocytes levels.
- Significant higher increase in probiotic group than placebo on day seven.



THE POWER OF BACILLUS SPORES



No refrigeration required



99.9% digestion survival rate



Can open capsule and mix in food and water



Maintains efficacy during antibiotic therapy



Produces 12 natural antibiotics

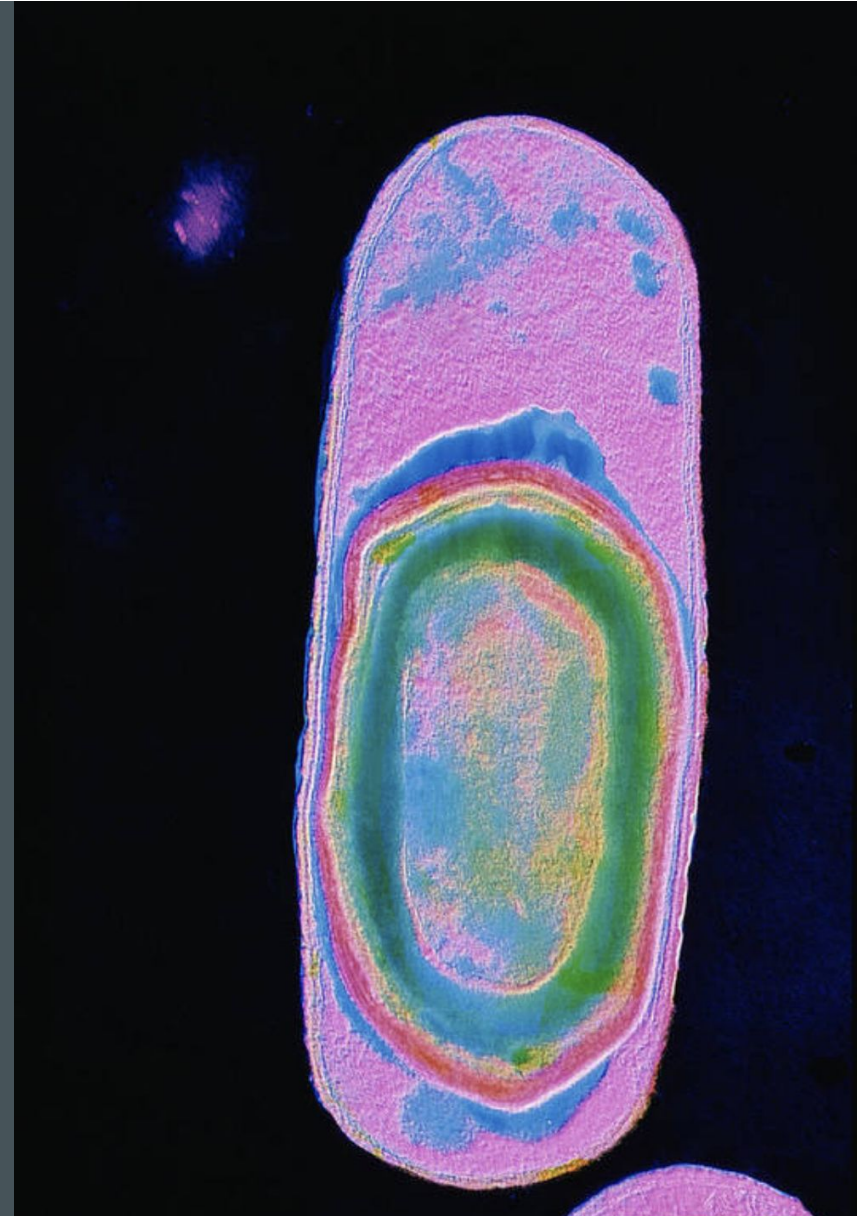


Produces vitamin K27, B vitamins, carotenoids, & digestive enzymes



BACILLUS SUBTILIS HU58

- Naturally produces 12+ antibiotics
- Supports immune function
- Supports K2 levels
- Shown to lower inflammatory IL6 and TNF-a
- Strong production of SCFAs



BACILLUS SUBTILIS HU58 & AAD

- 60 randomized patients took HU58 or a placebo for 7 days
- HU58 group demonstrated:
 - More well formed stools
 - Less stool frequency
 - Reduced TNF-a by 55%
 - Reduced IL-6 45%

Bristol Stool Chart








Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. Entirely Liquid

Figure 1: Bristol stool chart ^[11]

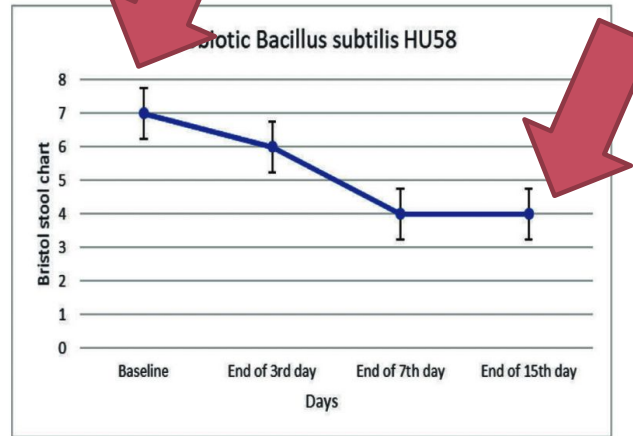


Figure 2: Stool consistency in *Bacillus subtilis* HU58 group

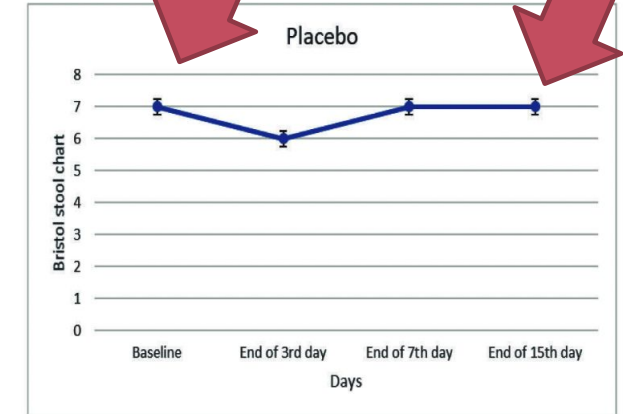
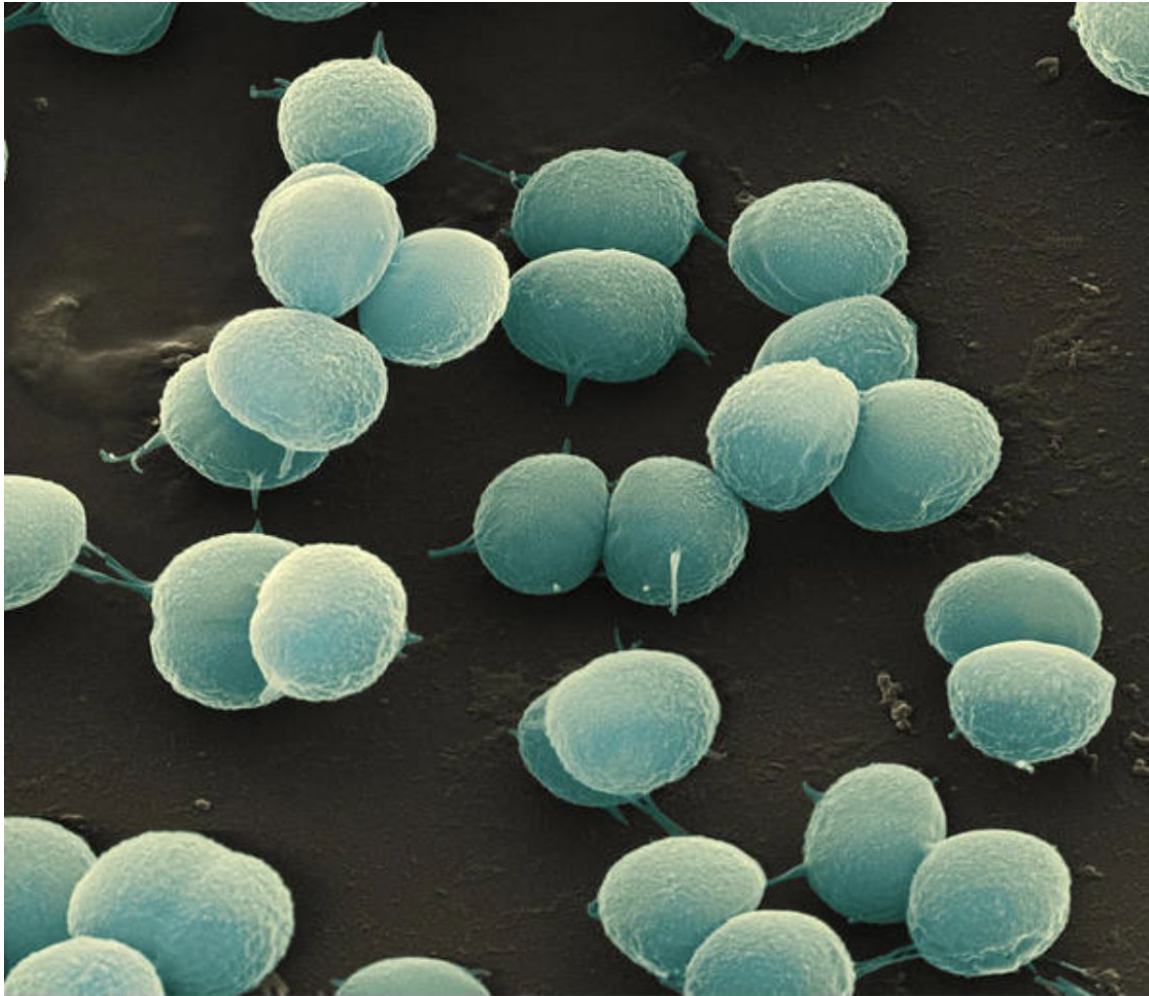


Figure 3: Stool consistency in placebo group

HU58 SIGNIFICANTLY IMPROVES STOOL CONSISTENCY FOLLOWING ANTIBIOTIC ASSOCIATED DIARRHEA



BACILLUS COAGULANS SC-208

- Produces lactic acid
- Produce enzymes that aid digestion and nutrient absorption
- Improve microbial diversity
- Increase absorption of BCAAs
- Improve intestinal peristalsis
- Reduce inflammatory metabolites

BACILLUS COAGULANS (SC-208) HU58 IN POST-ANTIBIOTIC ADMINISTRATION




microorganisms



Article

***Bacillus subtilis* HU58 and *Bacillus coagulans* SC208 Probiotics Reduced the Effects of Antibiotic-Induced Gut Microbiome Dysbiosis in an M-SHIME[®] Model**

Massimo Marzorati ^{1,2}, Pieter Van den Abbeele ², Sarah S. Bubeck ^{3,*} , Thomas Bayne ⁴, Kiran Krishnan ⁴, Aicacia Young ⁴, Dilip Mehta ⁵ and Anselm DeSouza ⁵

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⁵ Synergia Life Sciences, PVT Ltd., Universal Majestic, 1503, PL Lokhande Marg, Chembur, Mumbai, Maharashtra 400071, India; DilipMehta39@hotmail.com (D.M.); Kay2seven@gmail.com (A.D.)

* Correspondence: bubeckscientific@gmail.com

- Effective during antibiotic therapy
- Improved epithelial barrier function
- Enhances immune modulation
- Lowers inflammatory cytokines (TNF- α)
- Increased anti-inflammatory markers

BACILLUS
CLAUSII SC-109



Clinically shown to support immune function



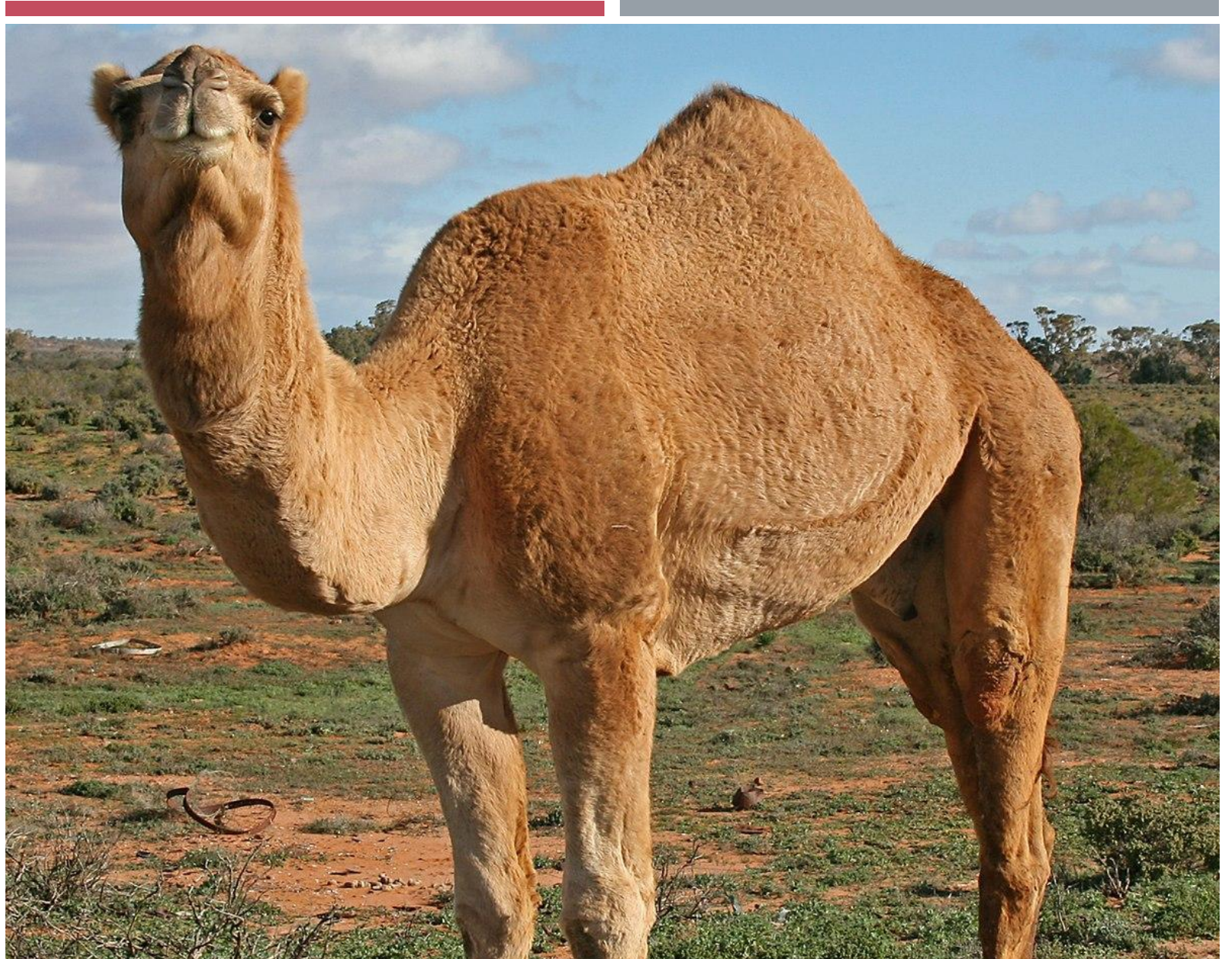
Studies demonstrate reduced length of upper respiratory infections



Improves cytokine balance

HISTORY OF SPORES

- Late 1940's – bacillus subtilis isolated and used as a prescription spore probiotic in Germany. Primarily used to treat dysentery, chronic upper respiratory infections and other immune dysfunctions in kids and adults.



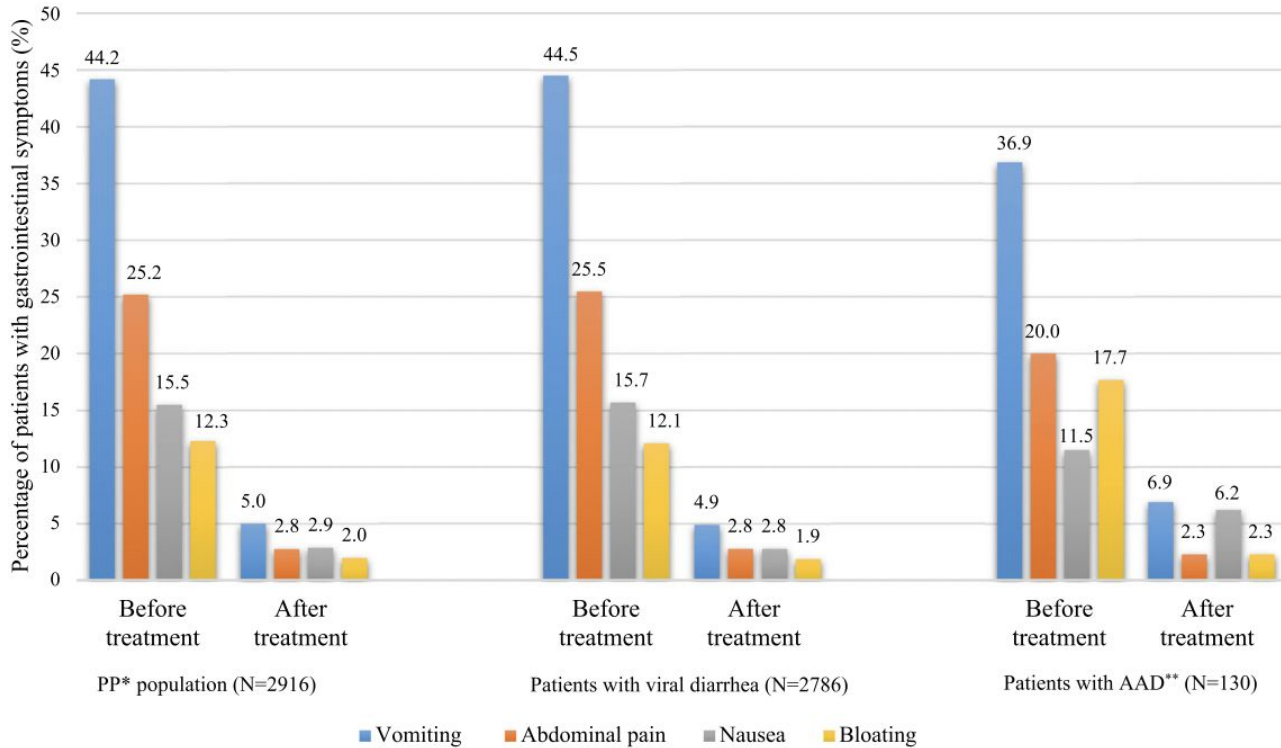
BACILLUS CLAUSII – POLY-ANTIBIOTIC RESISTANT

Able to colonize the intestine even in the presence of antibiotics including:

- Chloramphenicol
- Novobiocin
- Rifampin
- Neomycin
- Streptomycin
- Tetracycline

De Castro JA, Kesavelu D, Lahiri KR, Chaijitraruch N, Chongsrisawat V, Jog PP, Liaw YH, Nguyen GK, Nguyen TVH, Pai UA, Phan HND, Quak SH, Tanpowpong P, Guno MJ. Recommendations for the adjuvant use of the poly-antibiotic-resistant probiotic *Bacillus clausii* (O/C, SIN, N/R, T) in acute, chronic, and antibiotic-associated diarrhea in children: consensus from Asian experts. Trop Dis Travel Med Vaccines. 2020 Oct 23;6:21. doi: 10.1186/s40794-020-00120-4. PMID: 33110611. PMCID: PMC7583175

BACILLUS CLAUSII IN PEDIATRIC AAD



- 3178 children (median age of 2)
- Treated for 5 -7 days
- 52.6% had resolved diarrhea in 3 days
- Significant reduction in number of stools per day
- Reduced loose stools from 81.6% to 9.2%



■ Lifestyle: ADDITIONAL TOOLS TO SUPPORT THE MICROBIOME DURING ANTIBIOTIC THERAPY

■ Get outdoors as much as possible

- Sleep hygiene
- Stress reduction
- Avoid exposure to harmful chemicals
- Cook foods to appropriate temperature

■ Diet:

- Fiber-rich foods
- Fruits and vegetables
- Omega-3 rich foods
- Organic when possible

RESTORFLORA™ SUMMARY

- Is a unique combination of spore-based probiotics and commensal probiotic yeast that provides multiple protective benefits during antibiotic therapy.
- The synergistic effects of each ingredient helps provide luminal, trophic, and immunomodulatory effects that are important in the restoration of gut health following antibiotic therapy.
- The ingredients in RestorFlora been shown to reduce stool frequency and improve stool consistency in as little as 3 days.
- RestorFlora contains well-researched ingredients that get to the root of dysbiosis and diarrhea following a course of antibiotics.



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