ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI SCIENZE MEDICHE VETERINARIE

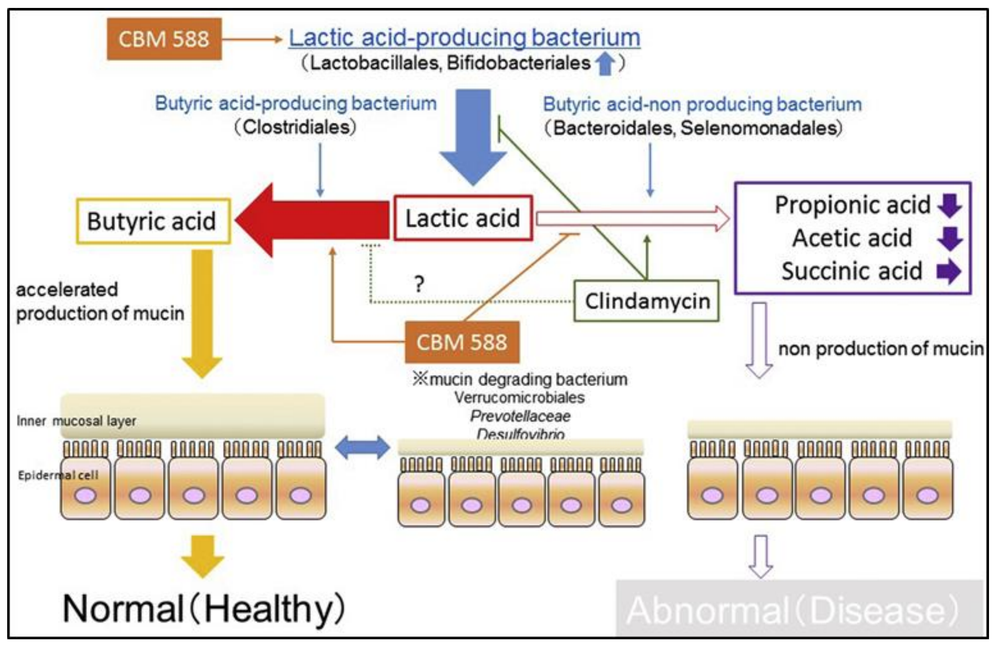
Dottorato di Ricerca in Scienze Veterinarie
Candidato: Diana G. Scorpio
Tutor: Maria Laura Bacci
Curriculum: Scienze di Base
Ciclo: XXXVI°

National Institute of Allergy and Infectious Diseases

Evaluating the Efficacy of a *Clostridium butyricum* Probiotic Strain in Treatment of Colitis and Diarrhea in Rhesus Macaques

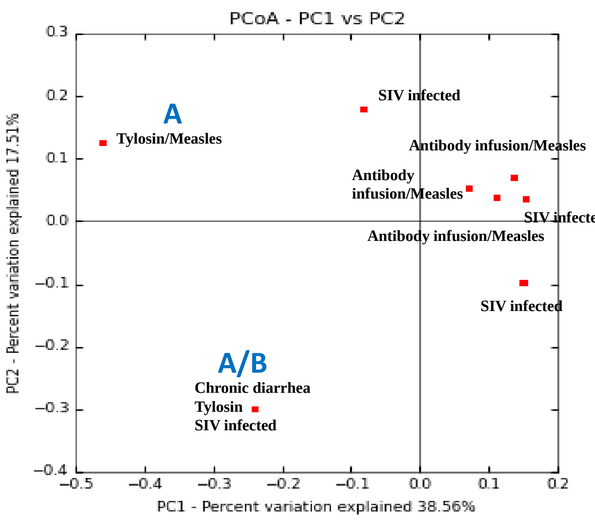
Introduction

- *Clostridium butyricum (*CBM 588), a gram-positive spore-forming bacterium, was first isolated from soil in Nagano, Japan in the 1960's. Unlike other *Clostridium* species, CBM 588 does not produce botulinum neurotoxins. CBM 588 has beneficial effects due to production of butyric acid essential to gut mucosal health.
- Non-human primates (NHP) are valuable models when studying inflammatory bowel disease in humans. Chronic diarrhea, alone or as a secondary condition in macaques, is very common and can contribute to severe weight loss and dehydration causing significant burden of disease in many colonies of primates.
- **Hypothesis**: CBM588 will minimize disruption of healthy gut microbiota, decrease duration of symptoms associated with diarrhea, and restore the balance of the beneficial bacteria within the gut microflora.
- **Aims**: To evaluate microbial disruption and translocation in NHP with chronic diarrhea following treatment and to provide an animal model of disease and therapy for human and veterinary applications. This evaluation will be accomplished using fecal microbiome sequencing, blood markers of inflammation, and measurements of GI mucosal compromise in feces and biopsy tissues.



Preliminary Data

Sample	Sex	Weight	Source	Notes
DS1.F3	F	2.36	New Iberia	**HIV trimer study; tylosin given Dec 2015; flagyl given Jan 2016 (Amoeba present);** measles positive titers
DS2.F4	F	4.38	New Iberia	Antibody infusion, measles positive titers
DS3.F5	F	3.68	New Iberia	Antibody infusion, measles positive titers
DS3.H3	M	8.4	Tulane	SIV infected Oct 2015
DS4.F6	F	3.48	New Iberia	Antibody infusion, measles positive titers
DS4.H4	M	8.04	PrimGen	SIV infected Oct 2015
DS5,F7	M	7.24	PrimGen	**Chronic diarrhea, multiple treatments with Tylosin through 2014; SIV infected Oct 2015**
DS6.F8	F	7.32	PrimGen	SIV infected Oct 2015
DS7.F9	M	9.06	PrimGen	SIV infected Oct 2015



Evidence for microbiome clustering (Principal Component Analysis- PCA):
Beta-diversity separation of microbiome as a function of treatment and underlying condition. Effect on bacterial diversity is evident when animals are treated with antibiotics like Tylosin (A) and separation of diversity goes even further when animals are SIV infected and have chronic diarrhea (B). This result gave further evidence that effect of existing diarrhea and antibiotics disrupts the GI microbiome. These results initiated the current investigation to see if CBM588 could restore normal microbiome equilibrium in NHP.

Materials and Methods

Enrollment Requirements:

- Rhesus macaques experiencing more than 1 week of chronic diarrhea not associated with any specific enteric pathogen (*Shigella, Salmonella, Yersinia, Campylobacter*, or intestinal parasites)
- Significant weight loss (5-10% decrease in body weight)
- Non-infectious disease or secondary to simian immunodeficiency virus infection (SIV)

Materials used:

- CBM 588: Miyarisan Pharmaceutical Co.
- Monkey Intestinal Fatty Acid Binding Protein ELISA kit
- Microbiome Sequencing 16S rRNA Illumina (IDEXX Laboratories)
- Colon biopsy and IHC (LPS core antibody)
- qPCR for CBM588 detection (data not shown)

Experimental design:

- 2 SIV infected animals with chronic diarrhea
- 4 animals with chronic diarrhea of unknown etiology
- Treated orally with either low ($10x10^8$) or high ($10x10^{10}$) CFU dose CBM588 twice daily
- Treatment was given for 24-28 weeks with biosampling done at select time points
- Clinical evaluation was done cage side daily and included fecal consistency scoring
- Animal studies were approved by the VRC Animal Care and Use Committee (NIH)

Results

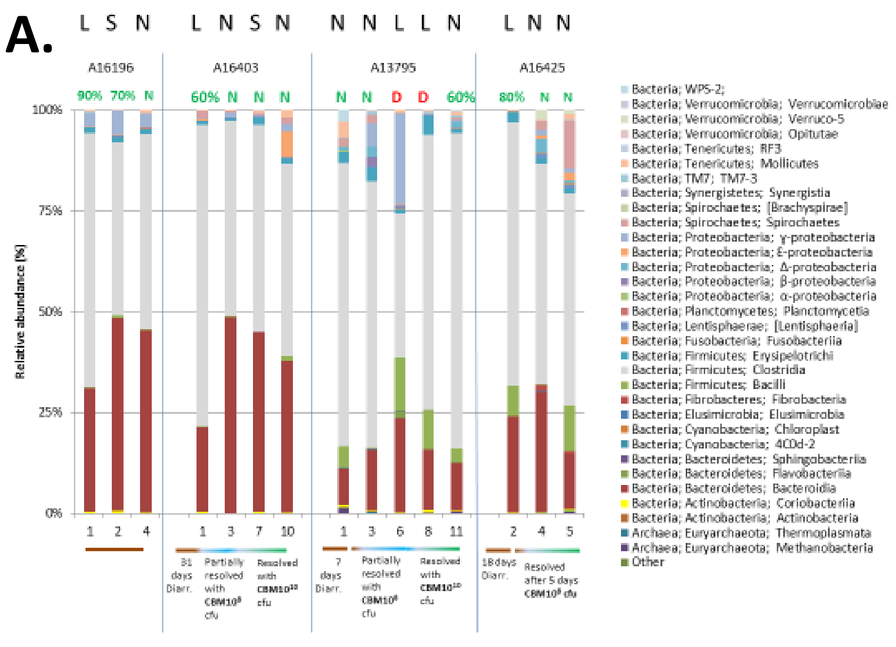


Figure A: Microbiome Sequencing Analysis (partial data, bioinformatic analysis still pending) Microbiome sequencing analysis of NHP fecal samples demonstrates improved species diversity over time with CBM 588 treatment. Diversity is plotted along with fecal consistency. Key: L, loose; S, soft; D, diarrhea; N, normal. Over time, clinical improvement was evident in most subjects and correlates with greater diversity of beneficial bacteria in the fecal samples.

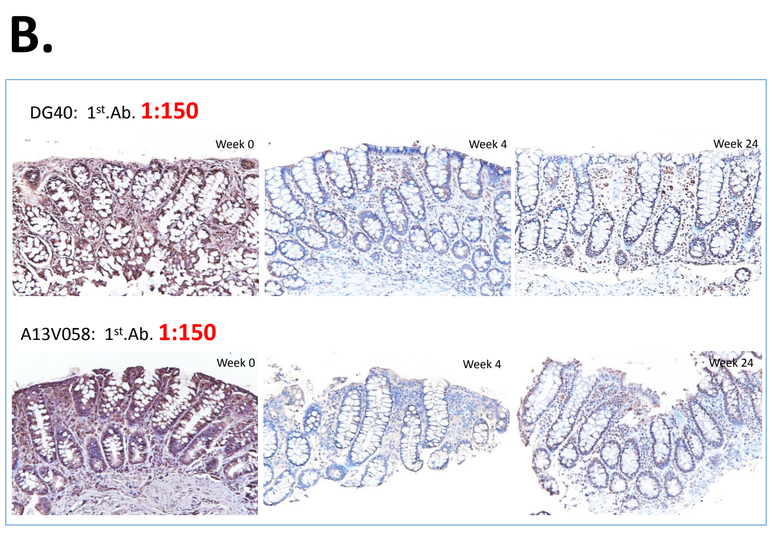


Figure B. LPS Core Protein via immunohistochemistry (IHC) detection. Using IHC and LPS Core Protein detection via antibody, deposition of LPS gram-negative bacterial protein is significantly abundant in damaged and diseased colonic mucosal biopsy tissue. Over weeks of CBM588 administration, this protein deposition begins to diminish. Greatest effect is notable after 4 weeks but this effect is still maintained and exhibits dramatic improvement up through week 24 of the study. The 2 animals represented above have chronic diarrhea due to long standing SIV infection. Antibody dilution 1:150.

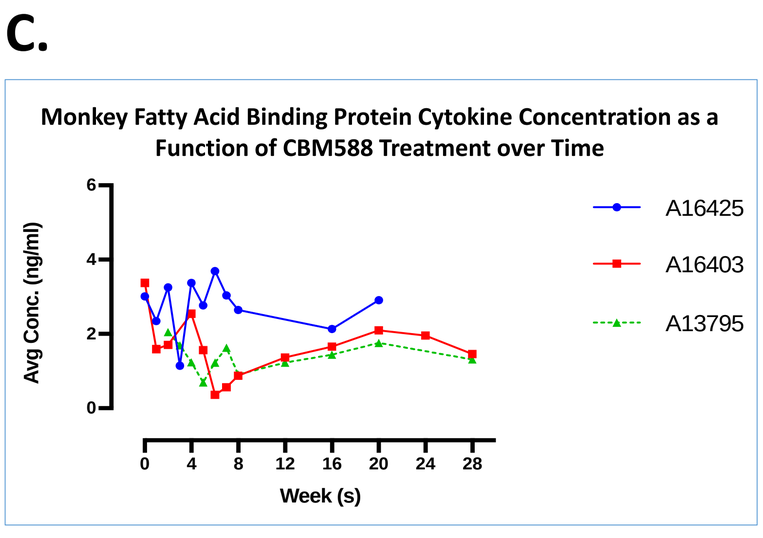


Figure C. Intestinal Fatty Acid Binding Protein (IFABP) Cytokine Concentrations Over Time. Intestinal fatty acid-binding protein (I-FABP) is specifically and abundantly present in epithelial cells of the mucosal layer of small intestinal tissue. I-FABP is also considered to be rapidly released into the blood circulation just after small intestinal mucosal tissue is injured. In the 3 subjects above, IFABP fluctuations in blood are quite dramatic in the first 0-8 weeks, followed by a diminished and more consistent trend through to end of study. This implies improvement in mucosal integrity and reduced gut leakage.

Conclusions

- Chronic diarrhea does result in changes to the fecal microbiome due to gut mucosa microbial disturbance and bacterial translocation.
- Infection with SIV in NHP does impact microbiome species diversity compared to other systemic conditions.
- CBM 588 has shown to be efficacious in NHP with chronic diarrhea of different etiologies. CBM 588 is safe and resulted in improved fecal consistency and gut health over time.
- **Future Directions**: We will continue to study the use of CBM 588 to treat chronic diarrhea in SIV-infected animals to minimize microbial translocation and systemic disease. Further studies including bioinformatic analysis and histopathology evaluation are currently underway. This treatment for gut dysbiosis will be invaluable for both the human and veterinary field.