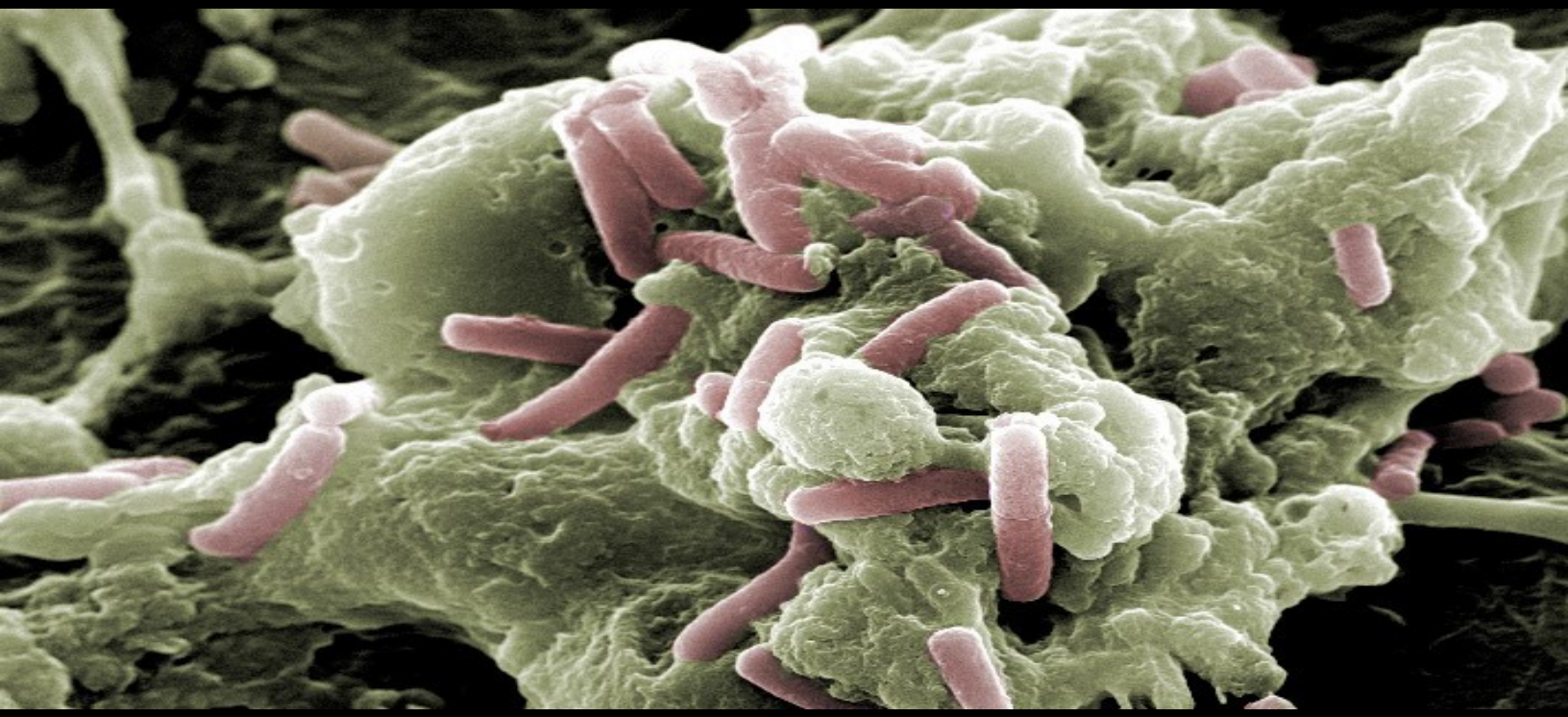
GCC 2nd Annual Antimicrobial Resistance & Gut Health Symposium



June 15, 2018

BioScience Research Collaborative
6500 Main St.
Houston, Texas

Gulf Coast Consortia
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Notes

2nd Annual Antimicrobial Resistance & Gut Health

Agenda

June 15, 2018

8:30	Breakfast and poster set up
8:55	Welcome and Introductory Remarks
9:00	Plenary: *Harnessing the Power of the Microbiome to Revolutionize Treatment of Disease: Phase II & III trials for recurrent C. difficile* **Ken Blount, Rebiotix**
9:30	*Developing a Novel Microbial Therapeutic for Clostridium difficile infection* **Jennifer Achtung, Baylor College of Medicine**
9:50	*Building Infectious Disease Classifiers using a Systems Biology Approach* **Qinglong Wu, Texas Children's Hospital**
10:10	*Understanding Clostridium difficile Epidemiology Through the Use of Whole Genome Sequencing; A Houston Perspective* **Bradley Endres, PhD, University of Houston**
10:30	Break
10:40	Plenary: *Endogenously Synthesized Antibiotics and Secondary Bile Acids Regulate the Structure of the Gut Microbiome: Implications for Clostridium difficile Infection* **Phillip Hylemon, Virginia Commonwealth**
11:10	*Understanding the Mechanism of Action of New Antibiotics against Clostridium difficile* **Eugénie Bassères, University of Houston**
11:30	*Using CRISPR-Cas9-mediated Genome Editing to Generate C. difficile Mutants Defective in Selenoproteins Synthesis* **Kathleen McAllister, Texas A&M**
11:50	Meet-the-Professor, **Phillip Hylemon** Lunch and poster session (Event Space)

1:10	*Precision Metabolomics™ – A Key Technology for Microbiome Research & Unlocking Microbiota Function in Human Health* **Robert Mohney, Metabolon**
1:20	Plenary: *Comparative Systems Biology Analysis of Microbial Pathogens* **Jonathan Monk, University of California San Diego**
1:50	*Identification of clinically significant Antimicrobial Resistance Genes in the Commensal Microbiota* **Samuel Shelburne, MD Anderson**
2:10	*Dietary Trehalose Enhances Virulence of Epidemic Clostridium difficile* **James Collins, Baylor College of Medicine**
2:30	Selected abstracts: Induction of *C. difficile* Sporulation by Next Generation Probiotics **James McLellan, Texas State University** Genome Scale Metabolic Modeling of *Clostridium difficile* **CJ Norsigian, UC San Diego**
2:50	Break
3:00-5:00	Workshop: *Building and Using Genome-scale Models of Metabolism to study Microbial Pathogens*

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Ken Blount, PhD
Chief Scientific Office, Rebiotix

Harnessing the Power of the Microbiome to Revolutionize Treatment of Disease: Phase II & III trials for recurrent C. difficile

About Dr. Blount:

Ken Blount, PhD, is the Chief Scientific Officer at Rebiotix, a clinical-stage company developing transformational Microbiome Restoration Therapeutics (MRT). Ken has directed innovative R&D teams at multiple companies and academic institutions, including as the co-founder of BioRelix, a venture-backed company that developed new antibiotics for novel mRNA target classes. His scientific expertise includes microbiome and antibiotic drug development, translational medical oncology, and RNA biology. At Rebiotix, Ken leads the evolution of new analytic tools to demonstrate microbiome restoration for Rebiotix' novel MRT portfolio, and he is heading the expansion of Rebiotix MRT platform to new therapeutic areas.

Abstract:

RBX2660 is a standardized, stabilized microbiota-based drug being developed by Rebiotix to prevent recurrent CDI. Here we will present the results from two Phase 2 controlled trials to evaluate the safety and efficacy of RBX2660 among patients with documented multi-recurrent CDI.

In a double-blinded Phase 2 clinical trial (PUNCH CD2), patients were randomized to receive either 2 doses of RBX2660, 1 dose of RBX2660 + 1 dose of placebo, or 2 doses of placebo. Patients in this study who failed blinded treatment could also receive up to two additional RBX2660 doses. The efficacy among patients who received >1 blinded RBX2660 treatment was 66.7% (n=83) compared to 45.5% for placebo-treated patients (n=42; p<.047). The efficacy among patients who also received >1 RBX2660 in the open-label phase was 77.8% (n=54).

In a subsequent open-label study (PUNCH SOS), patients received up to 2 doses of RBX2660. Outcomes from active treatments were compared to patients who only received standard-of-care antibiotic treatment in a matched control arm. Efficacy was 79.4% (n=136) for the RBX2660 treatment group compared to 51.8% in the control group (n=110; p<.001).

Sequencing analyses of fecal samples from participants in both trials demonstrated significant restoration of a healthier microbiome correlated with response to RBX2660. Collectively, these controlled Phase 2 clinical trials demonstrate safety of RBX2660 in rCDI patients and the effectiveness of RBX2660 in preventing rCDI. Rebiotix is currently enrolling a Phase 3 trial of RBX2660 for prevention of rCDI in participants with at least one recurrence which will also include assessment of microbiome changes after treatment.

Jennifer Achtung, PhD
Assistant Professor, Baylor College of Medicine
Developing a Novel Microbial Therapeutic for Clostridium difficile infection

About Dr. Achtung:
Dr. Achtung has been an Assistant Professor in the Alkek Center for Metagenomics and Microbiome Research and the Department of Molecular Virology and Microbiology at Baylor College of Medicine since August 2014, where her research interests have focused on interactions between gastrointestinal pathogens, the commensal microbiome, and the host. Prior to her time at Baylor, she completed post-doctoral training at Michigan State University with James Tiedje, studying the molecular ecology of environmental populations of Shewanella, and with Robert Britton, developing new tools to study interactions between gastrointestinal pathogens and human GI microbes. Jennifer completed her Ph.D. with Alan Grossman at the Massachusetts Institute of Technology studying the role that intercellular signaling plays in regulating horizontal gene transfer in Bacillus subtilis. In July, Jennifer will be starting her lab in the Department of Food Science and Technology at the University of Nebraska-Lincoln, where she will be working with colleagues in the Gut Health Initiative and Foods for Health Center.

Abstract:
The gastrointestinal pathogen Clostridium difficile is the most common cause of antibiotic-associated diarrhea and the leading cause of hospital-acquired infections. Because of the significant public health burden imposed by C. difficile infection, several different approaches for disease treatment are being pursued. Fecal microbiota transplantation (FMT) is one of the most effective treatments for recurrent CDI and administration of the subset(s) of bacteria responsible for restoring resistance to CDI is an attractive therapeutic target. Despite preliminary advances, no simplified consortia has progressed to a commercially-available therapeutic. After screening >100 simplified communities, we identified three communities that provided protection from C. difficile in experimental models. In addition to potential for these communities to be developed as therapeutics, these communities also provide unique tools to probe mechanisms through which the microbiome influences susceptibility to C. difficile infection and to test the extent to which interpersonal variation can impact the efficacy of microbiome-based therapy.

Qinglong Wu, PhD
Postdoctoral Associate, Texas Children's Hospital
Building Infectious Disease Classifiers using a Systems Biology Approach

About Dr. Wu:
Qinglong Wu received his B.Eng. (2012) and Ph.D. (2016) both in the field of food and nutritional sciences with a special emphasis on food microbiology. He has studied GABA-producing lactic acid bacteria during his graduate study at The University of Hong Kong, and later moved to Savidge Laboratory as postdoctoral associate in Texas Children's Microbiome Center for studying GABA-producing gut commensals that exacerbate *Clostridium difficile* infection. His current major focus are characterization of disease-associated gut commensals and analysis pipeline development for gut microbiota data.

Abstract:
Toxigenic Clostridium difficile infection (CDI) is listed by CDC as one of three urgent threats to public health. CDI is commonly misdiagnosed, with approximately 30% of cases reported as a functional GI disease misdiagnosis. Thus, clinical CDI diagnosis remains challenging for a number of reasons, including asymptomatic carriage of the pathogen. In our work, we developed supervised learning classifiers for the diagnosis of CDI based on our large collection of fecal microbiota and metabolome data of adult and pediatric patients with CDI or functional GI disorders (FGID), as well as control subjects without GI disease. Classification based on adult fecal microbiome data (16S) provided at least 90% accuracy for predicting CDI. Moreover, precision classification based on microbiota and metabolome data demonstrated over 95% accuracy for differentiating CDI from FGID and controls. In summary, supervised learning classification using a system biology approach provides precision diagnosis of CDI versus non-infectious enteric disease at a population scale level.

Bradley Endres, PhD
Postdoctoral Fellow, University of Houston

Understanding Clostridium difficile Epidemiology Through the Use of Whole Genome Sequencing; A Houston Perspective

About Dr. Endres:
Bradley Endres is originally from Menomonee Falls, Wisconsin. He completed his bachelors of science degree at the University of Wisconsin – Milwaukee majoring in Biochemistry and completed his PhD in Physiology at the Medical College of Wisconsin in 2015. Brad has been a postdoctoral fellow in Kevin Garey's lab at the University of Houston since 2015. He has mainly focused on developing two different research projects: 1) using next-generation sequencing technology to understand the epidemiological spread of infectious diseases and 2) using high-resolution microscopy to study antibiotics' mechanisms of action.

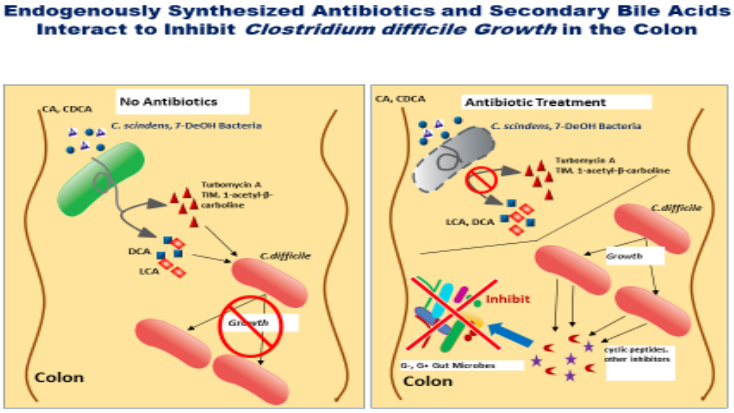
Abstract:
Clostridium difficile, a CDC threat level urgent pathogen is associated with significant antimicrobial resistance, which has lead to worldwide outbreaks. Specifically, the recent pandemic caused by ribotype 027 was the result of dissemination of two separate lineages resistant to fluoroquinolones called the fluoroquinolone resistant 1 (FQR1) and FQR2 lineages. Both lineages originated in North America, however, specific mapping within the US and Canada has yet to be done. We sought to determine whether these pandemic strains and specific lineages were also present in Houston, Texas. Given the international nature of Houston and denseness of hospital centers, we hypothesized that both lineages were present. To test the hypothesis, we analyzed whole-genome sequencing data from 76 ribotype 027 clinical isolates obtained from symptomatic, hospitalized patients with C. difficile infection in Texas and 33 previously sequenced worldwide strains. DNA was purified and sequenced on an Illumina NextSeq. R20291 (ribotype 027) was used as the reference genome for alignment and mapping and established FQR1/FQR2 strains were used to help identify the lineagesWhole-genome SNP analysis was performed using 2,841 SNPs, of which 900 non-synonymous mutations, 1,404 synonymous substitutions, and 537 intergenic changes were identified. Phylogenetic analysis separated the strains into two prominent groups, which grossly differed by 28 SNPs; the FQR1 lineage (n= 35 Texas-specific) and the FQR2 lineage (n=36 Texas-specific). Five samples were defined as pre-epidemic strains. Of note, phylogeny demonstrated unique clustering amongst the Houston strains indicating geographic source has defined the local 027 genetics. Other antimicrobial resistance genes were also present amongst the strains. In conclusion, we found that both FQR1 and FQR2 ribotype 027 epidemic lineages are present in the USA, have evolved over time, and represent a continued urgent public health threat.

Phillip Hylemon, PhD
Professor, Virginia Commonwealth University

Endogenously Synthesized Antibiotics and Secondary Bile Acids Regulate the Structure of the Gut Microbiome: Implications for Clostridium difficile Infection

About Dr. Hylemon:

Over the past several decades, Dr. Hylemon's laboratory has been interested in three different areas related to bile acid biology. 1) Enzymology and genetics of bile acid metabolism by gut bacteria and the regulation of the gut microbiome in health and disease. Discovery of tryptophan-derived antibiotics synthesized by different gut bacteria and their role in *Clostridium difficile* infection and the structure of the gut microbiome. 2) Bile acid activated cell signaling pathways in the liver and their role in regulating metabolism and cell growth. 3) In collaboration with his clinical colleagues, the role of bile acids in regulating the structure of the gut microbiome in patients with cirrhosis.



His laboratory has studied the physiology, metabolism, and genetics of human gut bacteria since the mid-1970's. In the late 1980's, my laboratory discovered a new 8 step biochemical pathway (bile acid 7α/7β-dehydroxylation) found in specific species of the genus *Clostridium* which is part of the human gut microbiota. This pathway produces the secondary bile acids (deoxycholic acid and lithocholic acid) from the primary bile acids (cholic acid and chenodeoxycholic acid), respectively. We subsequently cloned and characterized a large >11kb bile acid inducible (bai) operon in *Clostridium scindens* encoding 8 genes encoding enzymes/transporter in this unique biochemical pathway. They then individually cloned, expressed, and characterized each gene product (bile acid transporter and various enzymes in this pathway). They also discovered that specific bile acid 7α/7β-dehydroxylating human gut bacteria can convert glucocorticords into androgens. Recently, in collaboration with a group at Scripps Institute, they obtained the 3D structure and

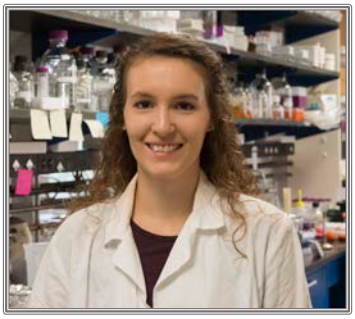
proposed a catalytic mechanism of bile acid 7α-dehydratase, the rate limiting enzyme in this pathway. In the mid-1990's, in collaboration with Dr. Paumgartner's laboratory in Munich, Germany, they showed that increased levels of intestinal bile acid 7α-dehydroxylating bacteria increased the cholesterol saturation index (CSI) of bile in gallstone patients. Treatment of these patients with an antibiotic markedly decreased the CSI, below 1.0 of these patients**.** More recently, in collaboration with J.S. Bajaj, M.D**.**, they reported evidence that bile acids help determine the structure of the human gut microbiome, and that secondary bile acids increases colonic inflammation in alcoholic cirrhosis patients Moreover, they showed that transfer of fecal samples from alcoholic cirrhotic patients to Germ-free mice mimics many of the pathophysiological features found in these patients. Finally, Dr. Hylemon's laboratory has discovered that C. *difficile* secretes cyclic dipeptides that inhibits other gut bacteria possibly allowing colonization of the colon. Moreover, we have discovered that *C. scindens* and *C. sordellii* secretes tryptophan derived antibiotics that inhibit *C. difficile* and their activities are enhanced by eoxycholic acid, synthesized from cholic acid by this bacterium.

Eugénie Bassères, PhD
Postdoctoral Fellow, University of Houston

Understanding the Mechanism of Action of New Antibiotics against Clostridium difficile

About Dr. Bassères :
Eugénie Bassères is a postdoctoral fellow at the University of Houston College of Pharmacy. Her main interests include Clostridium difficile pathogenesis at translational levels: from epidemiology to experimental design and treatment by understanding mechanisms of action of new antimicrobials. Dr. Bassères received a Master in Transmissible Diseases and Tropical Pathologies from the Aix-Marseille University and a PhD in Medical Sciences from Karolinska Institute in Stockholm.

Abstract:
An interesting new compound in the pipeline, ridinilazole, is a novel, non-absorbable narrow-spectrum antibiotic that is currently in clinical development for the treatment of C. difficile infection. Ridinilazole has been shown to have potent activity against multiple strains of C. difficile and has demonstrated efficacy in both in vitro gut and in vivo hamster models. Although it has been shown to be highly potent against C. difficile, the mechanism of action of ridinilazole has not been fully elucidated. Here are presented the approaches used in our laboratory to identify targets and/or pathways involved in ridinilazole response such as genomics, pharmacology, molecular biology and microscopy.



Kathleen McAllister
PhD Candidate, Texas A&M University

Using CRISPR-Cas9-mediated Genome Editing to Generate C. difficile Mutants Defective in Selenoproteins Synthesis

About Ms. McAllister:
Kathleen McAllister is a PhD Candidate in the Department of Biology at Texas A&M University. I grew up in Ocean Springs, Mississippi. She attended the University of South Alabama where she first started her scientific career by participating in undergraduate research investigating the starvation stress response in Salmonella enterica serovar typhimurium. After earning her Bachelors of Science in Biomedical Sciences, she moved to College Station, Texas to start her graduate studies at Texas A&M University. In 2013, she joined Dr. Joseph Sorg's laboratory which focuses on C. difficile spore germination. She currently work on understanding the physiology and metabolic processes in C. difficile as well as developing genetic tools for this organism.

Abstract:
Clostridioides difficile is a significant concern as an opportunistic pathogen and a major cause of antibiotic-associated diarrhea. Much of our understanding of C. difficile physiology has come in the last few years and coincided with the development of genetic tools for this organism. Though the current genetic tools for C. difficile have been beneficial, they each have negative aspects (i.e., time-consuming, multi-step processes, inefficient and polar/off-target effects). The clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) system has gained attention as a genetic tool in several organisms, and this system was recently shown to work efficiently in other Clostridia. To apply such a system to C. difficile for genetic modification, we created a CRISPR-Cas9 plasmid which contains all of the components necessary for the system to function. We targeted and made a clean deletion of pyrE, which encodes orotate phosphoribosyltransferase and can be used easily as a counter-selection, in C. difficile R20291. Using this system we achieved an efficiency of ~ 50% with no off-target effects. To investigate the role of selenoproteins in C. difficile Stickland metabolism, a primary source of energy in a small number of anaerobic bacteria, we applied our genetic tool to make a clean deletion of selD, a gene which encodes a selenophosphate synthetase, the first step of selenium incorporation into proteins. We found that selD has an important role in the growth of C. difficile where a selD mutant has a growth defect in protein-rich medium. This newly-developed C. difficile genetic system builds upon and improves upon the available

genetic systems. Because this system does not rely upon segregationally unstable plasmids or pre-existing/generated pyrE mutations, this system should increase the rate with which mutations can be made in C. difficile.

Jonathan Monk, PhD
Researcher, University of California San Diego

Comparative Systems Biology Analysis of Microbial Pathogens

About Dr. Monk:

Dr. Monk is a Researcher in the Systems Biology Research Group working on building genome-scale models of metabolism for microbial pathogens. His interests including using genome-scale models to compare metabolic network reconstructions of related microbes to interpret and classify their evolutionary differences as well as adaptations to specific niches. He applies genome-wide modeling to study microbial virulence and resistance factors. Furthermore, he is particularly interested in applying high-throughput techniques to evaluate, build and improve metabolic network reconstructions.

Abstract:

Genome-scale models of microbial metabolism have become a commonly used tool in the systems biology toolbox. These models can predict, based solely on an organism's genome sequence, its metabolic capabilities and unique phenotypes in different conditions and under unique perturbations. Furthermore, an array of in-silico methods have been developed that can be applied to these models to more deeply characterize an organism, re-engineer it and even to design effective ways to interrupt and kill it. This talk will focus on the creation and analysis of multiple genome-scale models of metabolism for different microbial pathogens. I will start with an overview of genome-scale modelling and techniques including flux balance analysis that can be used to predict an organism's metabolic capabilities. I will cover the process of building a comprehensive genome-scale model for Escherichia coli K-12 MG1655 as well as how these models are validated experimentally using gene-knockout studies and other techniques. Finally, I will describe applying these methods to build and analyze multiple genome-scale metabolic reconstructions of diverse Escherichia coli strains. Such analyses can be used to systematically elucidate strain-specific adaptations to nutritional environments. I will demonstrate that these models can be used to identify strain-specific pathogenic characteristics and unique metabolic capabilities that are related to infectious capabilities. Finally I will provide an overview of similar studies that are ongoing to study other microbial pathogens of interest including Shigella, Salmonella, S. aureus and C. difficile.

Samuel Shelburne, MD, PhD
Associate Professor, MD Anderson

Identification of clinically significant Antimicrobial Resistance Genes in the Commensal Microbiota

About Dr. Shelburne:

Sam Shelburne, MD, PhD is currently an Associate Professor in the Departments of Infectious Diseases and Genomic Medicine at MD Anderson Cancer Center in Houston, TX.

Training: Dr. Shelburne did his undergraduate work at Princeton University, his medical school training at the University of Texas Medical Branch, and his residency, chief medical residency, and infectious diseases training at Baylor College of Medicine in Houston, TX. He did a research fellowship (T32 and K08) under Dr. Jim Musser at the Methodist Hospital Research Institute in Houston, TX.

Current Activity: He is the Director of Genomic Infectious Diseases Research and is interested in factors that determine the incidence and clinical outcomes of bacterial infections. His work mainly focuses on the genomics and signal transduction of group A Streptococcus to cause serious human infections.

The Shelburne laboratory also researches:

- Invasive infections due to viridans group streptococci in cancer patients
- The epidemiology of invasive group B streptococcal infections in adults
- The impact of the microbiome on infections in immunocompromised patients
- Genetic factors driving antimicrobial resistance amongst bacteria causing human infections

Abstract:

The gastrointestinal (GI) microbiome is increasingly understood as a key site for the development of antimicrobial resistance for major bacterial pathogens causing serious human infections. Moreover, it is becoming clear that monitoring of the GI microbiome can help predict the development of infections in highly susceptible patients such as those with hematologic malignancy or in the intensive care unit. In this talk, we will review data regarding the ability to detect antimicrobial resistance elements in the GI microbiome both in healthy and hospitalized patients. Moreover, recent data will be presented regarding how monitoring of antibiotic resistance determinants in the GI microbiome might be used to predict the development of infections due to antibiotic resistant organisms and thus improve antimicrobial targeting.

James Collins, PhD
Postdoctoral Associate, Baylor College of Medicine

Dietary Trehalose Enhances Virulence of Epidemic Clostridium difficile

About Dr. Collins:
James Collins is a Postdoctoral Associate at Baylor College of Medicine. His work focuses on elucidating factors that enable epidemic *Clostridium difficile* ribotypes to become successful. Following a D. Phil at the University of Oxford, James developed his skills at the Alimentary Pharmabiotic Centre, University College Cork, Ireland; and Michigan State University before moving to Texas.

Abstract:
Clostridium difficile disease has markedly increased since the early 2000s, becoming a dominant nosocomial pathogen in North America and Europe. While this increase can be attributed to a small number of pathogenic ribotypes, little is known about the mechanism underlying their epidemic and hypervirulent nature.

Here we show that two phylogenetically distinct C. difficile ribotypes (RT027 and RT078) have independently acquired unique mechanisms to metabolize low concentrations of the disaccharide trehalose. The RT027 strains contain a single point mutation in the trehalose repressor (TreR) that increases this ribotype's sensitivity to trehalose by >500 fold. Furthermore, dietary trehalose increases virulence of a RT027 strain in a mouse model of infection, indicating that trehalose metabolism plays a role in disease severity. Remarkably, a RT027 strain (but not a non-RT027 strain) was capable of detecting trehalose in human ileostomy fluid of patients eating a "normal" diet. Ribotype 078 strains have independently acquired a cluster of four genes encoding proteins annotated to participate in trehalose metabolism and transport. One of these genes, a PTS permease, is both necessary and sufficient for growth on low concentrations of trehalose. Preliminary data suggests that other epidemic ribotypes, common in Asia and Europe, have also evolved mechanisms for enhanced trehalose use.

James McLellan
Texas State University

Induction of C. difficile Sporulation by Next Generation Probiotics

About Mr. McLellan:
Mr. McLellan became interested in molecular microbiology while attending Texas State University in San Marcos where he trained with Manish Kumar Ph.D. He worked on developing high-throughput drug screening of peptide deformylase inhibitors. He presented his work at the Fall Undergraduate Research Symposium at the University of Texas at Austin in 2017. James' research interest is the effect that small molecules have on the genetic regulation of endospore formation in *Clostridium difficile*. James received his B.S. from Texas State University December of 2017 and will begin a Ph.D. program in Cell and Molecular Biology at the University of Texas at San Antonio in the Fall of 2018.

Abstract:
Clostridium difficile infection (CDI) is the leading cause of hospital acquired, antibiotic associated diarrhea in the United States. According to Centers for Disease Control and Prevention (CDC), nearly half a million-people suffered from CDI in a single year and the cost of treatment of CDI rose to nearly 4.8 billion dollars. The mode of transmission of CDI hinges upon the ingestion of metabolically dormant spores which then colonize the gastrointestinal tract following a depletion of the normal gut flora commonly occurring after the administration of broad spectrum antibiotics. Furthermore, due to the persistence of these spores, one in five individuals treated for CDI experiences recurrent infection (rCDI). Recent research into "next generation" probiotics (NGPs) capable of producing chemical compounds that are bactericidal but non-inhibitory to normal gut bacteria may improve the overall efficacy of fecal matter transplants (FMT) through the integration of defined microbial communities including NGPs. However, the effect of these NGPs on sporulation is unknown, and an increase in sporulation could lead to increased chances of transmission and rCDI. We hypothesized that inhibitory metabolic by-products of selected NGPs including 3-HPA (reuterin) and mycocin from D. hansenii can increase the sporulation frequency of C. difficile in vitro. Our data demonstrate the increased sporulation in presence of spent media of D. hansenii and other NPG strains. Furthermore, using RT-PCR we are measuring the gene expression of sigH, alternative transcription factor that controls the sporulation. It could be serious implications as upregulation of

endospore formation is likely to increase transmission events and rCDI in both hospital and home care situations.

CJ Norsigian
UC San Diego
Genome Scale Metabolic Modeling of Clostridium difficile

About Mr. Norsigian:
Charles J. Norsigian is a Ph.D. student in the Systems Biology Research Group at the University of California, San Diego conducting his thesis research in Bioengineering under the advisement of Dr. Bernhard Palsson. He received his B.S. degree in Biomedical Engineering in 2016 from the University of Virginia. His research interests center on building and applying metabolic network reconstructions to study the evolutionary landscapes of infectious pathogens.

Abstract:
Clostridium difficile is a pathogen of high clinical interest as it continues to be a persistent hospital borne infection. As a means of better understanding the linkage between genotype and phenotype of this organism we constructed a new genome-scale reconstruction of *C. difficile* 630 that builds and improves upon previous efforts. We deploy constraint-based reconstruction and analysis along with flux-balance analysis to investigate metabolic capabilities. We utilize the reconstruction to analyze conserved metabolic function in the *C. difficile* core genome and to build strain-specific models of other strains from their genome sequences.

First	Last	Institution	Title	Poster #
Ying-Shiuan	Chen	IBT	*Dietary Spinach Increases Gut Microbiome Diversity Concurrent with Tumor Suppression in a Rat Model of Colorectal Cancer*	1
Dongmei	Chen	MDACC	*Quxie Capsule Inhibits the Colon Tumor Growth Partially Mediated by Modulating the Gut Microbiome and Myosin11*	2
Sara	Dann	UTMB	*Polymicrobial Bacterial Infection Increases Host Susceptibility to Intestinal Inflammation*	3
Mindy	Engevik	BCM	*Human Intestinal Enteroid Monolayers as a Physiologically Relevant Model to Study Clostridium difficile Toxin Activity*	4
Nicholas	Hummel	Rice	*Identification of Host Defense Pathways Utilized in Liquid Killing of C. elegans*	5
James	McLellan	Texas State	*Induction of C. difficile Sporulation by Next Generation Probiotics*	6
CJ	Norsigian	UCSD	*Genome Scale Metabolic Modeling of Clostridium difficile*	7
Alexey	Revtovich	Rice	*Interplay between Mitochondria and Diet Mediates Pathogen Resistance in C. elegans*	8

Quxie Capsule Inhibits the Colon Tumor Growth Partially Mediated by Modulating the Gut Microbiome and Myosin11

Dongmei Chen[1,2,3], Yufei Yang[3], Peying Yang[2]

[1]Graduate School, Beijing University of Chinese Medicine

[2]Department of Palliative, Rehabilitation and Integrative Medicine, The University of Texas MD Anderson Cancer Center

[3]Department of Oncology, Xi-Yuan Hospital of China Academy of Chinese Medical Sciences

Corresponding author: Peying Yang, Department of Palliative, Rehabilitation and Integrative Medicine, The University of Texas MD Anderson Cancer Center, 6767 Bertner Avenue, Houston, TX, E-mail: pyang@mdanderson.org

Objective: Traditional Chinese Medicine (TCM), such as the Quxie Capsule (QXC), has been routinely used in colorectal cancer treatment at Xiyuan Hospital in China. However, the mechanism(s) underlying the effects of QXC in colon cancer still remain unclear, which hampers its optimal use for the treatment of colon cancer. The purpose of this study is to understand how TCM, especially QXC, can exert anti-tumor effects via alteration of gut microbiota and related pathways.

Methods: The antitumor efficacy of QXC was tested in both the CT-26 syngeneic Balb/c mouse colon cancer models that were using antibiotics (an alternative to germ-free mice) and in the models that were not using antibiotics. Intestinal microbiome was tested by 16sRNA sequencing. Signaling protein expression in tumor tissues was measured by a Reverse Phase Proteomic Array (RPPA).

Results: QXC gavaged to mice carrying CT26 mouse colon tumors without antibiotics for 2 weeks significantly reduced the average tumor weight (0.92 ± 0.15g) compared to that of the vehicle control treated mice (1.569 ± 0.1689, $p < 0.05$). The antitumor effect of QXC was reduced in antibiotics treated mice. 16SRNA gene sequencing showed that Firmicutes (F), Bacteriodetes (B) and Proteobacteria were the dominant gut bacterial phyla in all the mice without antibiotics treatment which is consistent with results published in the previous studies. At the phylum level, the ratio of Firmicutes over Bacteriodetes(F/B) decreased 2.03 fold in tumor-bearing mice compared to non-tumor mice ($p<0.05$). QXC resulted in an increase of Firmicutes and reductions of Bacteriodetes, that led to a 2.0-fold increase in the ratio of F/B

compared to that prior to QXC treatment ($p<0.05$). In the antibiotics treated mice, the OTUs dramatically decreased ($p<0.05$) after antibiotics treatment, suggesting that most bacteria were eliminated by an antibiotics cocktail. Both alpha diversity and beta diversity in antibiotic and QXC co-treated mice were similar to that of the vehicle control and antibiotic co-treated group. RPPA data showed that the protein Myosin 11 (MYH11), which had been reportedly downregulated in fiber-deprived colon mucosa and colorectal cancer, was significantly elevated in QXC treated tumor tissue compared to that of control. In contrast, Myosin 11 expression was lower in the tumor tissue of antibiotics and QXC co-treated mice compared to that of antibiotics and vehicle control co-treatment group. Further, the levels of apoptotic-regulating proteins such as Fas, Bim and cleaved caspase-3 were elevated in QXC treated tumor tissue as opposed to that of the control group.

Conclusion: The Quxie Capsule inhibited the growth of colon tumors which could be due to its ability to rebalance the intestinal microbiota and upregulate the myosin11 level in the syngeneic colon cancer model.

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Dietary Spinach Increases Gut Microbiome Diversity Concurrent with Tumor Suppression in a Rat Model of Colorectal Cancer

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The gut microbiome influences human health and pathological conditions, such as obesity, cancer, and inflammatory responses. Intervention in the gut microbiome thus provides an avenue to affect prevention and therapeutic outcomes. This investigation had two main objectives. First, we sought to profile the microbiome composition in the polyposis in rat colon (Pirc) preclinical model of human familial adenomatous polyposis (FAP). Second, we ascertained how administration of chlorophyll-rich spinach in the diet would influence the gut microbiome. After 6.5 months of spinach intervention, 16S sequencing of cecum contents (n=28 rats) was performed, and temporal changes in the fecal microbiome also were tracked over the first several hours, days and weeks. Dietary spinach increased cecum microbiome diversity in both Pirc and wild type rats, and was correlated with tumor suppression in the Pirc model. Unweighted UniFrac analysis indicated low abundance bacteria were significantly altered by dietary spinach. According to linear discriminant analysis, 23 bacterial operational taxonomic units (OTUs) were significantly altered in the Pirc model, and 28 OTUs were shifted by dietary spinach intervention. Several OTUs associated with *Lachnospiraceae* were decreased in Pirc and reversed after spinach intervention, whereas OTUs associated with *Ruminococcaceae* had the opposite trend. PICRUSt and STAMP were used as predictive tools for metagenome changes in the cecum microbiome. Spinach increased butanoate and alpha-linolenic acid metabolism, and decreased TCA cycle and Pathways in Cancer. Taxonomic changes also were compared in fecal *versus* cecum samples; some OTUs responded promptly and others more gradually to dietary spinach administration. Notably, fecal microbiome diversity was increased by dietary spinach, and reflected major changes in unweighted UniFrac. In summary, dietary spinach increased the diversity in both cecum and fecal microbiomes, coincident in tumor suppression in the GI tract. *Lachnospiraceae, Ruminococcaceae,* and other less abundant bacterial populations appeared to play important roles in colorectal cancer progression. Further investigation of cause-and-effect is warranted, and the functional metabolites implicated for prevention in human FAP and other colorectal cancer susceptible populations. Work supported in part by NIH grants P01 CA090890 and R01 CA122959, the John S. Dunn Foundation, and a Chancellor's Research Initiative.

Polymicrobial Bacterial Infection Increases Host Susceptibility to Intestinal Inflammation

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Background: There is a critical need for new approaches to combat *Clostridium difficile* infection (CDI). Most of what is known about CDI is based on studies that view symptomatic disease as being "monomicrobial" in nature considered to be dominated by the virulence factors of CDI alone without contributions by other microbes. However, with the advent of new diagnostic methodologies several studies have detected *C. difficile* in the presence of other enteric pathogens. The significance of co-infections in CDI is unknown. **Methods:** Stool specimens from children (3-18 years of age) and adults (>18 years of age) studied as part of a multi-center U01 research trial were analyzed for virulence determinants using systems biology methods and a FilmArray Gastrointestinal Panel (BioFire Diagnostics, Salt Lake City, UT) which detects 21 common enteric pathogens. Select pathogens were then tested in a murine model of CDI to determine the impact of co-infection on disease susceptibility and outcome. **Results:** Of 357 pediatric patients, 315 (88%) had antibiotic-associated diarrhea, and based on symptoms and routine toxin PCR, 177 (50%) were diagnosed with non-recurrent CDI, 31 (8%) with recurrent CDI (rCDI), and 107 (30%) were negative for *C. difficile* toxin (AAD). The remainder of patients 42 (12%) did not develop GI symptoms and served as controls. FilmArray analyses identified the presence of additional pathogens in 55/177 (31.1%) with primary CDI, 20/31 (64.5%) with rCDI, 53/107 (49.5%) with AAD, and 5/42 (11.9%) controls. Enteropathogenic *E. coli* (EPEC) and rotavirus were the most common enteric pathogens associated with rCDI. Coinfections with EPEC were not observed in adults. In a murine model of co-infection, rotavirus improved clinical symptoms, despite an increase in *C. difficile* burden. By contrast, co-infection with *Citrobacter rodentium*, a robust model of EPEC, resulted in greater clinical disease and mortality than singly infected mice. Co-infected mice had a significantly greater *C. difficile* bacterial burden compared to controls, and impaired ability to control bacterial replication and toxin production. Surprisingly, histopathological analysis of co-infected mice showed reduced mucosal damage and inflammation early after infection, which was associated with marked reductions in chemokines involved in the recruitment of protective neutrophil and macrophage responses. In support of this observation, administration of a CXCL1 dimer significantly protected co-infected mice from clinical disease during the early stage of infection. **Conclusion:** Our results indicate that co-infection with another pathogen can have markedly different clinical outcomes on CDI. Importantly, co-infection with EPEC may place pediatric CDI patients at greater risk of disease recurrence because of pathogen-induced impairment in protective innate immunity against *C. difficile*. Thus, co-infection in CDI may significantly impact the diagnosis and management of these patients.

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Human Intestinal Enteroid Monolayers as a Physiologically Relevant Model to Study *Clostridium difficile* Toxin Activity

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Background: *Clostridium difficile* is a spore-forming microbe that has emerged as an important nosocomial pathogen globally. *C. difficile* infection (CDI) involves alterations of the host microbiota, germination of *C. difficile* spores, and production of toxins A and B that are primarily responsible for the clinical manifestations. Both toxins bind cell surface receptors, are endocytosed, and inactivate GTP-binding proteins. This leads to an influx of calcium (Ca^{2+}) and collapse of the actin cytoskeleton, which can be assayed by cell rounding. Current *C. difficile* model systems have used immortalized cell lines to assess toxin-mediated cell rounding. However, the biological relevance of these model systems is limited by variability in response to *C. difficile* toxins, making standardization of activity challenging. To date, no study has examined the distribution of known *C. difficile* toxin receptors in the human intestine or determined the sensitivity of the human epithelium to *C. difficile* toxins. We hypothesized that human intestinal enteroids (HIEs), as the most physiologically relevant *in vitro* model system, expresses the important native toxin receptors and provide an ideal model to dissect *C. difficile* toxin activity. **Methods & Results**: We generated biopsy-derived HIE cultures stably expressing a GFP-based calcium sensor (GCaMP6s) to monitor cytosolic Ca^{2+} and LifeAct-Ruby labeling F-actin to monitor actin cytoskeleton rearrangement by live cell imaging. For comparison we also generated stably expressing LifeAct-Ruby and GCaMP6s cell lines, Hela and Vero. By qPCR we found that jejunal and colonic HIEs expressed far higher concentrations of the known toxin receptors compared to Vero cells. The highest expressing receptor for HIEs was the toxin B receptor FZD7 (~60,000 fold increase in jejunum and colon), followed by toxin A receptor GP96 (~500 fold increase in jejunum, ~3,500 fold increase in colon). Live imaging revealed toxins from pathogenic *C. difficile* strains elicited robust Ca^{2+} signaling in cell lines and HIEs, while non-toxigenic strains did not. Increased intracellular Ca^{2+} correlated with actin cytoskeleton rearrangement in cell lines and HIEs. Interestingly, the HIEs were less sensitive to toxins derived from several *C. difficile* strains (R20291, 196, 630, M68, M929972) or purified toxins when compared to the cell lines, particularly the Vero and HeLa cells. HIE's rounded in response 1 µg toxin A or 1 µg toxin B within 3 hours. Of note, HIEs both rounded at 1 ng of toxin A and B, although HIEs rounded were more sensitive to toxin A at the lower concentrations. **Conclusions**: We found that HIEs have high expression of toxin receptors, and yet decreased sensitivity to *C. difficile* toxins when compared to traditionally used cell lines. We speculate that this may reflect components that are present in the HIEs and absent in immortalized models, such as mucins and secreted peptides. Our work demonstrates the need for more robust models of *C. difficile* infection and that the use of HIEs provides a new biologically relevant system for dissecting the intricate signaling between toxins and the host epithelium.

Identification of Host Defense Pathways Utilized in Liquid Killing of *C. elegans*

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Antibiotic-resistant infections cause an average of 23,000 deaths per year. Unfortunately, the rate of discovery of new antibiotics has dwindled significantly in recent years with only 3 new antibiotic classes being introduced into the market since the year 2000. Because of this, novel methods of combating infection are desperately needed. Past antibacterial treatments have focused upon the bacterial pathogen itself, with the goal of limiting growth. However, another equally important determinant of the lethality of infection is the host defense response. Given an adequate understanding of the defense responses mounted by infected hosts against specific pathogens, it should be possible to design pharmaceuticals that upregulate expression of host defenses that are critical for resisting fatal infections by multidrug resistant bacteria.

Pseudomonas aeruginosa is a gram negative opportunistic pathogen commonly infecting patients with cystic fibrosis. Previously, we performed a high-throughput chemical screen to identify small molecules that rescued the nematode *Caenorhabditis elegans* from infection by *P. aeruginosa*. Worms were exposed to the pathogen for 48 hours in the presence of small molecule or a DMSO control. Worms were scored via an automated analysis to determine compound-mediated resistance. Of the hits identified, 6 (LK16, LK32, LK34, LK35, LK38 and LK56) were determined to be potential stimulators of host defense pathways. Importantly, these compounds did not significantly affect bacterial growth or production of known virulence factors. Using microarray analysis, bioinformatic clustering, and infection assays with other pathogens, we have begun to characterize the potential genetic pathways responsible for rescue by these immune stimulators. Using leads from our analyses, we will use reverse genetics to identify genetic pathways each compound uses to increase host survival. Thus far, we have identified promising genetic leads for compound LK56, which appears to use a known host defense pathway mediated by SKN-1/Nrf. Three other compounds, LK34, LK35, and LK38, appear to have significant overlap of downstream effectors, suggesting the possibility of a shared pathway. We will also assay our hits for potential hormetic mechanisms by studying long-term compound exposure. By identifying the chemical-genetic underpinning of these effects, we hope to learn how host defense pathways can be stimulated to promote innate immunity, attenuate virulence, and improve human health.

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TITLE: Induction of *C. difficile* Sporulation by Next Generation Probiotics

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ABSTRACT: *Clostridium difficile* infection (CDI) is the leading cause of hospital acquired, antibiotic associated diarrhea in the United States. According to Centers for Disease Control and Prevention (CDC), nearly half a million-people suffered from CDI in a single year and the cost of treatment of CDI rose to nearly 4.8 billion dollars. The mode of transmission of CDI hinges upon the ingestion of metabolically dormant spores which then colonize the gastrointestinal tract following a depletion of the normal gut flora commonly occurring after the administration of broad spectrum antibiotics. Furthermore, due to the persistence of these spores, one in five individuals treated for CDI experiences recurrent infection (rCDI). Recent research into "next generation" probiotics (NGPs) capable of producing chemical compounds that are bactericidal but non-inhibitory to normal gut bacteria may improve the overall efficacy of fecal matter transplants (FMT) through the integration of defined microbial communities including NGPs. However, the effect of these NGPs on sporulation is unknown, and an increase in sporulation could lead to increased chances of transmission and rCDI. We hypothesized that inhibitory metabolic by-products of selected NGPs including 3-HPA (reuterin) and mycocin from *D. hansenii* can increase the sporulation frequency of *C. difficile in vitro*. Our data demonstrate the increased sporulation in presence of spent media of *D. hansenii* and other NPG strains. Furthermore, using RT-PCR we are measuring the gene expression of *sigH*, alternative transcription factor that controls the sporulation. It could be serious implications as upregulation of endospore formation is likely to increase transmission events and rCDI in both hospital and home care situations.

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Genome Scale Metabolic Modeling of *Clostridium difficile*

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Clostridium difficile is a pathogen of high clinical interest as it continues to be a persistent hospital borne infection. As a means of better understanding the linkage between genotype and phenotype of this organism we constructed a new genome-scale reconstruction of *C. difficile* 630 that builds and improves upon previous efforts. We deploy constraint-based reconstruction and analysis along with flux-balance analysis to investigate metabolic capabilities. We utilize the reconstruction to analyze conserved metabolic function in the *C. difficile* core genome and to build strain-specific models of other strains from their genome sequences.

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Interplay between Mitochondria and Diet Mediates Pathogen Resistance in *C. elegans*

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Diet is a crucial determinant of organismal biology. The interaction between the host, its diet, and its microbiota are as critical to determining the health of an organism as its genome.

Using a variety of genetic and biochemical means, we assess the differences in pathogen and abiotic stress sensitivity in *C. elegans* that has been reared on two different diets: the standard laboratory food stuff, *E. coli* OP50 or the commonly used *E. coli* strain HT115, which is normally used for RNAi-mediated gene knockdown. These assays include survival assays, lifespan measurements, microarrays, fluorescence microscopy, and measurements of mitochondrial health.

We demonstrate the dramatic impact of a subtle shift in diet on the ability of *Caenorhabditis elegans* to survive pathogenic or abiotic stress. Interestingly, this shift occurs independently of canonical host defense pathways, arising instead from improvements in mitochondrial health. We reveal that the most common *C. elegans* food source (*E. coli* OP50) results in a subclinical vitamin B12 deficiency that compromises mitochondrial homeostasis. Increasing B12 supply, by feeding on *E. coli* HT115 or by supplementation with methylcobalamin, restored mitochondrial function and conferred resistance to multiple pathogens and stressors. This is due to B12's role as a cofactor for methylmalonyl-CoA mutase, an enzyme required for propionate breakdown. Our study forges a mechanistic link between a dietary deficiency (nutrition/microbiota) and a physiological consequence (host sensitivity), using the host-microbiota-diet framework. The ubiquity of B12 deficiency (~10-40% of US adults) highlights the importance of our findings.