AGENDA

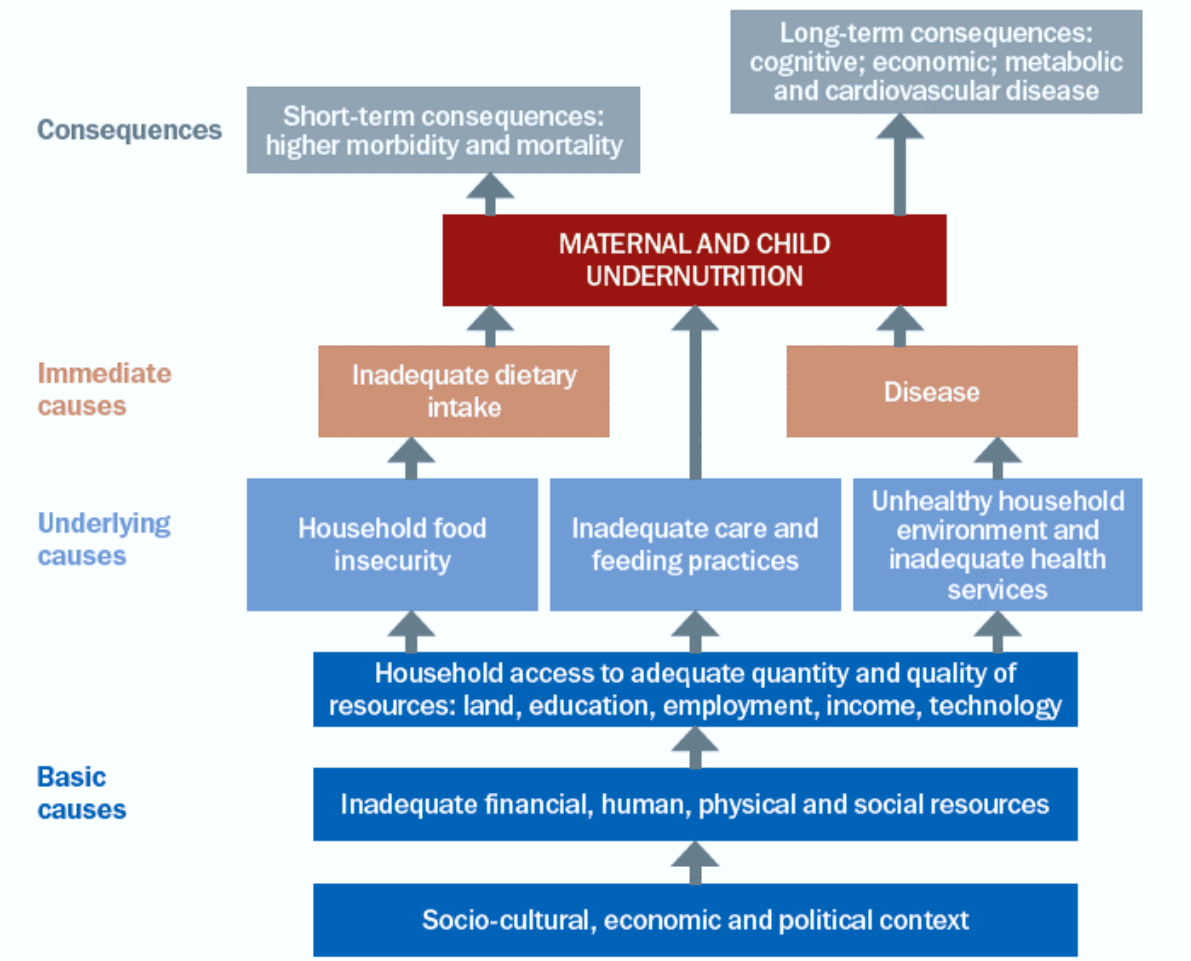
Time	Title	Speaker/Moderator
11:20-11:32	Establishing Context and Need (12 min)	Srivalli Krishnan & Chris Damman Gates Foundation
11:32-11:38	Fermented Foods Impact on Inflammation and Microbiome Diversity (6 min)	Erica Sonnenburg Stanford University
11:38-11:44	Inflammation and Microbiome in Mother Infant Dyads (6 min)	Najeeha Iqbal VITAL Pakistan Trust
11:44-11:50	Fermented Foods GC Call (6 min)	Ravi Sheth Gates Fellow
11:50-11:56	Sequencing Capacity Development & Coordination of a Collaborative Network (6 min)	Aashish Jha NYU Abu Dhabi
11:56-12:20	Q&A (24 min)	Shelby Montgomery & Brendan Thomason Gates Foundation

FERMENTED FOODS GRAND CHALLENGE

October 21, 2020

Srivalli Krishnan
Bill & Melinda Gates Foundation

A MULTI-SECTORAL EFFORT FOR IMPROVED NUTRITION



Reference
UNICEF. Improving Child Nutrition. New York, United Nation's Children's Fund (UNICEF), 2013.

- Due to the multisectoral causes of undernutrition, multisectoral approaches to improve nutrition are needed. These approaches span food, health, and social protection sectors.
- Good nutrition also depends on adequate practices, particularly for women and children, and thus requires demand-side interventions.
- Income growth alone is not sufficient to address undernutrition.
- Diet quality is fundamental to good health and nutrition. Many micronutrients and macronutrients are necessary, not just one.

HISTORY OF CONSUMPTION OF FERMENTED FOODS IN INDIA/ASIA

From North to South, East to West – Traditional Indian foods have been fermented since 1000 BC

WHY TRADITIONAL FOODS WERE FERMENTED?

- Relatively simple process and can be done within household
- Can preserve seasonal foods for a longer time
- Economical process for preserving foods
- Increases flavor and digestibility
- Improved nutritional benefits

Categories of fermented foods in India:

(i) Cereal-based (with/without pulses) fermented foods
(ii) Cereal/pulse and buttermilk-based fermented food
(iii) Cereal-based fermented sweets and snacks
(iv) Milk-based fermented foods
(v) Vegetable, bamboo shoot (BS) and unripe fruits-based fermented foods
(vi) Meat-based fermented foods
(vii) Pulse (legume)-based fermented foods.

FROM STRATEGY TO ACTION AND SCALE

- Cutting edge technologies for crop/ farm diversification
- Critical themes with long term view
- Transformative approaches

- Innovative delivery approaches (ICT, platforms)
- Business-like approach (market analysis to feed supply)

Research and Discovery for India/Asia

Improving access to products and services

Learning and Evaluation

Partnerships to scale

- Risk taking appetite
- How to define success
- Incorporate measurement and learning agenda

- Government- Federal and State
- Policy level
- Private sector
- Research institutions

SCALING UP EFFORTS FOR FOOD SYSTEMS

- How can fermented foods be introduced as part of social safety nets/ school feeding/ MDM programs?
- How can we promote consumption of fermented foods for critical lifecycle periods?
- What are the behaviors that promote/ inhibit consumption of fermented foods?
- Can these foods offer long term nutrition solutions to developing country challenges?

Improve affordability

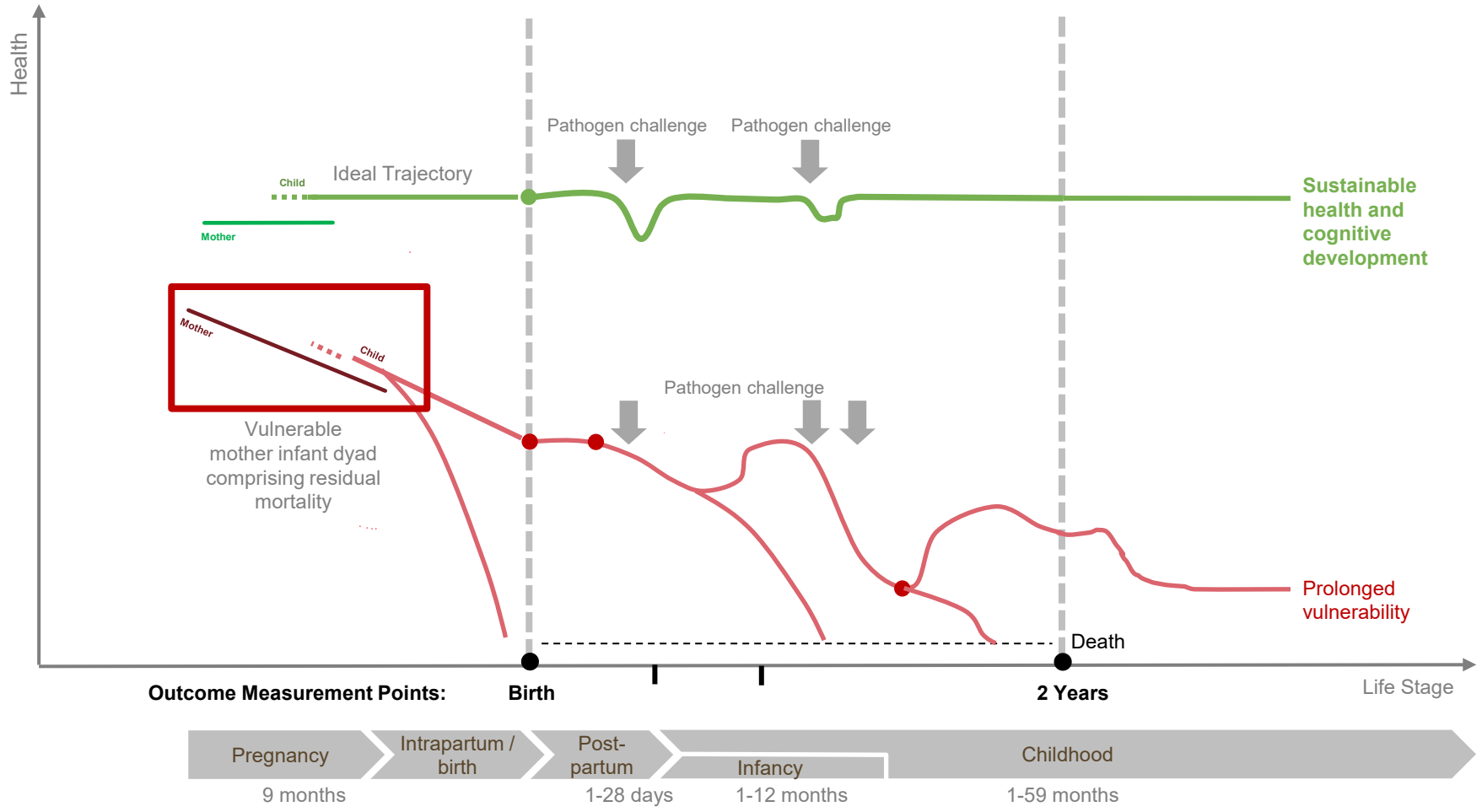
Promote household production and consumption

Improve market accessibility

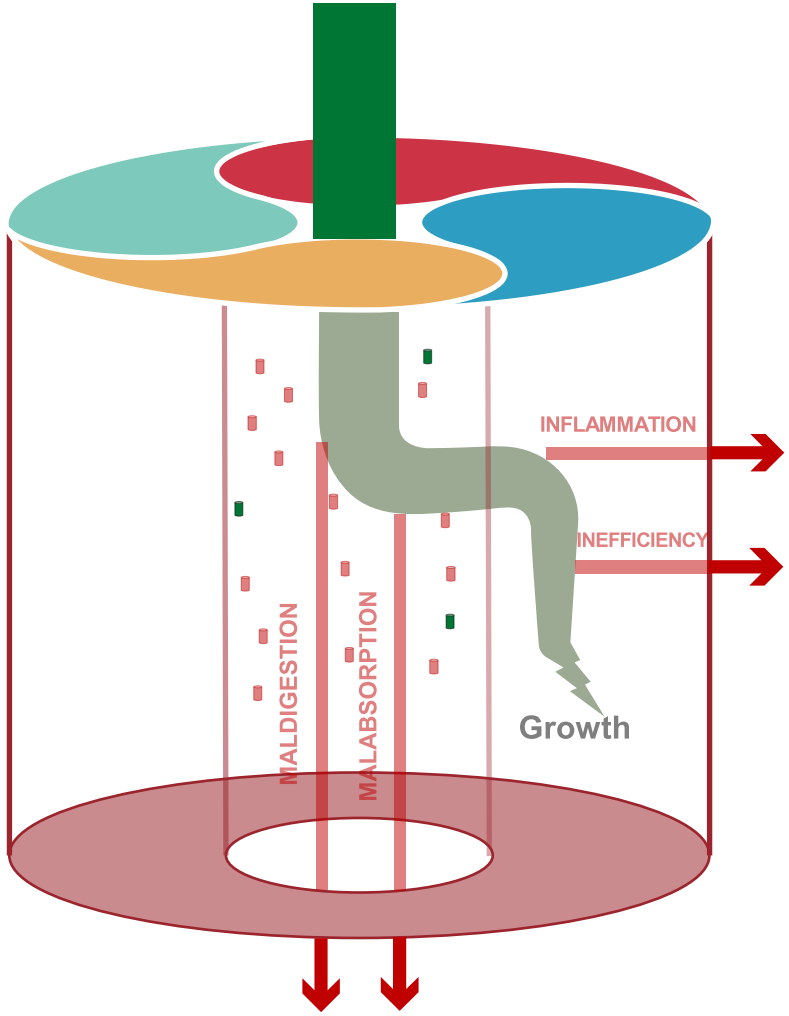
Improve local availability

SOME DISCOVERY & TOOLS PERSPECTIVES

GROWTH AND RESILIENCE FRAMEWORK



A GASTROENTEROLOGIST'S VIEW OF THE WORLD



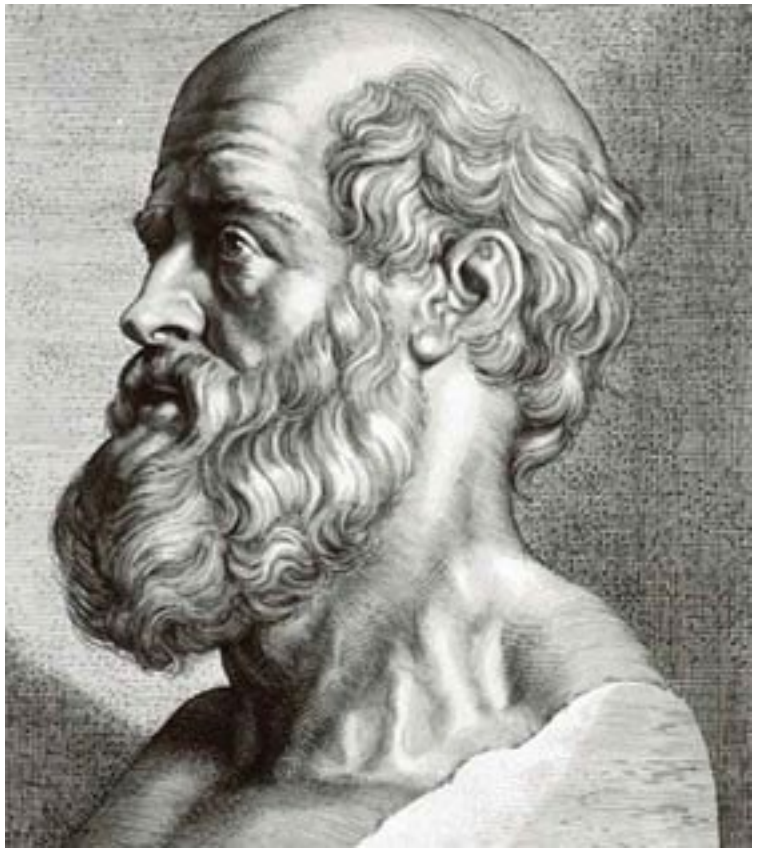
We are tubes:

- That have evolved to maximize energy absorption and transformation
- The gut lumen creates a controlled environment for host factors *and microbes* to break down and transform foods into more nutritious and absorbable nutrients
- Malnutrition represents not just the wrong foods but the wrong bugs and the impact of both on the efficiency of capturing food energy (maldigestion, malabsorption, inflammation, metabolic inefficiency) for growth!

THE HEALING POWER OF FOOD & MICROBES

and microbes

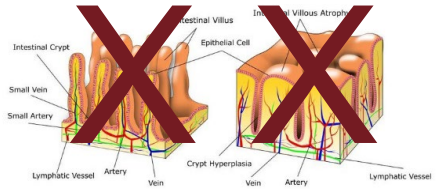
"Let food by thy medicine"-
Hippocrates (400 BC)



NEXT GENERATION FOODS & MICROBIAL INTERVENTIONS

MALABSORPTION

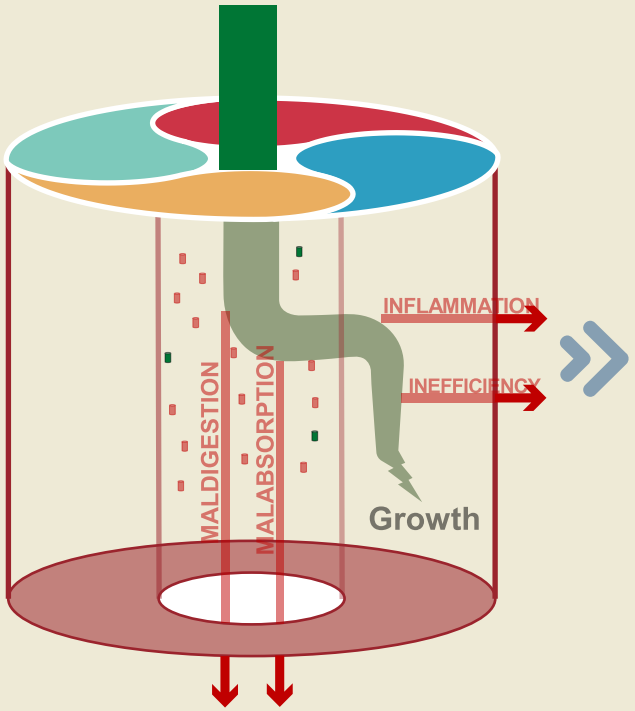
↓surface area & leaky gut



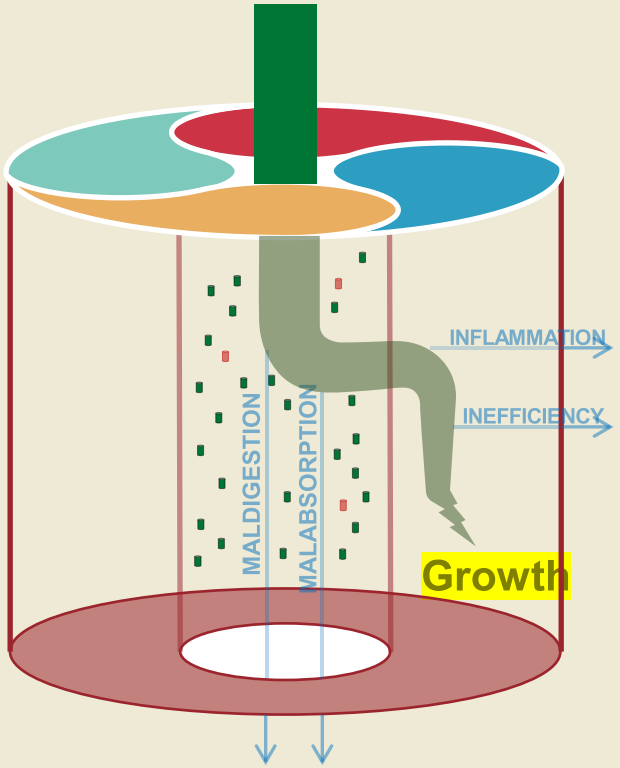
MALDIGESTION

missing microbes &
SCFA calories from fiber

Ultra-Processed & Bland Foods



Fermented Foods & Microbiome Enriching Interventions

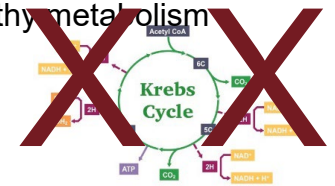


INFLAMMATION

bacterial translocation,
immune activation & energy

INNEFICIENCY

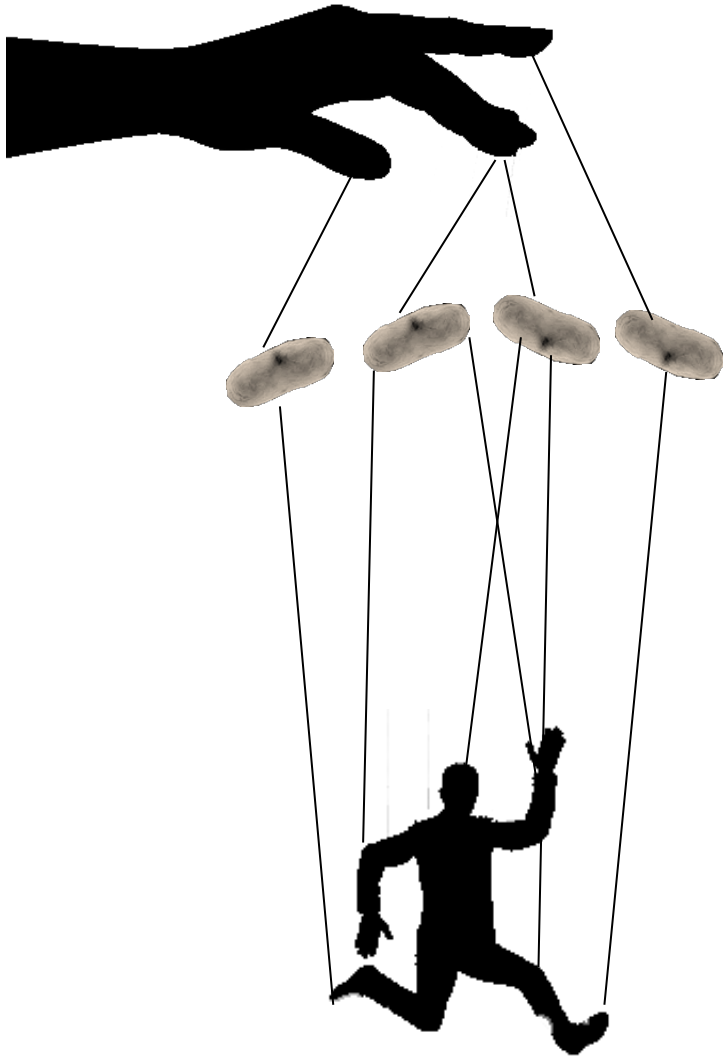
missing microbes & vitamins for healthy metabolism



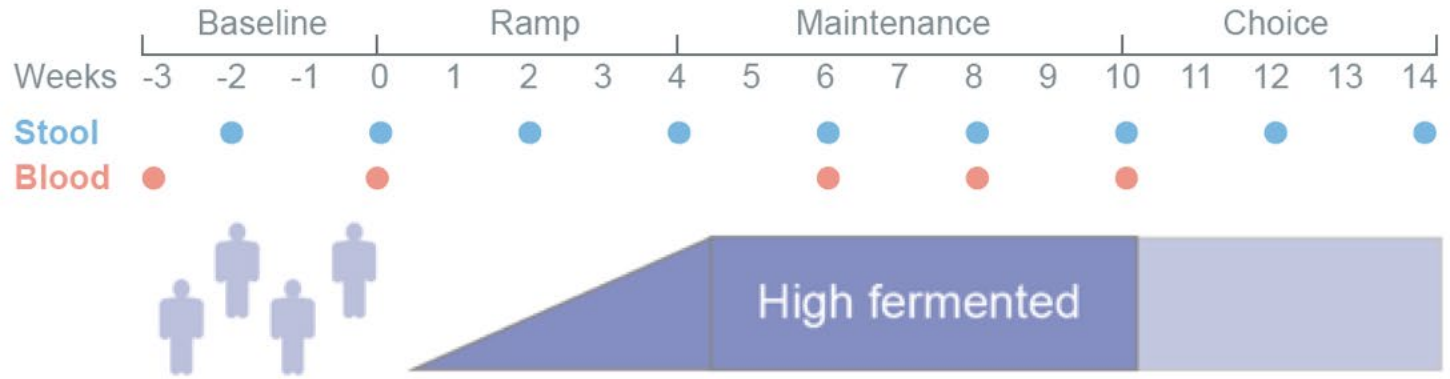
Pilot Trial:
Fermented Foods Impact on Inflammation and Microbiome Diversity

Erica D. Sonnenburg, PhD
Senior Research Scientist
Department of Microbiology and Immunology
Stanford University School of Medicine

How can we manipulate the gut microbiota to improve health?



Can we change **immune** status and improve **health** with **diet**-induced **microbiome** alterations?

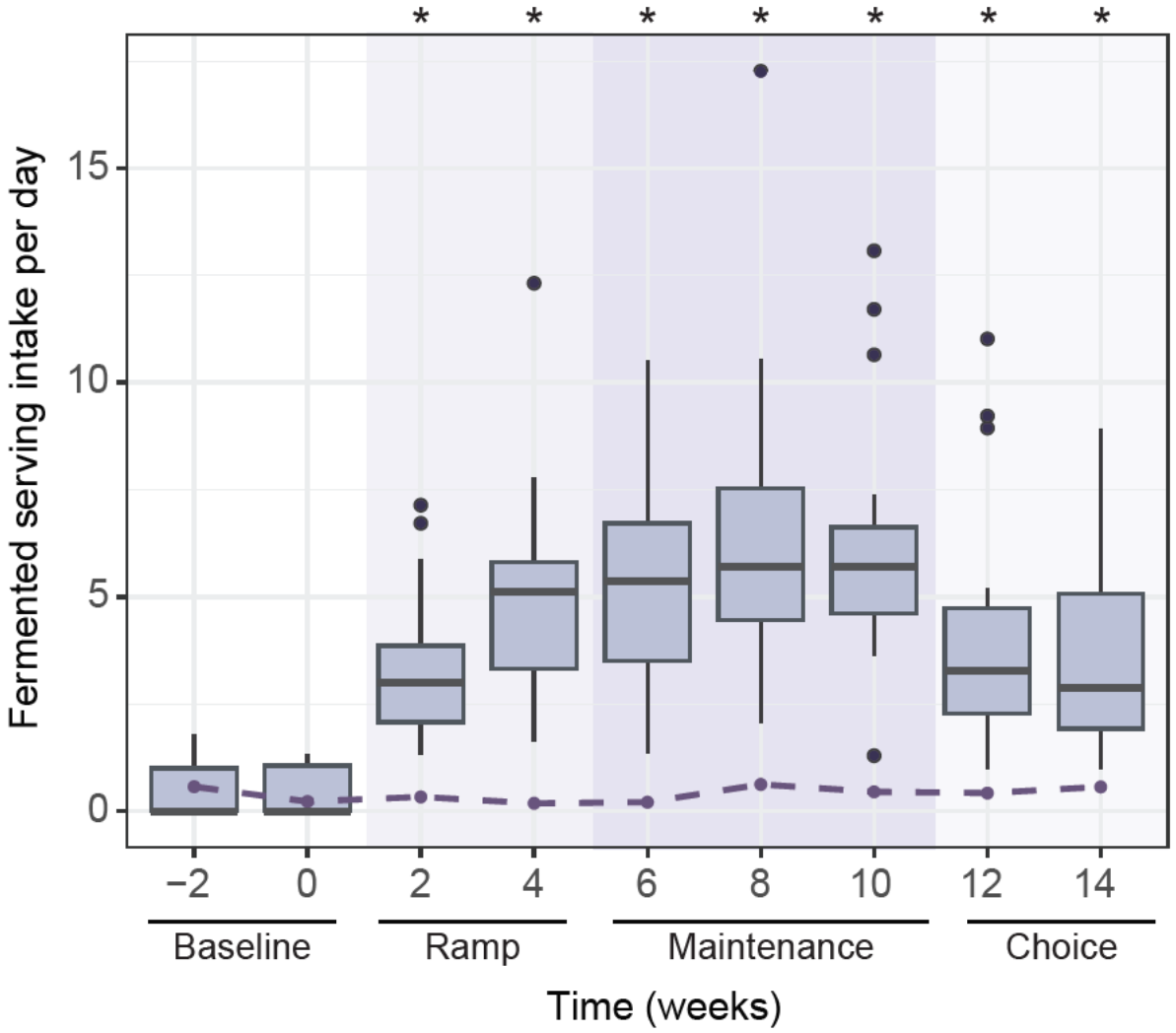


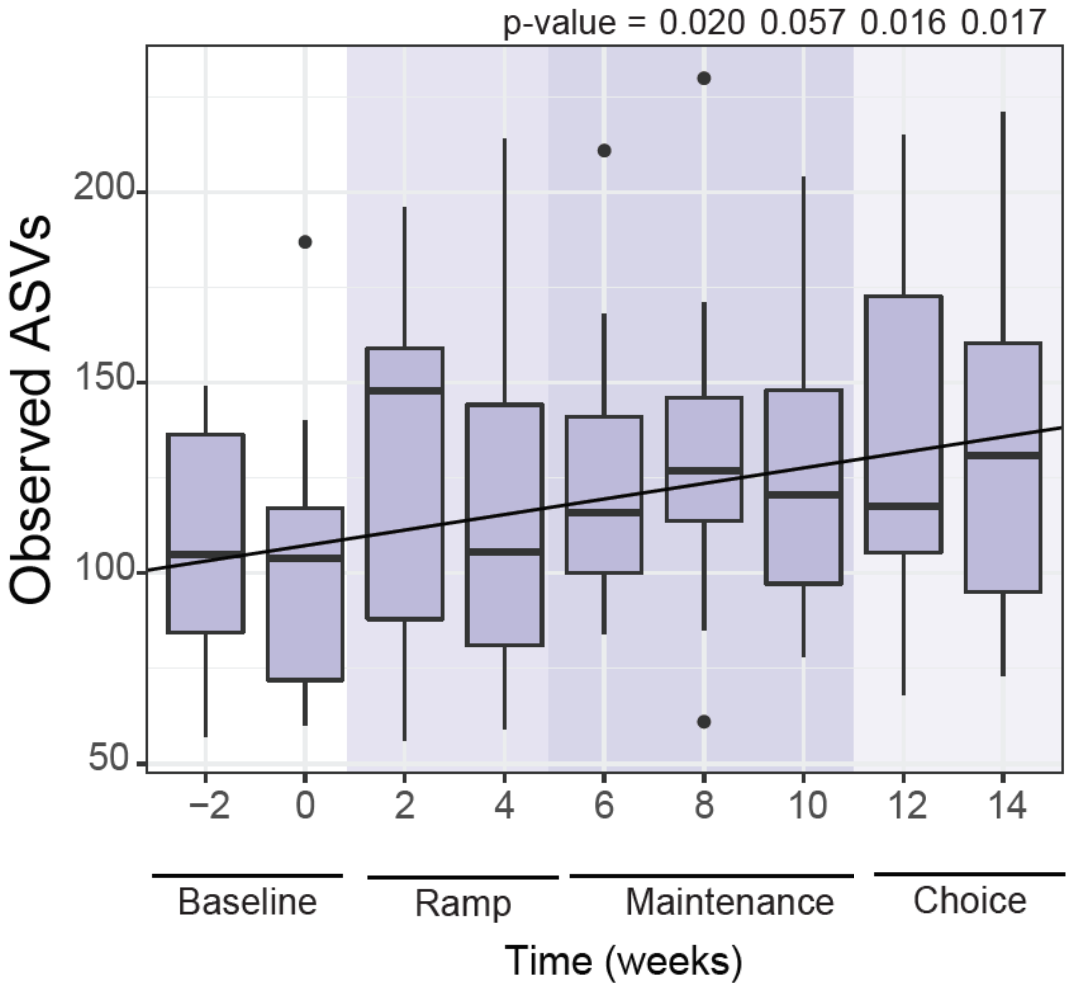
Microbiome profiling: 16S, Metagenomics, SCFA

Immune profiling: CyTOF, Phosphoflow, O-link

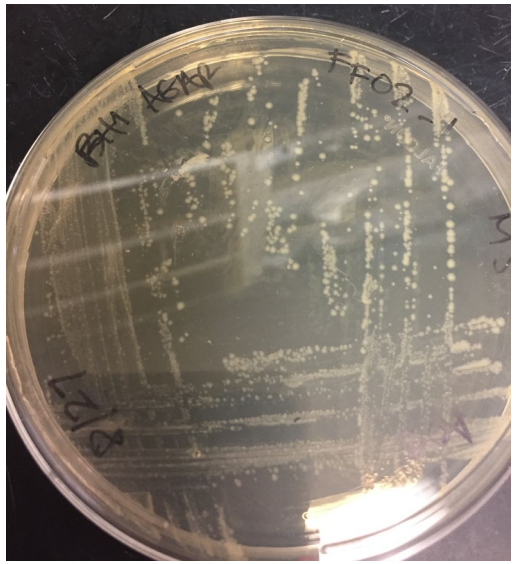
In collaboration with Christopher Gardner (Stanford)

Participants increased fermented foods intake and microbiome diversity



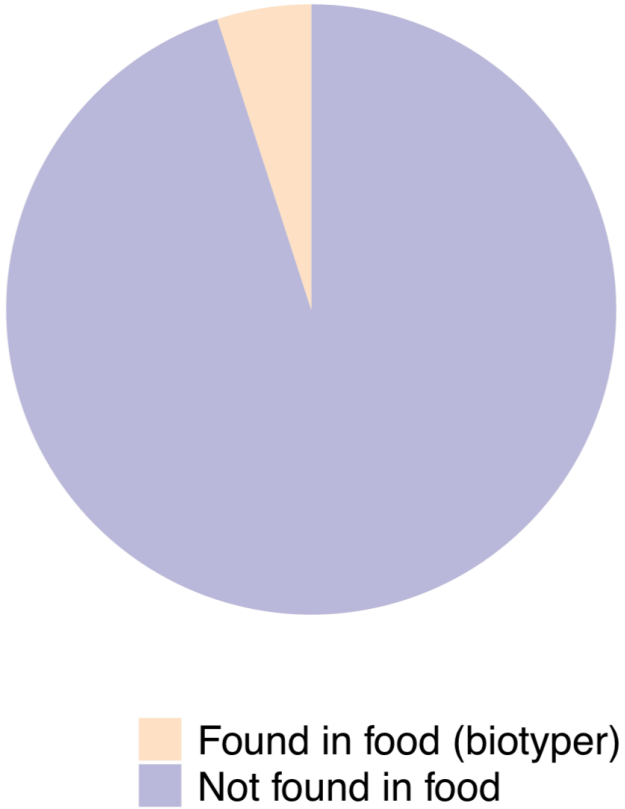


Fermented food had an indirect effect on microbiota diversity



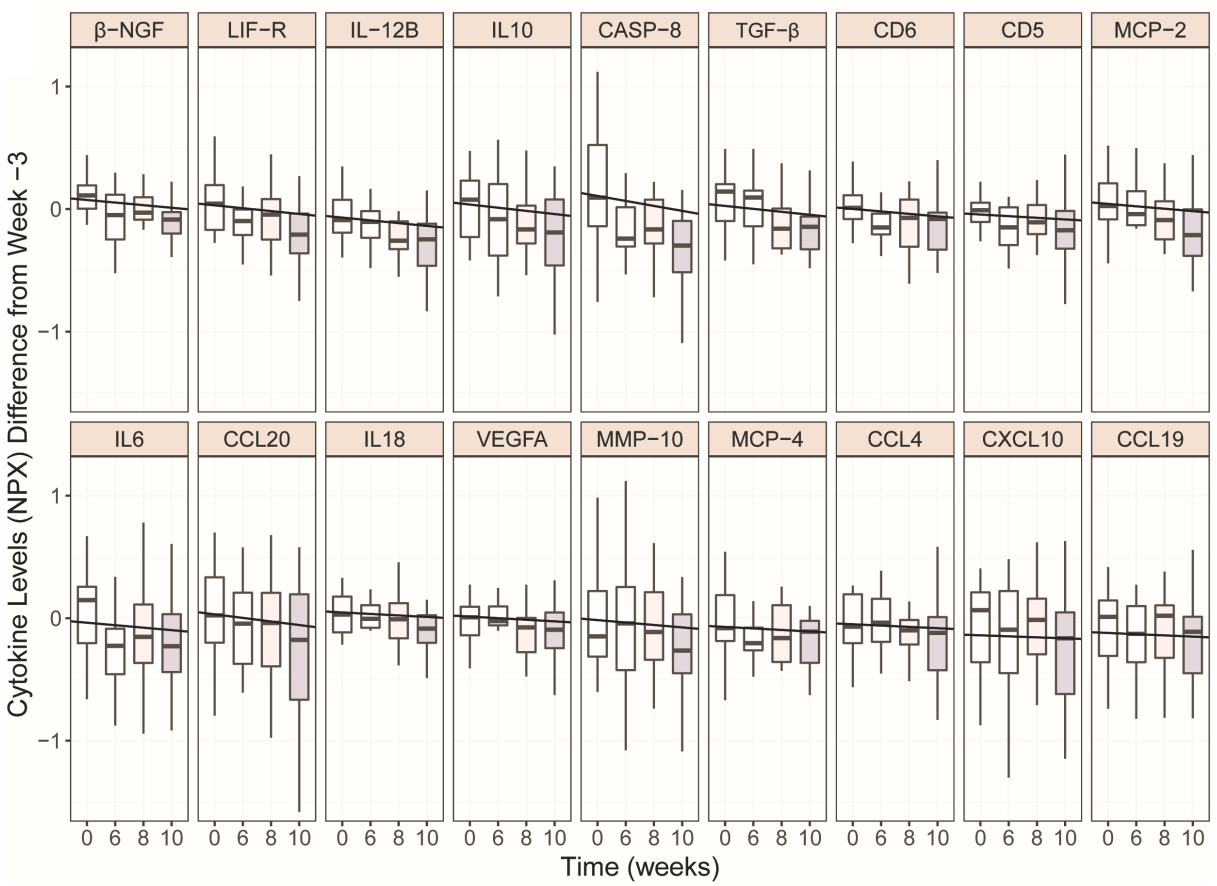
Yoghurt	*Streptococcus mitis*
	Streptococcus pneumoniae
	Streptococcus salivarius thermophilus
	Paenibacillus lactis
	Micrococcus luteus
	Bifidobacterium animalis
	Lactobacillus rhamnosus
	Lactobacillus delbrueckii
	Lactobacillus paracasei
	Lactobacillus rhamnosus
	Lactobacillus plantarum
Kefir	*Lactobacillus rhamnosus*
	Lactobacillus paracasei
	Lactobacillus delbrueckii
	Lactococcus lactis
Sauerkraut	*Lactobacillus plantarum*
	Micrococcus luteus
	Lactobacillus paracasei
Cottage cheese	*Lactobacillus lactis*
	Lactobacillus paracasei
Kimchi	*Bacillus pumilius*
	Lactobacillus sakei
	Lactobacillus curvatus
Kombucha	*Paenibacillus lactis*
	Lactobacillus brevis
Gut Shots	*Lactobacillus plantarum*
	Lactobacillus paraplantarum

New ASVs pooled across cohort

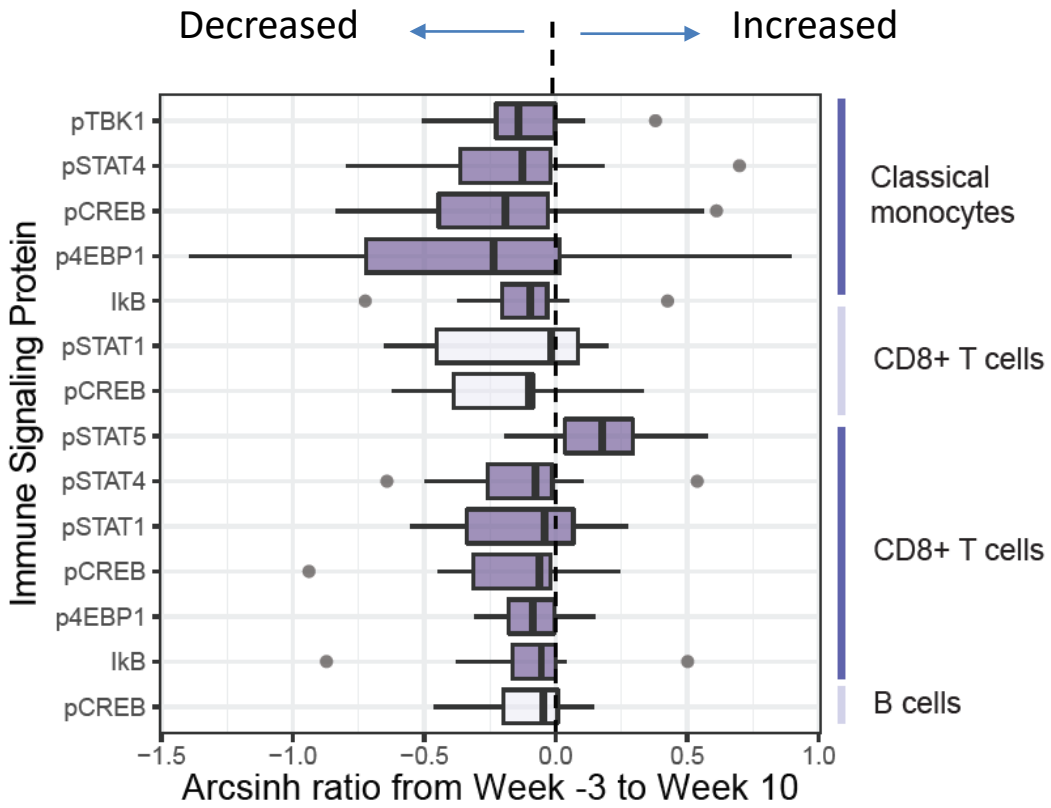


Fermented food consumption decreased inflammatory cytokines and signaling

cytokines/chemokines



Inflammatory signaling



Summary

Gut microbiome-targeted diets may be a low cost, scalable approach for improved health across populations

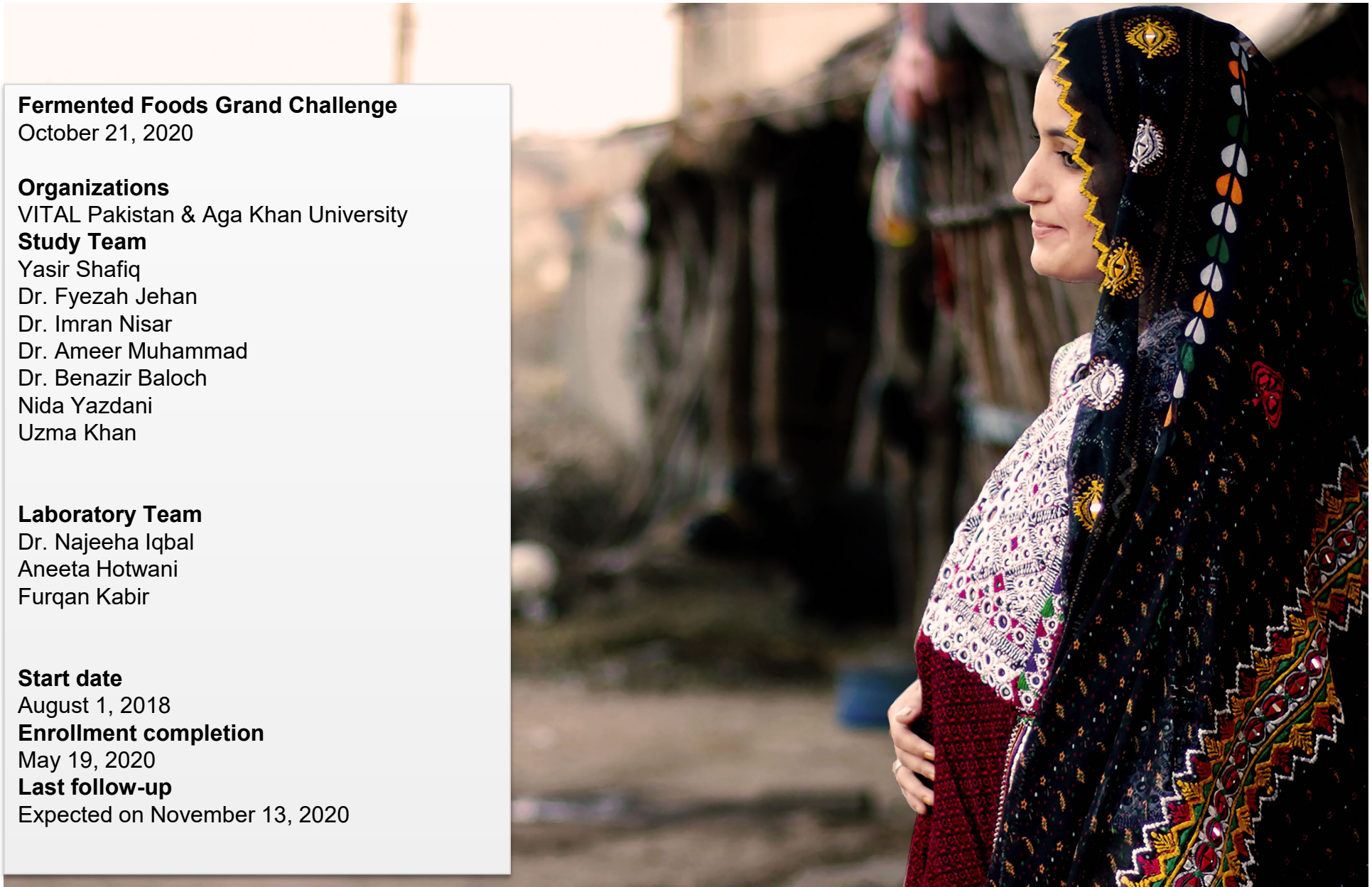
In a cohort of healthy US adults, **fermented foods**:

- Increase diversity of the gut microbiota
- Decrease markers of inflammation

What are the yet-unrecognized health benefits of fermented foods?

→ We need more well-designed human trials that employ –omics technologies

Nutritional support for lactating women with or without azithromycin for infants compared to breastfeeding counselling alone in improving the 6-month growth outcomes among infants Pakistan



Fermented Foods Grand Challenge
October 21, 2020

Organizations
VITAL Pakistan & Aga Khan University
Study Team
Yasir Shafiq
Dr. Fyezah Jehan
Dr. Imran Nisar
Dr. Ameer Muhammad
Dr. Benazir Baloch
Nida Yazdani
Uzma Khan

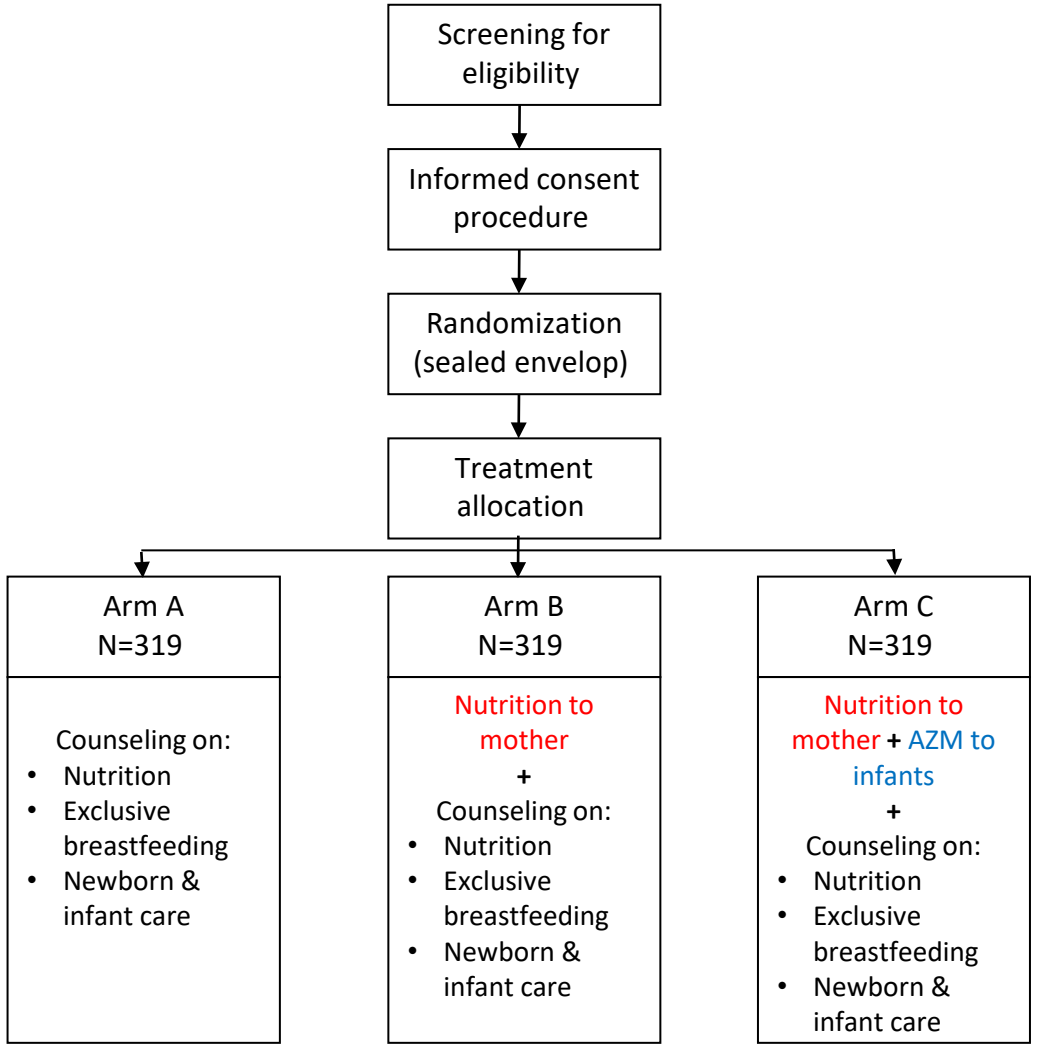
Laboratory Team
Dr. Najeeha Iqbal
Aneeta Hotwani
Furqan Kabir

Start date
August 1, 2018
Enrollment completion
May 19, 2020
Last follow-up
Expected on November 13, 2020

METHODOLOGY

Open-labelled, community-based randomized controlled trial (blinded at outcome assessment) – enrolling mother within first week of birth having MUAC < 23.0

Trial design and procedures



Interventions

Balanced energy-protein supplement to LW

Locally produced, ready-to-use (Protein source: Chickpea based, peanuts, & skimmed milk)

10.5 gram protein and 400 Kcal/ per 75 gram sachet

Dose of 2 sachets per days to women until 180 days, starts from day of enrollment

Azithromycin (AZM) to infant

Suspension

20 mg/Kg single dose at day 42

TRIAL PROCEDURE

After enrollment, all mothers and infants are being followed until 180 days

Follow-up schedule

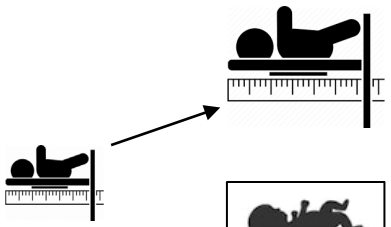
Newborn/infant assessment for danger sign

&

Breastfeeding recall

Daily → Alternate → 72 hours → Weekly

Anthropometry



Maternal weight, MUAC, Height*

Infant's weight, MUAC, Length & Head circumference

Baseline | Day 27 | Day 56 | Day 85 | Day 114 | Day 143 | Day 179

*At enrollment

Specimen collection

Schedule: Day 40-41, Day 56

Collection type

Infant blood (All infant): Hemoglobin (Hb), Transferrin, Ferritin, Proteomics, AGP & CRP

Subset cohort:
- Breast milk: Macro/ Micronutrients; HMOs / Microbiome, immunoglobulin
- Maternal blood: Hb, Ferritin, Transferrin, AGP, CRP
- Maternal & Infant stool: Microbiome, enteropathogens, B.Infantis, Calprotectin, Lipocalin-2, MPO

AIM OF STOOL BIOMARKERS ANALYSIS

Stool Biomarkers: Currently data is available on n=80 participants

1 Observe differences between maternal and infant biomarkers

To understand differences in baseline state between maternal and infant biomarkers.

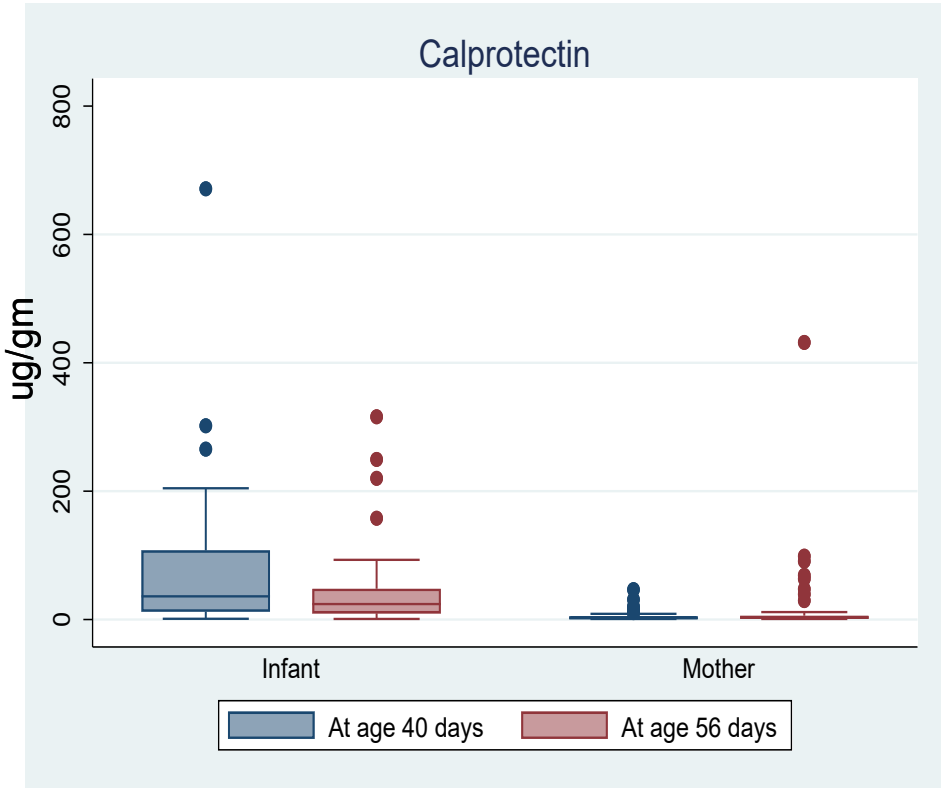
2 Determine which biomarkers associate with good clinical outcomes

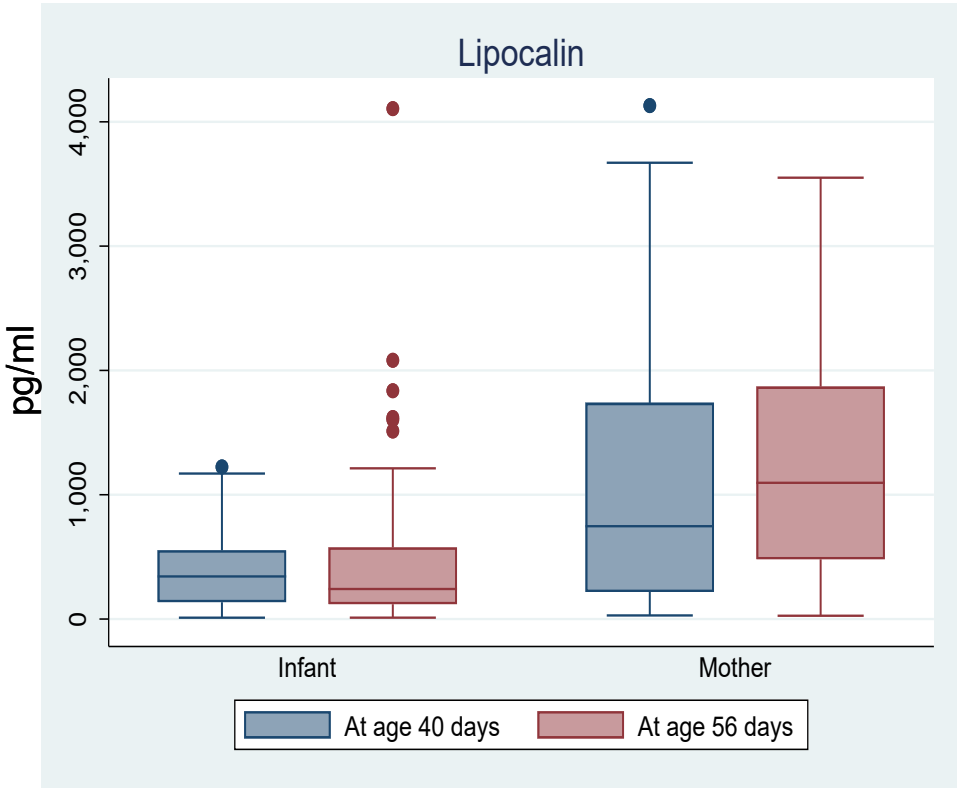
These include both maternal (anthropometric status, breast milk composition) status and pediatric (growth and neurodevelopment) outcomes.

3 Determine the relationship between biomarkers and study arm

To understand association between biomarkers and any clinical effect observed by intervention arm.

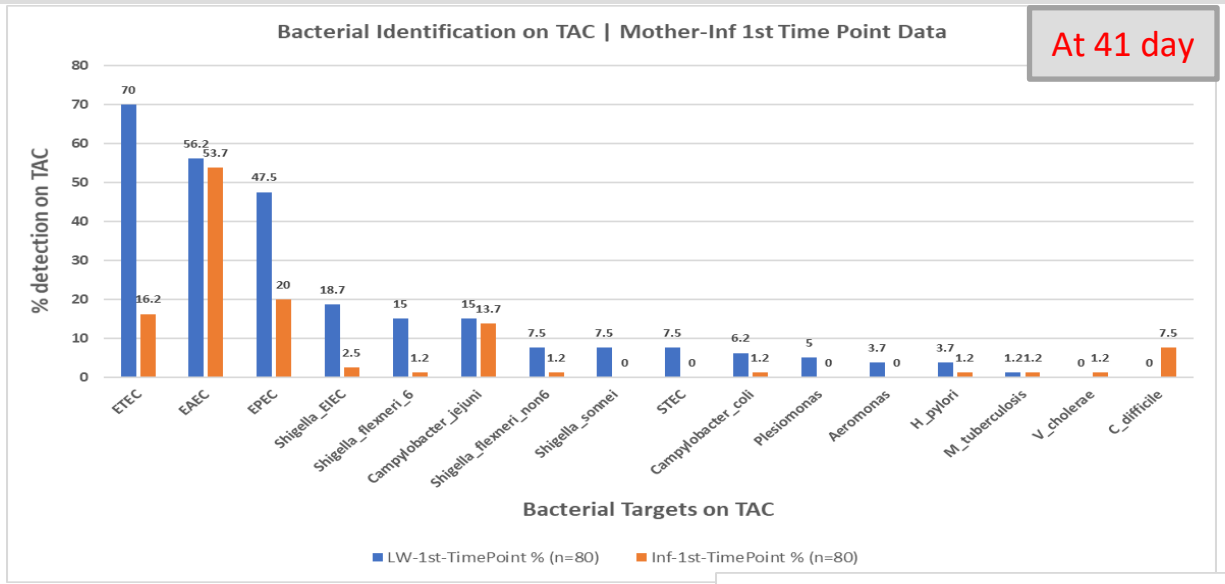
Comparison of fecal biomarkers in mothers and infants

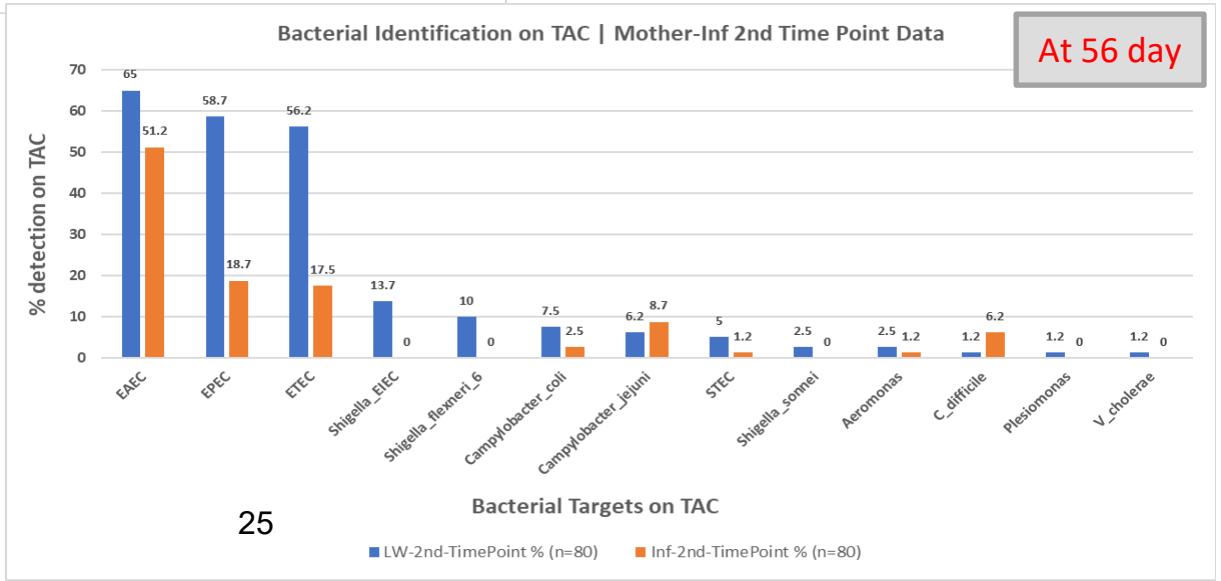




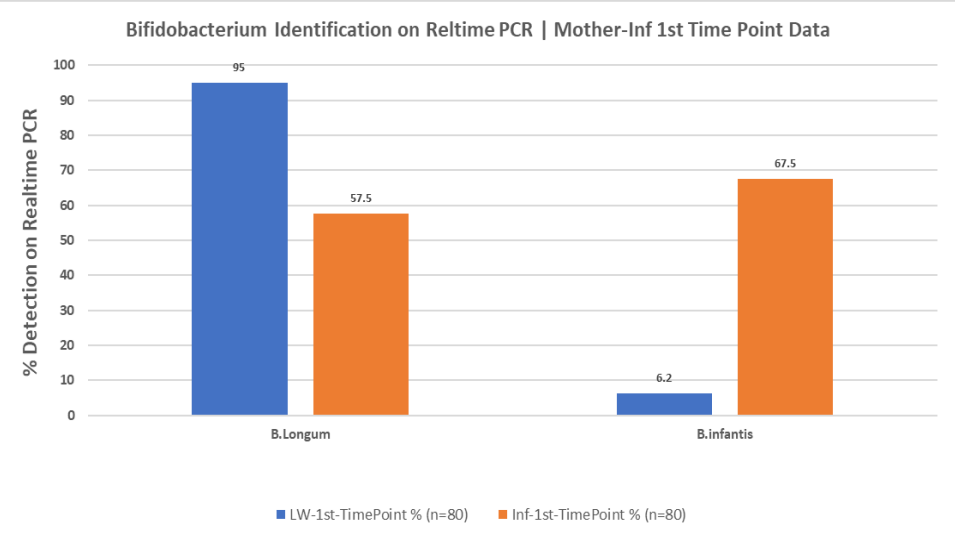
STOOL BIOMARKERS

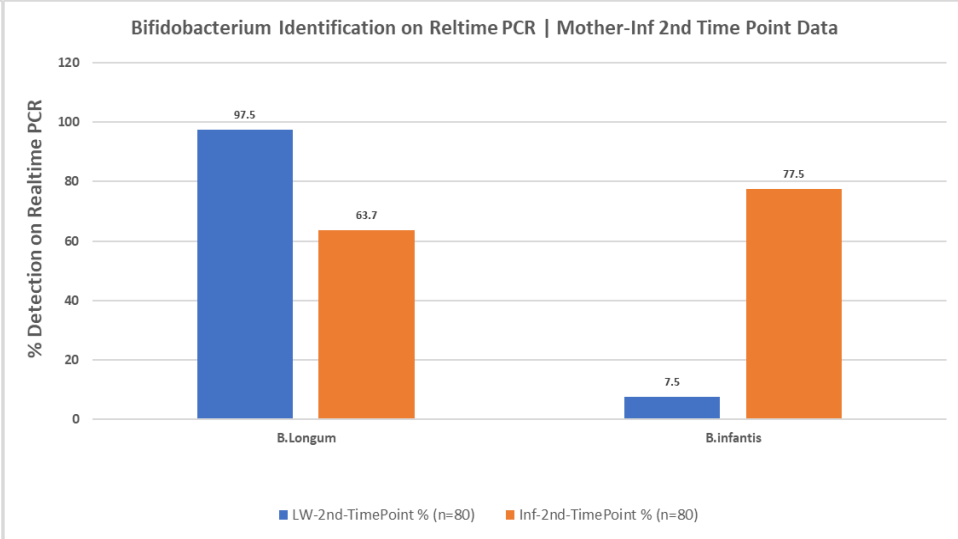
Bacterial' Panel Identification via TAC





Targeted Bifidobacterium' Identification via Realtime PCR





Metagenomic Analysis

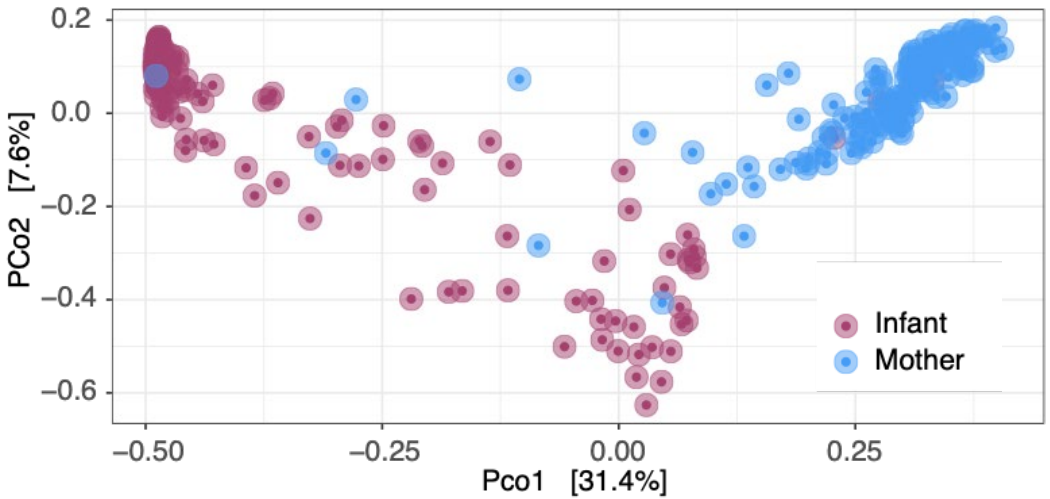


Figure 1. Principal Coordinate Analysis (PCoA) of mother and infant samples.

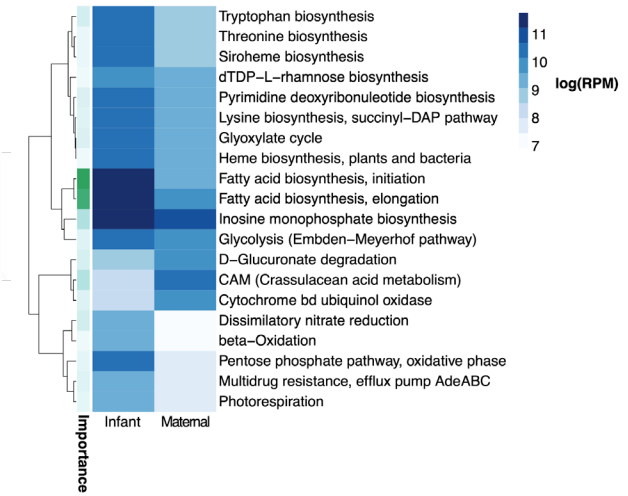


Figure 2. Machine learning highlights functions that distinguish infants across treatment arms.

Sonnenburg Lab

CONCLUSIONS & NEXT STEPS

- There are key differences in enteropathogen load, inflammatory markers, and microbiome profiles in mother infant dyads
- Future analysis will focus on determining which maternal biomarkers predict good clinical outcomes in infants (growth and neurodevelopment) and associate with better maternal health (anthropometric status and breast milk composition)
- Future analysis will also focus on arm wise analysis to determine which markers association with intervention
- These analysis will consist of a hypothesis-driven approach using multiple linear regressions as well a hypothesis-agnostic approach using supervised machine learning

THANKS

Grand Challenges: Preserving Culture

Ravi Sheth, PhD

Hertz-Gates Fellow (2018)

Fermentation is an ancient practice deeply intertwined with human biology & culture

Nearly all iconic foods are fermented & fermentation is pervasive across human cultures

1+1=3

Raw foods + microbes = something entirely new

Human ancestors predicted to adapt to fermentation 10M years ago[1]

1. Carrigan, PNAS 2014

Microbes can improve the qualities of food across multiple distinct axes

Improve the preservation and stability of foods by excluding pathogens (through lowering pH, bacteriocin production, removing simple sugars) [1]

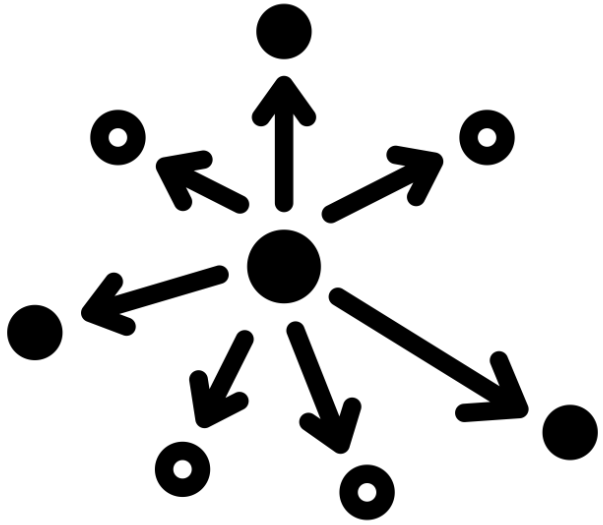
Improve macro- and micro-nutrient quality and bioavailability (e.g., B vitamins) [2]

Remove anti-nutrients (mycotoxins; phytates, which decrease iron availability) [3]

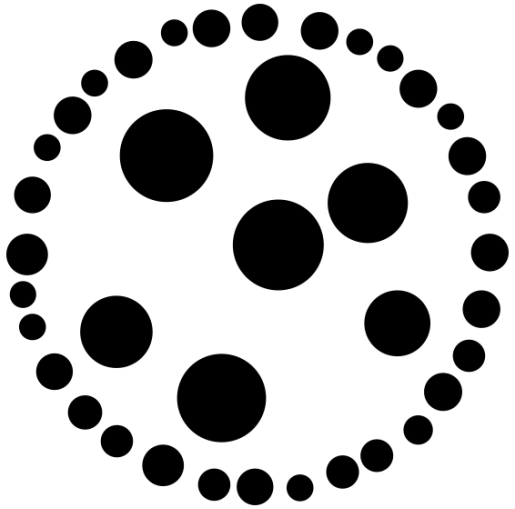
Transform taste, flavor and texture [4]

1. De Vuyst L, J Mol Microbiol Biotechnol 2007; 2. Walther Advances in Nutrition 2013;
3. Reddy Food Research International 1994; 4. Marshall Int. Journ. Food Sci Tech 2001

COVID-19 reveals strains on centralized food processing driven by chemistry



Centralized chemical-driven
food processing supply chains



Decentralized, distributed supply chains
uniquely enabled by scalable biology

Most traditional fermentation processes remain uncharacterized with modern tools

Salt & lactic acid bacteria based fermentations

Fungal fermentations

Many fermentation processes are not considered fermentation!

The challenge

Beyond many of the well-known examples of microbial fermentation, the vast majority of fermentation processes around the world remain uncharacterized and their potential human health benefits are unknown.

These ancient practices may hold the key to impactful and locally targeted nutritional interventions that combine tradition and science to tackle malnutrition.

Rigorous scientific evaluation has been limited and characterization to understand potential benefits could be pursued to validate and underscore the importance of preserving this cultural heritage.

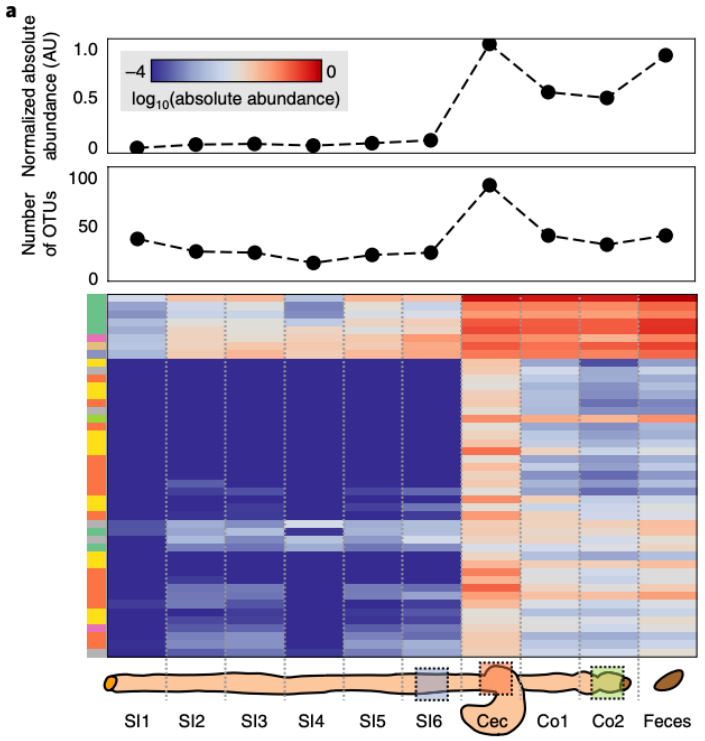
Grand Challenges Call

This call seeks to fund pilot studies that investigate the biological effect of traditional locally fermented foods on key microbiome, gut, and health biomarkers in local populations. The goal is to **provide investigators in Sub-Saharan Africa and South Asia with the resources to build local capacity to investigate fermented foods as novel maternal nutrition interventions**. Ultimately, the goal is to empower local communities to develop geography and culture specific interventions powered by fermentation, in country.

Identification of a local (geographic/cultural) fermented food for study	Pilot study design for longitudinal intervention study for understanding the effect of the fermented food in a naïve (no, or limited, fermented food consumption) population (women of reproductive age)	Biological sample biobanking and characterization before and after food intervention

Sequencing as an democratizing & enabling scientific tool

A microscope for delineating and measuring microbes



Identifying and studying microbes at unprecedented resolution

Sheth, Nature Biotech 2019

جامعة نيويورك أبوظبي
NYU | ABU DHABI

AASHISH R JHA, PHD
ASSISTANT PROFESSOR OF BIOLOGY
NEW YORK UNIVERSITY ABU DHABI

DEVELOPING GENOMICS CAPACITY COLLABORATIVE NETWORK & RESEARCH

DIVERSITY OF FERMENTED FOODS

Fermented grains	Fermented vegetables	Cultured dairy










DIVERSITY OF FERMENTED FOODS

Fermented grains	Fermented vegetables	Cultured dairy










How do we sequence microbiota of diverse food types?

STANDARDIZING MICROBIOMICS OF FERMENTED FOODS

Understanding

- Comprehensive surveys
- Culturally sensitive
- Modes of consumption
- Optimal uses

Sequencing

- 16S and/or ITS
- Marker gene region
- Sequencing platforms
- Sequencing depth

Continuation

- Metagenomics
- Metabolomics
- Metaproteomics
- Strain isolations
- Cytokine responses
- Other health effects
- Nutritional trials
- Other research
- Commercialization

Sampling & Processing

- Collection methods
- Sample storage
- Extraction methods

Data analysis

- Reference libraries
- Statistics
- Machine learning

COLLABORATION NETWORK FOR GENOMICS CAPACITY

Stanford

NYUAD

- Develop methods
- Optimize methods
- Create SOPs

Awardee

- Continuation
- Future studies

- Share SOPs
- Virtual trainings
- Support continuation
- Assist in future studies

SUMMARY

- Harmonizing sample collection, processing, sequencing, and data analysis allows us to integrate data across experiments and laboratories.
- Stanford and NYUAD will develop standard operating protocols (SOPs).
- NYUAD will conduct virtual trainings to assist awardees in project design, sampling, sample processing, amplicon sequencing, and data analysis in-country.
- Awardees can develop future collaborative projects with each other, Stanford, and NYUAD.

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THANK YOU!