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

October 21, 2020

Srivalli Krishnan
Bill & Melinda Gates Foundation
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

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

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

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

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

- Research and Discovery for India/Asia

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

- Improving access to products and services

- Learning and Evaluation

- Partnerships to scale
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
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!
“Let food by thy medicine” - Hippocrates (400 BC)
NEXT GENERATION FOODS & MICROBIAL INTERVENTIONS

Ultra-Processed & Bland Foods

- Malabsorption: Surface area & leaky gut
- Maldigestion: Missing microbes & SCFA calories from fiber

Fermented Foods & Microbiome Enriching Interventions

- Inflammation: Bacterial translocation, immune activation & energy
- Inefficiency: Missing microbes & vitamins for healthy metabolism

SCFA: Short Chain Fatty Acids

Growth
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?
Participants increased fermented foods intake and microbiome diversity.
Fermented food had an indirect effect on microbiota diversity

<table>
<thead>
<tr>
<th>Food</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt</td>
<td>Streptococcus mitis, Streptococcus pneumoniae,</td>
</tr>
<tr>
<td></td>
<td>Streptococcus salivarius thermophilus, Paenibacillus lactis</td>
</tr>
<tr>
<td></td>
<td>Micrococcus luteus, Bifidobacterium animalis,</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus rhamnosus, Lactobacillus delbrueckii,</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus paracasei, Lactobacillus rhamnosus,</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus plantarum</td>
</tr>
<tr>
<td>Kefir</td>
<td>Lactobacillus rhamnosus, Lactobacillus paracasei,</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus delbrueckii, Lactococcus lactis</td>
</tr>
<tr>
<td>Sauerkraut</td>
<td>Lactobacillus plantarum, Micrococcus luteus,</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus paracasei</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>Lactobacillus lactis, Lactobacillus paracasei</td>
</tr>
<tr>
<td>Kimchi</td>
<td>Bacillus pumilus, Lactobacillus sakei, Lactobacillus</td>
</tr>
<tr>
<td></td>
<td>curvatus</td>
</tr>
<tr>
<td>Kombucha</td>
<td>Paenibacillus lactis, Lactobacillus brevis</td>
</tr>
<tr>
<td>Gut Shots</td>
<td>Lactobacillus plantarum, Lactobacillus paraplastarum</td>
</tr>
</tbody>
</table>

New ASVs pooled across cohort

- Found in food (biotype)
- Not found in food
Fermented food consumption decreased inflammatory cytokines and signaling.

cytokines/chemokines

Inflammatory signaling

Decreased ➔ Increased

Classical monocytes
CD8+ T cells
CD8+ T cells
B cells

Immune Signaling Protein

Arcsinh ratio from Week -3 to Week 10
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
Open-labelled, community-based randomized controlled trial (blinded at outcome assessment) – enrolling mother within first week of birth having MUAC < 23.0

**METHODOLOGY**

**Screening for eligibility**

**Informed consent procedure**

**Randomization (sealed envelop)**

**Treatment allocation**

**Trial design and procedures**

- **Arm A**
  - N=319
  - Counseling on: Nutrition, Exclusive breastfeeding, Newborn & infant care

- **Arm B**
  - N=319
  - Nutrition to mother + Counseling on: Nutrition, Exclusive breastfeeding, Newborn & infant care

- **Arm C**
  - N=319
  - Nutrition to mother + AZM to infants + Counseling on: Nutrition, Exclusive breastfeeding, Newborn & infant care

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

**Follow-up schedule**

Newborn/infant assessment for danger sign & Breastfeeding recall

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>Infant's weight, MUAC, Length &amp; Head circumference</td>
</tr>
<tr>
<td>Alternate</td>
<td>Maternal weight, MUAC, Height*</td>
</tr>
<tr>
<td>72 hours</td>
<td>INFANT blood (All infants)</td>
</tr>
<tr>
<td>Weekly</td>
<td>Hb, Ferritin, Transferrin, AGP, CRP</td>
</tr>
</tbody>
</table>

**Anthropometry**

- Maternal weight, MUAC, Height*
- Infant's weight, MUAC, Length & Head circumference

**Specimen collection**

<table>
<thead>
<tr>
<th>Collection type</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant blood (All infants)</td>
<td>Day 40-41, Day 56</td>
</tr>
<tr>
<td>Maternal blood</td>
<td>Hb, Ferritin, Transferrin, AGP, CRP</td>
</tr>
<tr>
<td>Maternal &amp; Infant stool</td>
<td>Subset cohort, HMOs / Microbiome, immunoglobulin</td>
</tr>
<tr>
<td>Breast milk</td>
<td>Macro/ Micronutrients</td>
</tr>
<tr>
<td>Subset cohort</td>
<td>Microbiome, enteropathogens, B. Infantis, Calprotectin, Lipocalin-2, MPO</td>
</tr>
</tbody>
</table>

*At enrollment
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**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Infant</th>
<th>Mother</th>
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<tbody>
<tr>
<td>Calprotectin</td>
<td>24 pg/ml</td>
<td>24 pg/ml</td>
</tr>
<tr>
<td>Lipocalin</td>
<td>1,000 pg/ml</td>
<td>1,000 pg/ml</td>
</tr>
</tbody>
</table>

- **Calprotectin** concentration in infants and mothers at ages 40 and 56 days.
- **Lipocalin** concentration in infants and mothers at ages 40 and 56 days.
Bacterial’ Panel Identification via TAC

At 41 day

At 56 day
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
• 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.

Raw foods + microbes = something entirely new.

Human ancestors predicted to adapt to fermentation 10M years ago¹.

¹ Carrigan, PNAS 2014
Microbes can improve the qualities of food across multiple distinct axes

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

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

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

4. Transform taste, flavor and texture [4]

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 are considered fermentations, whereas fungal fermentations are a separate category. 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
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

<table>
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<th>Understanding</th>
<th>Sequencing</th>
<th>Continuation</th>
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<td>▸ Comprehensive surveys</td>
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<tr>
<td>▸ Culturally sensitive</td>
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<tr>
<td>▸ Modes of consumption</td>
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<td>▸ Optimal uses</td>
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<td>▸ Marker gene region</td>
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<tr>
<td>▸ Commercialization</td>
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### Sampling & Processing
- Collection methods
- Sample storage
- Extraction methods

### Data analysis
- Reference libraries
- Statistics
- Machine learning
COLLABORATION NETWORK FOR GENOMICS CAPACITY

- Develop methods
- Optimize methods
- Create SOPs

- 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.
THANK YOU!

AASHISH R JHA
TWITTER: @NEPALIAASHISH
EMAIL: JHAAR@NYU.EDU