Selectively Changing the Microbiome of the Rhizosphere

Karsten Zengler

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Co-founder **Isolation Bio** (San Carlos), developing high-throughput platform for **microbiome research**.

Co-founder **Native Microbials** (San Diego), developing microbial solutions for your **animals**.

Co-founder and SAB member **Allive Biosciences** (San Diego), improving health by **reducing inflammation**.

SAB member **DiscitisDX** (La Jolla), developing diagnostics for intervertebral **disc surgery**.

SAB member **Triton Algae Innovations** (San Diego), introducing new ingredients for **future foods**.

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**Former SAB member**

**Joyn Bio** (Boston)

**ProdermIQ** (San Diego)

**Syngip** (Vaals, The Netherlands)
Microbiome Sciences
Progress in Microbiome Research

(my personal view)

Why?

How?

What?

Who?

2025
Progress in Microbiome Research

**Why?**
...are they doing it?

**How?**
...are they doing it?

**What?**
...are they doing?

**Who?**
...is there?

Who else lives there?
Transplant organisms?
Build/create?
Predict?
Open Questions in Microbiome Research

...how do communities response to perturbations?
Open Questions in Microbiome Research

...how do communities response to perturbations?

...can we predict outcomes?
Open Questions in Microbiome Research

…how do communities respond to perturbations?

…can we predict outcomes?
Processes in the Rhizosphere
How do we study microbial communities?
**Microbiome Science Tools**

- **DNA**
- **mRNA**
- **Proteins**
- **Metabolites**

**Genomics**
- What is possible

**16S rRNA**
- Who is there

**Transcriptomics**
- What appears to be happening

**Proteomics**
- What makes it happen

**Metabolomics**
- What is happening

**% microbiome studies**
- (last 10 years, >68,000 articles)

- 16S rRNA (66%)
- Metagenomics (27%)
- Metatranscriptomics (2%)
- Metaproteomics (1%)
- Metabolomics (4%)
Microbiome science is mostly descriptive & correlation-based
Microbiome science is mostly descriptive & correlation-based

...often NOT predictive
Microbiome science is mostly descriptive & correlation-based

...establish causation and make it predictive!
What defines the phenotype?

Translation is the most expensive process in the cell.
What defines the phenotype?

- Genomics
- Transcriptomics
- Metabolomics
- Proteomics
- 16S rRNA
- Regulation
- DNA
- Proteins
- Metabolites
- TRANSLATION

Al-Bassam et al. Nature Communication 2018
What defines the phenotype?

- Genomics
- Transcriptomics
- Metabolomics
- Proteomics
- 16S rRNA
- Translational Efficiency
- Proteins
- Metabolites

Al-Bassam et al. *Nature Communication* 2018
What defines the phenotype?

- Genomics
- Transcriptomics
- Metabolomics
- Proteomics
- Translatomics (Ribonucleotide-Seq)
- 16S rRNA

Regulation

Translational Efficiency

Proteins

Metabolites

Al-Bassam et al. Nature Communication 2018
Translatomics – Ribo-Seq

Stop Translation \(\rightarrow\) Digest mRNA \(\rightarrow\) Sequence
Translational Efficiency (TE)

Translational Efficiency (TE) refers to how many mRNAs are being translated.

The cell controls its phenotype through translational efficiency (resource allocation).
Resource Allocation

Resource allocation defines a person’s preferences
Resource Allocation

- Amino Acid Synthesis
- Division
- Sugars
- DNA
- RNA
- N-fix
- Fatty Acid Metabolism
- Motility

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Resource allocation defines a cell’s preferences.
TE in Communities

Translational Efficiency

\[ TE = \frac{\text{MetaRibo-Seq}}{\text{MetaRNA-Seq}} \]

Microbes Preferences

Functional microbiome classification (Guilds)

Control – Change – Rational Design
Method Validation
### Synthetic Community (SynCom)

#### 16 strains
Isolated from switchgrass rhizosphere

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Strain</th>
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<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysobacter</td>
<td>OAE881</td>
<td>Bosea</td>
<td>OAE506</td>
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<tr>
<td>Burkholderia</td>
<td>OAS925</td>
<td>Methylobacterium</td>
<td>OAE515</td>
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<td>Variovorax</td>
<td>OAS795</td>
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<td>OAP107</td>
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<td>OAE908</td>
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<td>OAS944</td>
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<td>OAS809</td>
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<td>Mucilaginibacter</td>
<td>OAE612</td>
<td>Marmorica</td>
<td>OAE513</td>
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<td>OAE497</td>
<td>Brevibacillus</td>
<td>OAP136</td>
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<tr>
<td>Bradyrhizobium</td>
<td>OAE829</td>
<td>Paenibacillus</td>
<td>OAE614</td>
</tr>
</tbody>
</table>
Experimental Design

16 strains SynCom

0.1X R2A medium
7 days, 30 °C
4 biological replicates

Multi-omics

Metagenomics
Metatranscriptomics
Metatranslatomics
➢ **Excellent reproducibility within -omics**

➢ **Distinct profiles between -omics**
Guilds and Microbial Niche Determination (MiND)
16 strains, 275 metabolic pathways (KEGG)
Average 4 replicates

TE = \frac{\text{MetaRibo-Seq}}{\text{MetaRNA-Seq}}
Guilds vs Phylogeny

Guilds based on TE

- Arthrobacter
- Brevibacillus
- Lysobacter
- Burkholderia
- Chitinophagaceae
- Rhodococcus
- Variovorax
- Bradyrhizobium
- Rhizobium
- Methylobacterium
- Bosea
- Chitinophaga
- Mucilaginibacter
- Paenibacillus

Phylogenetic tree based on 16S rRNA

- Burkholderia
- Variovorax
- Lysobacter
- Methylobacterium
- Bosea
- Bradyrhizobium
- Rhizobium
- Rhodococcus
- Mycobacterium
- Marmoricola
- Arthrobacter
- Paenibacillus
- Brevibacillus
- Chitinophagaceae
- Mucilaginibacter
- Chitinophaga

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Guilds vs Phylogeny

**TE**
- Brevibacillus
- Arthrobacter
- Lysobacter
- Niastella
- Burkholderia
- Variovorax
- Rhodococcus
- Bradyrhizobium
- Rhizobium
- Methylobacterium
- Bosea
- Mucilaginibacter
- Chitinophaga
- Paenibacillus

**Ribo-Seq**
- Marmoricola
- Bosea
- Methylobacterium
- Bradyrhizobium
- Variovorax
- Rhodococcus
- Brevibacillus
- Rhizobium
- Paenibacillus
- Niastella
- Mucilaginibacter

**RNA-Seq**
- Mucilaginibacter
- Niastella
- Chitinophaga
- Brevibacillus
- Lysozyme
- Bradyrhizobium
- Rhizobium
- Paenibacillus

**Genome Presence/absence**
- Brevibacillus
- Paenibacillus
- Arthrobacter
- Marmoricola
- Mycobacterium
- Rhodococcus
- Chitinophaga

**16S sequences**
- Burkholderia
- Variovorax
- Lysobacter
- Methylobacterium
- Bosea
- Bradyrhizobium
- Rhizobium
- Rhodococcus
- Mycobacterium
- Marmoricola
- Arthrobacter
- Paenibacillus
- Niastella
- Mucilaginibacter
- Chitinophaga

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Pathway Prioritization: SynCom vs. Axenic Culture

Organisms prioritize different pathways when in a community!
Can guilds predict intervention outcomes?
Modifying community composition
Response to the Removal of Microbes

Metabolic Guilds based on TE

- Mucilaginibacter
Response to the Removal of Microbes

Metabolic Guilds
based on TE

- Mucilaginibacter
Calculating Competition Score
Response to the Removal of Microbes

- Single-strain dropout
- Accurately predicted competitor increase

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Response to the Removal of Microbes
Response to the Removal of Microbes

- Single-strain dropout
- 7 days
- Accurately predicted competitor increase

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### Detection of competition:

100% sensitivity
74% specificity
Hypothesis

Bacteria in the Same Guild are Competitors
Antimicrobials

- Beta-Lactam resistance
- Multidrug resistance transporter
- Multidrug resistance efflux pump
- Rax Type 1 secretion system
- RTX toxin transport system
- Antimicrobial peptide resistance
Antimicrobials

a) Spread plate

- Burkholderia
- Chitinophaga
- Muclaginibacter
- Rhizobium

x16 (all SynCom strains)

b) Spot-on-lawn

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Antimicrobials

E = Chitinophaga
- Modifying community composition
PROBIOTIC INTERVENTION

ADDITION
Response to the Addition of Microbes

Burkholderia/Rhizobium

- Brevibacillus
- Arthrobacter
- Lysobacter
- Niastella
- Burkholderia
- Variovorax
- Rhodococcus
- Bradyrhizobium
- Rhizobium
- Methylobacterium
- Bosea
- Mucilaginibacter
- Chitinophaga
- Paenibacillus

- Arthrobacter
- Bosea
- Bradyrhizobium
- Brevibacillus
- Burkholderia
- Chitinophaga
- Lysobacter
- Marmorica
- Methylobacterium
- Mucilaginibacter
- Mycobacterium
- Niastella
- Paenibacillus
- Rhizobium
- Rhizobium
- Rhodococcus
- Variovorax
Response to the Addition of Microbes

Burkholderia/ Rhizobium

- Brevibacillus
- Arthrobacter
- Lysobacter
- Niastella
- Burkholderia
- Variovorax
- Rhodococcus
- Bradyrhizobium
- Rhizobium
- Methylobacterium
- Bosea
- Mucilaginibacter
- Chitinophaga
- Paenibacillus

SynCom

- Arthrobacter
- Bosea
- Bradyrhizobium
- Brevibacillus
- Burkholderia
- Lysoberacter
- Marmoricola
- Methylobacterium
- Mucilaginibacter
- Mycobacterium
- Niastella
- Paenibacillus
- Rhizobium
- Rhodococcus
- Variovorax
Response to the Addition of Microbes

Burkholderia/Rhizobium

- **Brevibacillus**
  - **Arthrobacter**
  - **Lysobacter**
  - **Niastella**
  - **Burkholderia**
  - **Variovorax**
  - **Rhodococcus**
  - **Bradyrhizobium**
  - **Rhizobium**
  - **Methylobacterium**
  - **Bosea**
  - **Mucilaginibacter**
  - **Chitinophaga**
  - **Paenibacillus**
Response to the Addition of Microbes

Mucilaginibacter/Chitinophaga

- Brevibacillus
- Arthrobacter
- Lysobacter
- Niaastella
- Burkholderia
- Variovorax
- Rhodococcus
- Bradyrhizobium
- Rhizobium
- Methylobacterium
- Bosea
- Mucilaginibacter
- Chitinophaga
- Paenibacillus

Arthrobacter
Bosea
Bradyrhizobium
Brevibacillus
Burkholderia
Lysobacter
Marmoricola
Methylobacterium
Mucilaginibacter
Niaastella
Mycobacterium
Niastella
Paenibacillus
Rhizobium
Rhodococcus
Variovorax
PROBIOTIC INTERVENTION

✓ Modifying community composition
✓ Modifying community composition

Adding metabolites
PREBIOTIC INTERVENTION

Adding metabolites
PREBIOTIC INTERVENTION

Adding metabolites
PREBIOTIC INTERVENTION

Adding metabolites
Importer Proteins

88 in metaG data
Microbial Niche Determination (MiND)

1. Microbial niches - Predict substrate preferences

a. TE for ribose import proteins

Brevibacillus, Arthrobacter, Lysobacter, Nostella, Burkholderia, Variovorax, Rhodococcus, Bradyrhizobium, Rhizobium, Methylobacterium, Bonea, Microbacterium, Chitinophaga, Paenibacillus, Mycobacterium

+ Ribose 5 g/L

3. Experimental validation: Prebiotic interventions

log(TE) 0 2 4 6
Microbial Niche Determination (MiND)
Microbial Niche Determination (MiND)

1. Microbial niches - Predict substrate preferences

2. Microbial guilds - Predict competition

3. Experimental validation: Prebiotic interventions
Microbial Niche Determination (MiND)

1. Microbial niches - Predict substrate preferences

a. TE for ribose import proteins

<table>
<thead>
<tr>
<th>Microbes</th>
<th>RbsA</th>
<th>RbsB</th>
<th>RbsC</th>
</tr>
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<tbody>
<tr>
<td>Brevibacillus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthrobacter</td>
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<td>Lysobacter</td>
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<tr>
<td>Niastella</td>
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<tr>
<td>Burkholderia</td>
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<tr>
<td>Variovorax</td>
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<tr>
<td>Rhizobium</td>
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<td></td>
<td></td>
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<tr>
<td>Methylbacterium</td>
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<td></td>
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<td>Bacea</td>
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<td>Chitinophaga</td>
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<tr>
<td>Paenibacillus</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Marmorcola</td>
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<td></td>
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</tr>
<tr>
<td>Mycobacterium</td>
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</tr>
</tbody>
</table>

2. Microbial guilds - Predict competition

d. Competition for Resources

3. Experimental validation: Prebiotic interventions

e. Ribose 5 g/L

h. Paenibacillus

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Microbial Niche Determination (MiND)

1. Microbial niches - Predict substrate preferences
   a. TE for ribose import proteins
      - RbsA
      - RbsB
      - RbsC
   b. TE for glutathione import protein
      - GsiA
   c. TE for putrescine import proteins
      - PuuP
      - PotA

2. Microbial guilds - Predict competition
   - Brevibacillus
   - Arthrobacter
   - Lysobacter
   - Burkholderia
   - Parvovorax
   - Rhodococcus
   - Bradyrhizobium
   - Rhizobium
   - Methylbacterium
   - Baseo
   - Mucilaginibacter
   - Chitinophaga
   - Paenibacillus
   - Marmoricola
   - Mycobacterium

3. Experimental validation: Prebiotic interventions
   e. Ribose 5 g/L
   - Control
   - Paenibacillus
   - Burkholderia
   f. Glutathione 1 mM
   - Control
   - Chitinophaga
   g. Putrescine 10 mM
   - Control
   - Rhodococcus
   - Paenibacillus
   h. Ribose (g/L)
   - Control
   - Burkholderia
   i. Glutathione (mM)
   - Control
   - Chitinophaga
   j. Putrescine (mM)
   - Control
   - Rhodococcus
   - Paenibacillus
Predicting Response to 11 Metabolites*

<table>
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<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Target:</strong></td>
<td>54%</td>
<td>83%</td>
<td>79%</td>
</tr>
<tr>
<td>(increase)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Secondary Target</strong></td>
<td>93%</td>
<td>65%</td>
<td>70%</td>
</tr>
<tr>
<td>(decrease)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fructose, Galactose, Ribose, Trehalose, Xylose, Maltodextrin, Glutamate, Glutathione, Putrescine, Spermidine, Sulfate+Thiosulfate
Axenic vs. Community Growth

very hard to predict!

12 isolates metabolize ribose axenically
5 try to grow with ribose in the community
2 isolates succeed
- Modifying composition (Probiotics)
- Adding metabolites (Prebiotics)
Complex → Complicated
SynCom

Complex

Soil

Complicated
Experimental Setup

Soil sampling

33 conditions tested
Probiotic consortium

- Probiotic
- Combinations

Soil sample in 0.1x R2A

Growth 7 days at 30°C
Soil

Fructose

Ribose

Soil + SynCom + Fructose 5 g/L (RPKM)

Soil + SynCom 5 g/L (RPKM)

SynCom strains:
- Arthrobacter
- Bosea
- Bradyrhizobium
- Brevibacillus
- Burkholderia
- Chitinophaga
- Methyllobacterium
- Mucilaginibacter
- Niastella
- Paenibacillus
- Rhizobium
- Rhodococcus
- Variovorax
Soil (only SynCom Members Shown)
## Targeted Interventions in Soil

<table>
<thead>
<tr>
<th>Intervention in soil</th>
<th>Total number of tested conditions</th>
<th>Number of conditions in which primary targets increased</th>
<th>Number of conditions in which secondary targets decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotic Single Strain</td>
<td>7</td>
<td>4/7 (57%)</td>
<td>4/4 (100%)</td>
</tr>
<tr>
<td>Prebiotic</td>
<td>7</td>
<td>6/7 (86%)</td>
<td>6/6 (100%)</td>
</tr>
<tr>
<td>Prebiotic + Probiotic Single Strain</td>
<td>10</td>
<td>8/10 (80%)</td>
<td>6/8 (75%)</td>
</tr>
<tr>
<td>Prebiotic + Probiotic Consortium</td>
<td>7</td>
<td>7/7 (100%)</td>
<td>7/7 (100%)</td>
</tr>
</tbody>
</table>
synCom in Soil  Soil
Guilds based on TE in soil
Guilds based on TE in soil

- **Clostridium**
- **Bosea**
- **Azospirillum**
- **Streptomyces**

Competition score against **Clostridium**: ▲
Soil – Substrate Addition

+ Ribose

- Substrate Addition

- Soil

+ Ribose 5 g/L (CPM)

- Soil Control (CPM)

- Clostridium

- Bosea

- Azospirillum

- Streptomyces

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Soil – Substrate Addition

- **+ Ribose**
  - **Soil Control (CPM)**
  - **+ Ribose 5 g/L (CPM)**

- **+ Putrescine**
  - **Soil Control (CPM)**
  - **+ Putrescine 10 mM (CPM)**

- **Clostridium**
- **Bosea**
- **Azospirillum**
- **Streptomyces**
Log-Fold Change Under Many Conditions

Azospirillium

Bosea

Streptomyces

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Log-Fold Change Under Many Conditions

**Non-Sugars**
(Glutathione, putrescine, sulfate/thiosulfate)

**Sugars**
(Fructose, ribose, maltose, trehalose)

![Graph showing log-fold change for different conditions and microorganisms]
Competition versus Collaboration

>19% of all interactions are based on collaboration

81% of all interactions were explained by competition
Open Questions in Microbiome Research

...how do communities respond to perturbations? ✓

...can we predict outcomes? ✓
Broadly Applicable Method

Complex

Complicated
Predicting Community Function
- Translational Efficiency
- Predicting Metabolic Niches and Guilds
- Identifying Interactions (Competition)
- Designing Interventions

Changing/Engineering Microbiomes
- Organism-Level, i.e. Probiotics
- Metabolite-Level, i.e. Prebiotics
- Scalable Technology, i.e. Soil, Stool

- Patent filed
Microbial Niche Determination (MiND) can predict outcomes in complex communities
Microbial Niche Determination (MiND) can predict outcomes in complex communities.

MiND and guild association identifies intervention strategies to selectively alter the microbiome.
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