



Microbiome

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Objectives

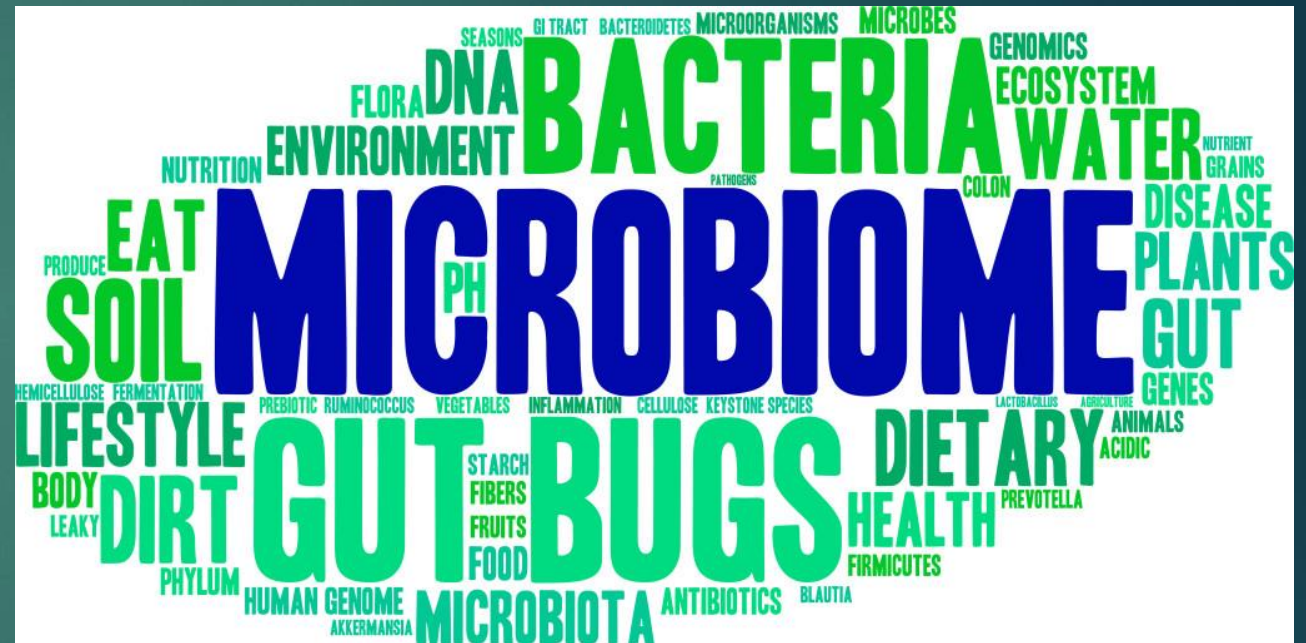
- ▶ Demonstrate an understanding of microbiomes and the relevance of microbiome studies
- ▶ Describe some methodologies utilized and result visualizations in microbiome research
- ▶ Explain basic techniques and conduntion of microbiome research

Microbiome Outline

- ▶ Applications to real world
- ▶ Human microbiome
- ▶ Methodologies and associated result reporting
- ▶ Insect microbiome and antimicrobial activity
 - ▶ Case study

Microbiome Relevance

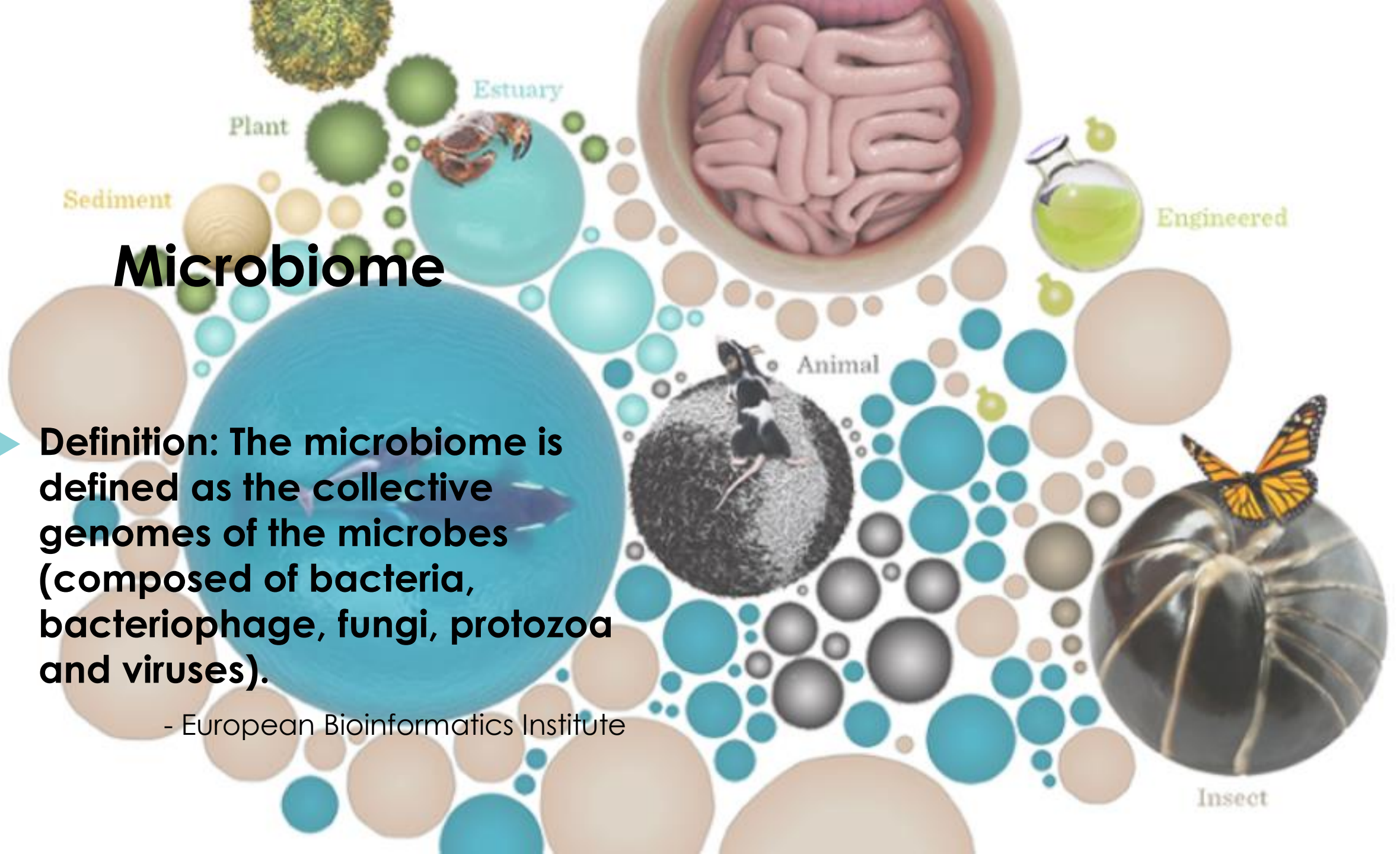
- ▶ Health
 - ▶ Personalized Medicine
 - ▶ Diet
 - ▶ Stress
 - ▶ Smoking and Drinking
 - ▶ Microbial Resistance
 - ▶ Fomite/Microbe Transmission
 - ▶ Transplant
- ▶ Forensic Science
 - ▶ Crime Scenes
- ▶ Environmental Studies



Microbiome

- **Definition: The microbiome is defined as the collective genomes of the microbes (composed of bacteria, bacteriophage, fungi, protozoa and viruses).**

- European Bioinformatics Institute



Human Microbiome Project (HMP) 2007: Findings



- ▶ # Microbial cells = 10 x Human cells
- ▶ # Microbial genes (8M) = Hundreds of times > Human genes
- ▶ Humans rely on microbes – housekeeping, ecosystem

HMP - metagenomics



- ▶ HMP- Collective genome sequences (NGS)
 - 3.5 Terabases metagenome sequence
 - Healthy metagenomes reference data
 - metabolic properties of healthy microbiomes
- ▶ Diversity (high/low) – Which is good, which is bad?
 - Depends on the sites
- ▶ Composition Vs Metabolic capabilities (MC) – Which is important?

Microbiome analysis

- ▶ Compare DNA in the samples – Healthy Vs diseased ones.
- ▶ Look for the differences, associations
- ▶ Make reasonable assumptions – What these genes do?

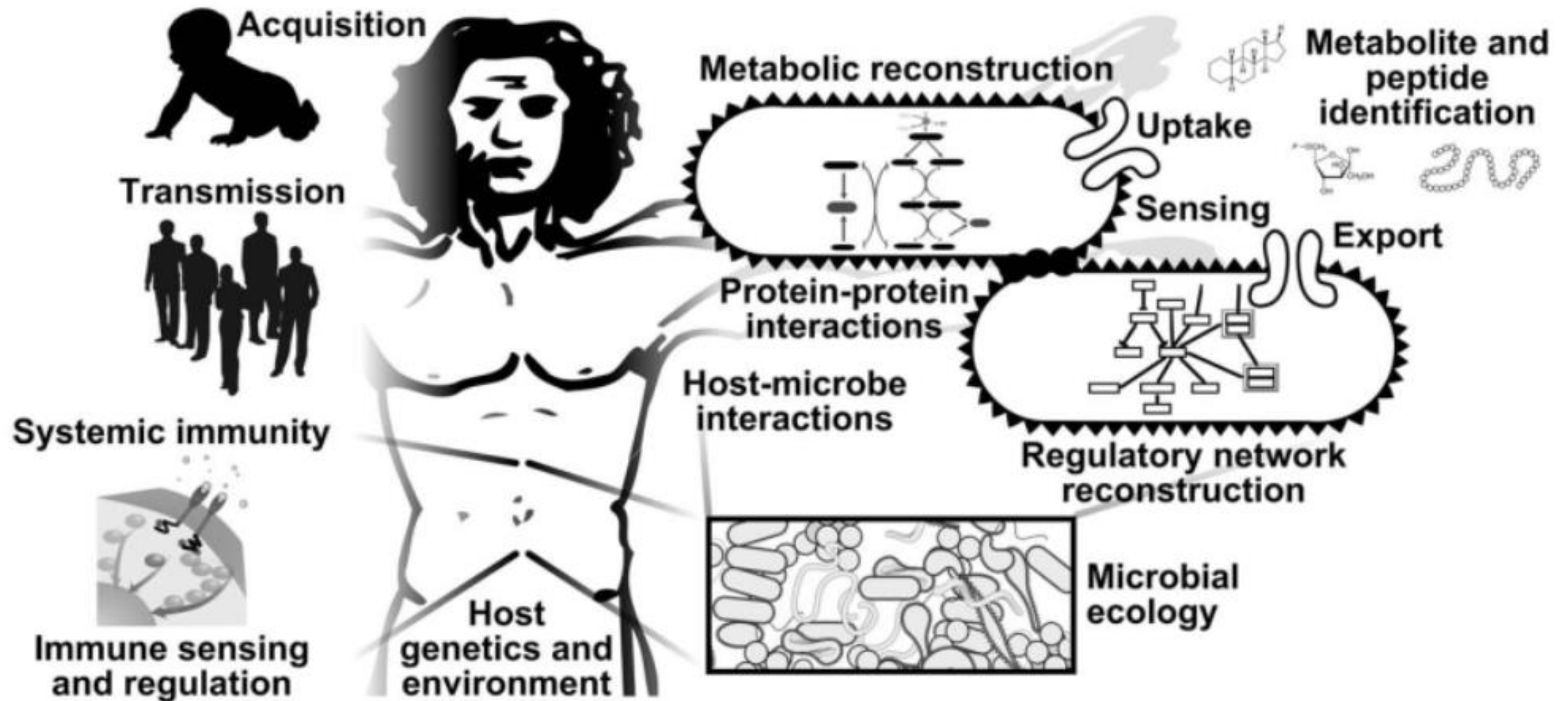


Figure: Topics in the study of the human microbiome with outstanding computational biology challenges.

(Gevers et.al., 2012)

Human microbiome analysis - continued

- ▶ Culture-independent analysis of the human microbiome using metagenomics approach
- ▶ Analysis:
 - i. specific marker genes
 - ii. shotgun sequencing (of the entire microbial community)
- ▶ Taxonomy profiling
 - i. reference sequences
 - ii. de novo clustering of the specific region of sequences
- ▶ Functional profiling
 - sequence similarity

(A) Taxonomy Profiling

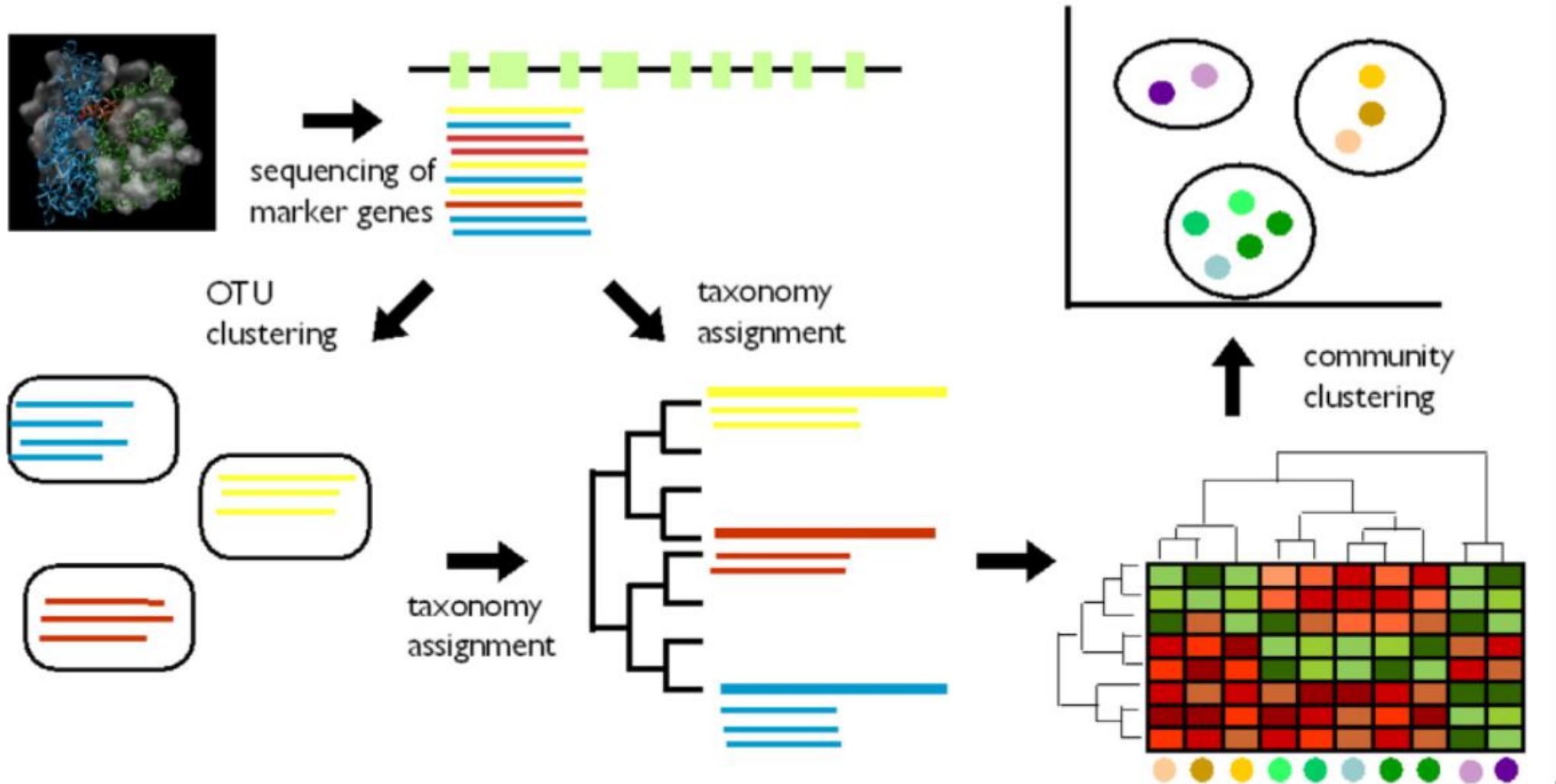
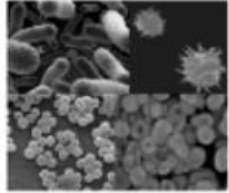


Figure: Frameworks for deciphering human microbiome based on bioinformatics approaches- (A) Taxonomy is profiling by using marker genes such as 16S ribosomal RNAs. (Kim 2015)

(B) Functional Profiling

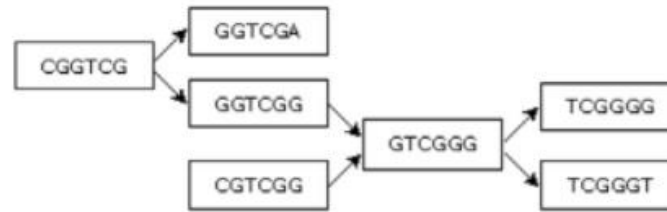
shotgun sequencing



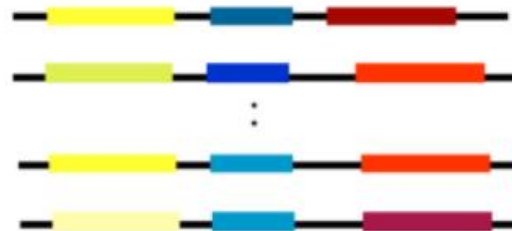
read mapping

de novo assembly

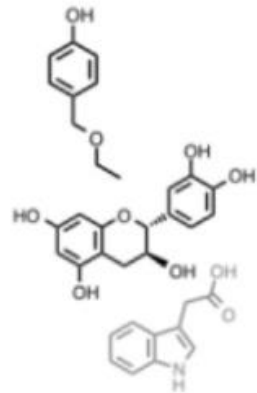
reference guided assembly



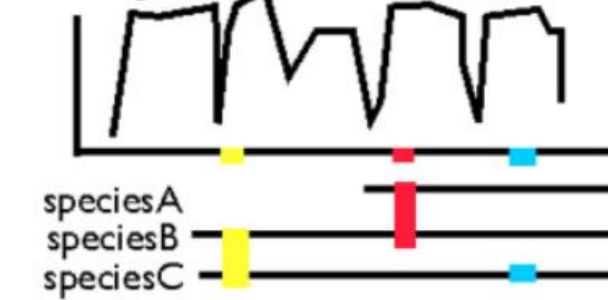
gene prediction



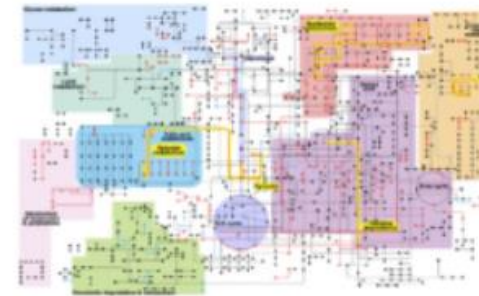
metabolite profiling



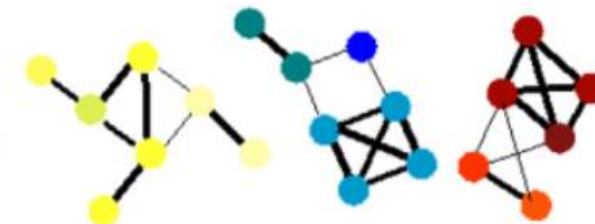
coverage



evolutionary analysis



metabolic profiling



network analysis

Figure: Frameworks for deciphering human microbiome based on bioinformatics approaches - (B) Functions that are enriched in the community are profiling by using predicted genes and read mapping on the protein reference databases. Assembly is carried out by using a de novo approach or a reference-guided approach. Predicted genes are clustered by using sequence similarity and gene annotation in order to infer their function (Kim 2015)

Ideologies



“Everything is everywhere, the environment selects”

-Lourens Baas Becking

"If we know what makes a microbiome function, we can figure out how to keep it healthy, or fix it when it's disrupted."

- Rob Finn

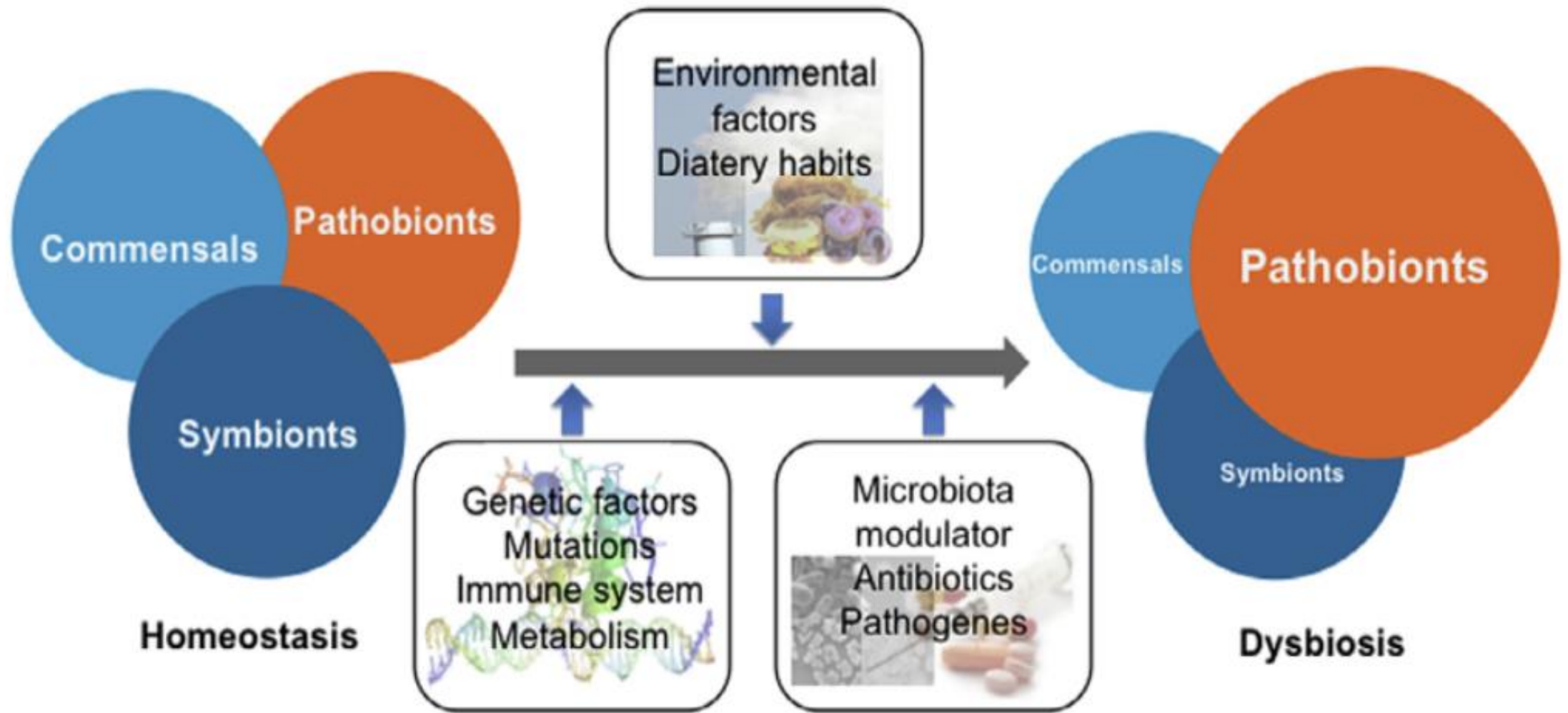


Figure: Changes of the microbial community composition. Environmental and genetic factors, and microbial modulators affect the homeostasis of human microbiome, which is associated with the development of several diseases

(Kim 2015)

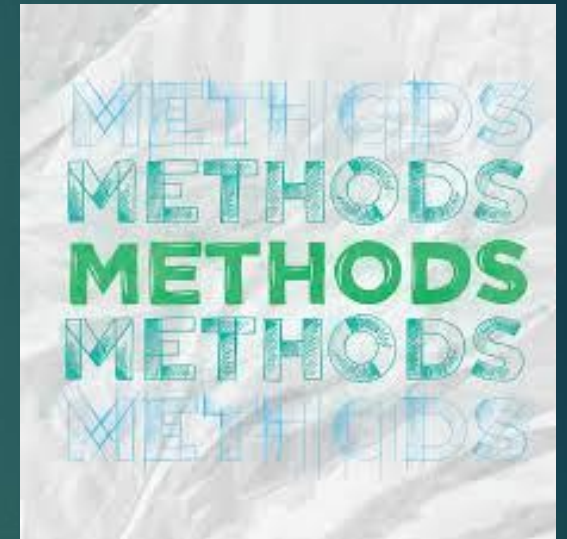
Health

- ▶ Diet: High protein → *Bacteroides* , High Carb. → *Prevotella*
- ▶ Obese vs Lean individuals: ↑ *Firmicutes*, ↓ *Bacteroidetes* in their gut microbiomes
- ▶ Enterotoxigenic *Bacteroides fragilis* (ETBF) triggers colitis and induces colonic tumors in multiple intestinal neoplasia (Min) mice. (Wu 2009)
- ▶ Fecal Transplant – for *C. difficile* infection
 - Problem- health care facilities over \$796 M
 - Antibiotic resistant
 - Role of microbiome – Rx: Fecal transplant



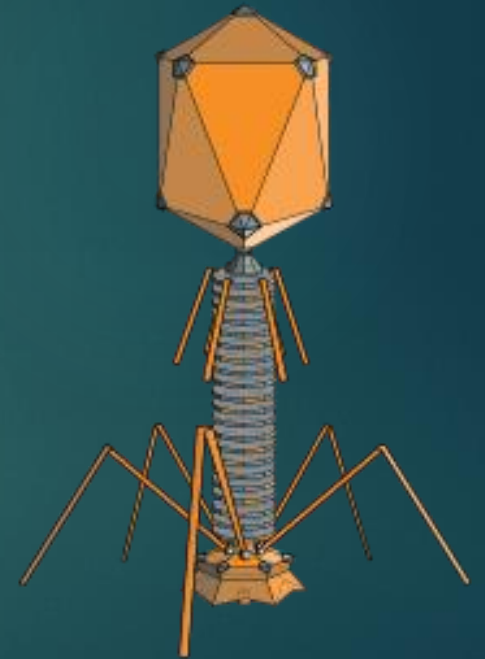
Methodologies

- ▶ Metagenomics
 - ▶ Sequence all nucleic acid
- ▶ Metatranscriptomics
 - ▶ Demonstrate transcripts
- ▶ Metaproteomics
 - ▶ Discover proteins (both host and microbe)
- ▶ Multi-omics approaches
 - ▶ Combination of methods to supplement data and results



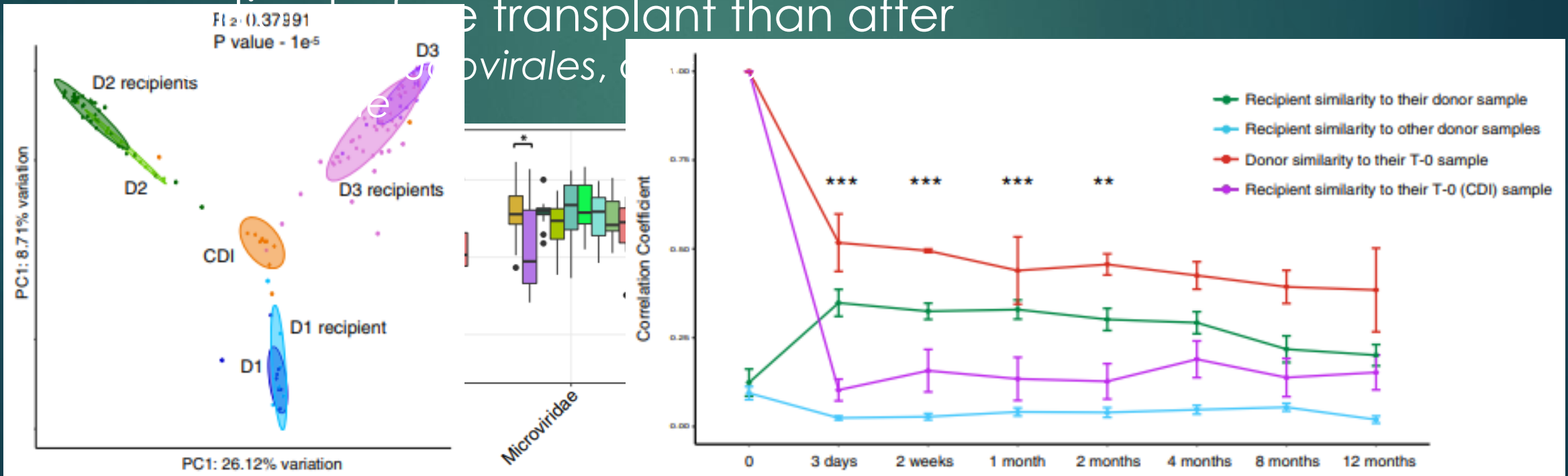
Methodologies: Metagenomic Approach

- ▶ Sequenced *Clostridium difficile* infection (CDI) fecal transplant recipients and donors looking at bacteriophage presence
 - ▶ Bacteriophage: viruses that infect bacteria
 - ▶ 14 recipient from 3 universal donors
 - ▶ Illumina pair-end reads
- ▶ Trimmed adapters and removed low quality reads
 - ▶ Cutadapt and Trimmomatic
- ▶ Classification of reads
 - ▶ Kaiju (uses amino acid similarity)
- ▶ Assembly of reads into contigs
 - ▶ MetaSPAdes



Methodologies: Metagenomic Approach

- Viromes of recipients (after transplant) similar to donor
 - Principal coordinate analysis
 - Persisted over next year
- Found that certain bacteriophages were present in higher



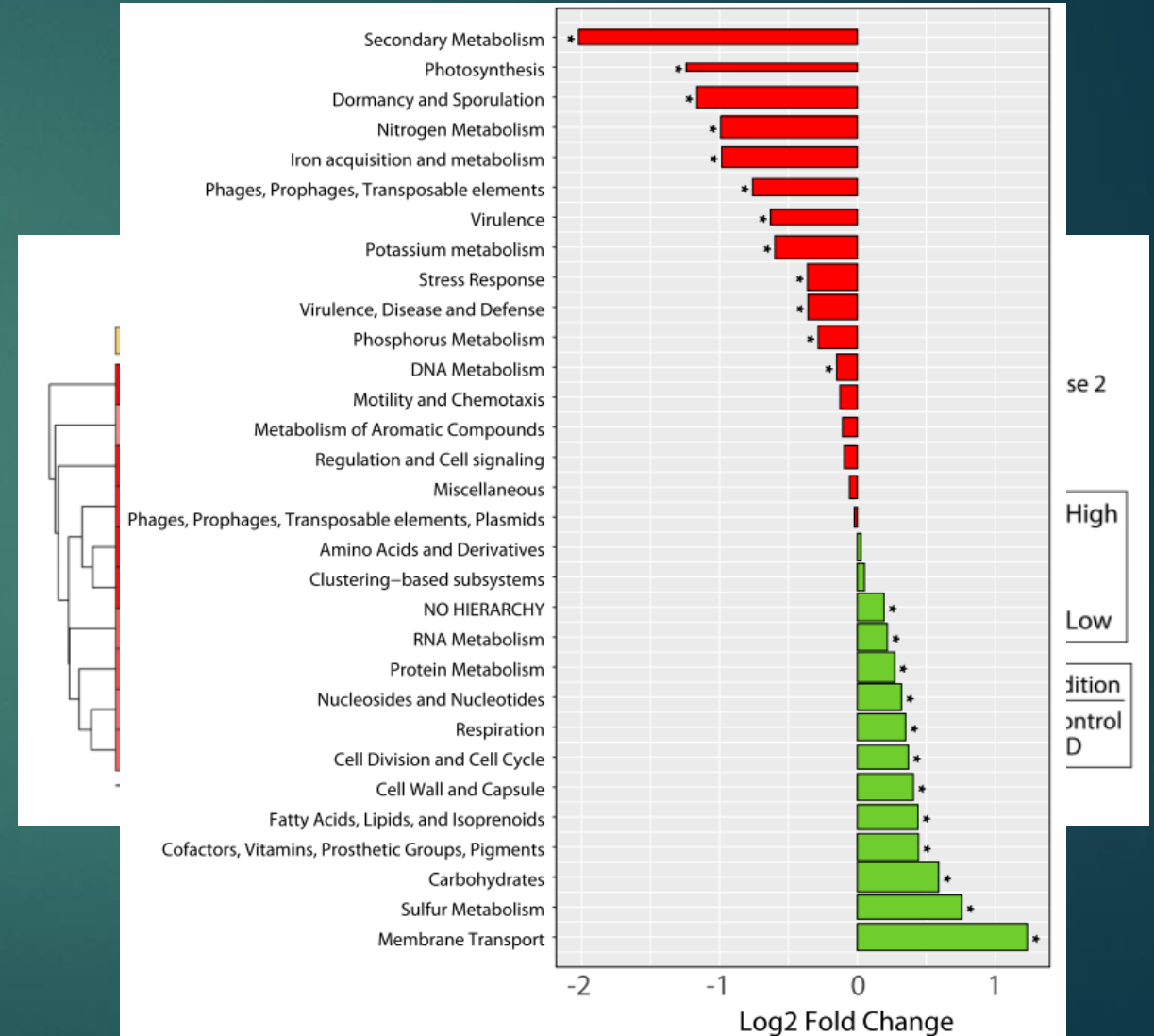
Methodologies: Metatranscriptomic Approach

- ▶ Sequenced RNA from 12 healthy and 12 idiopathic chronic diarrhea (ICD) macaques
 - ▶ Illumina pair-end reads
- ▶ Removed low quality reads followed by trimming and paired end alignment
 - ▶ SAMSA (unique for RNA-seq), Trimmomatic and PEAR
- ▶ Annotated reads through custom macaque transcript database
 - ▶ DIAMOND aligner

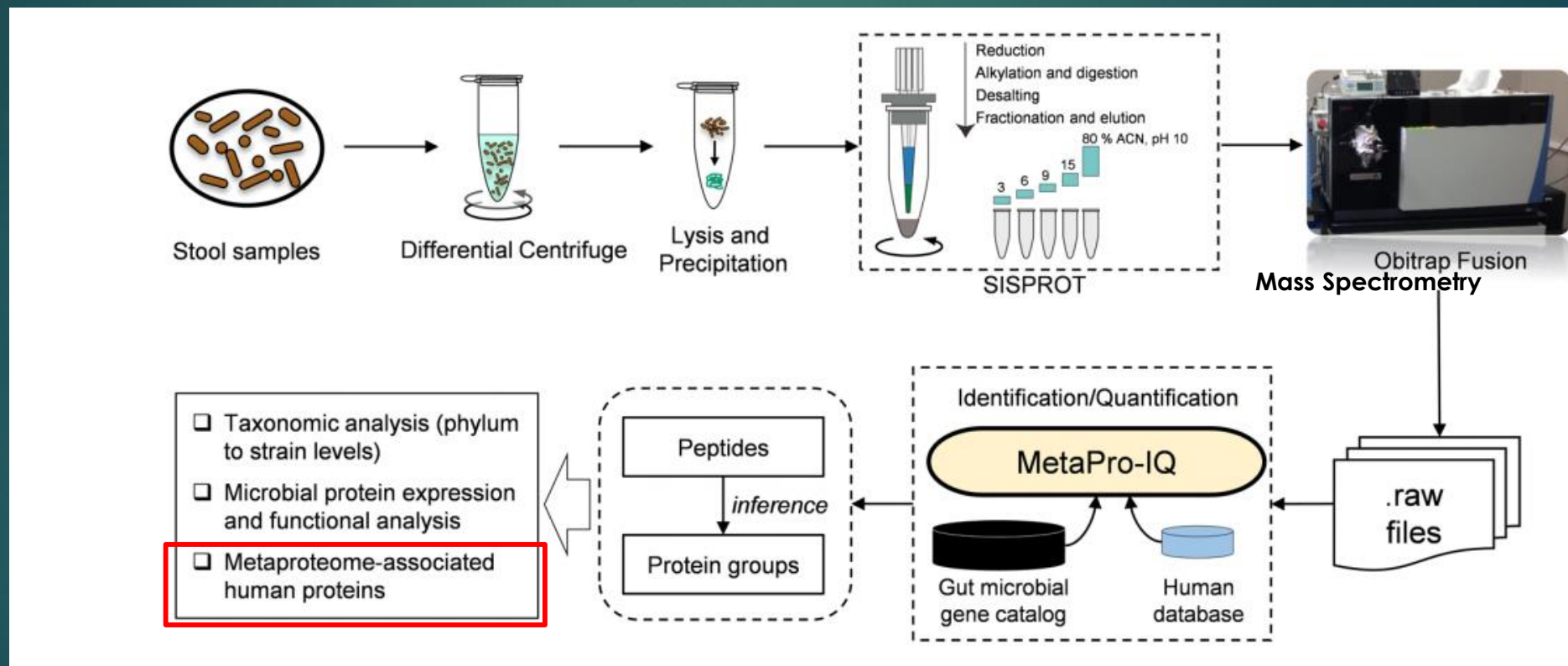


Methodologies: Metatranscriptomic Approach

- ▶ Higher level of certain transcripts in ICD macaques than control
 - ▶ Ten transcripts upregulated
 - ▶ All at least 32 times higher in ICD
- ▶ Transcripts involved in certain metabolic pathways demonstrated increased or decreased expression

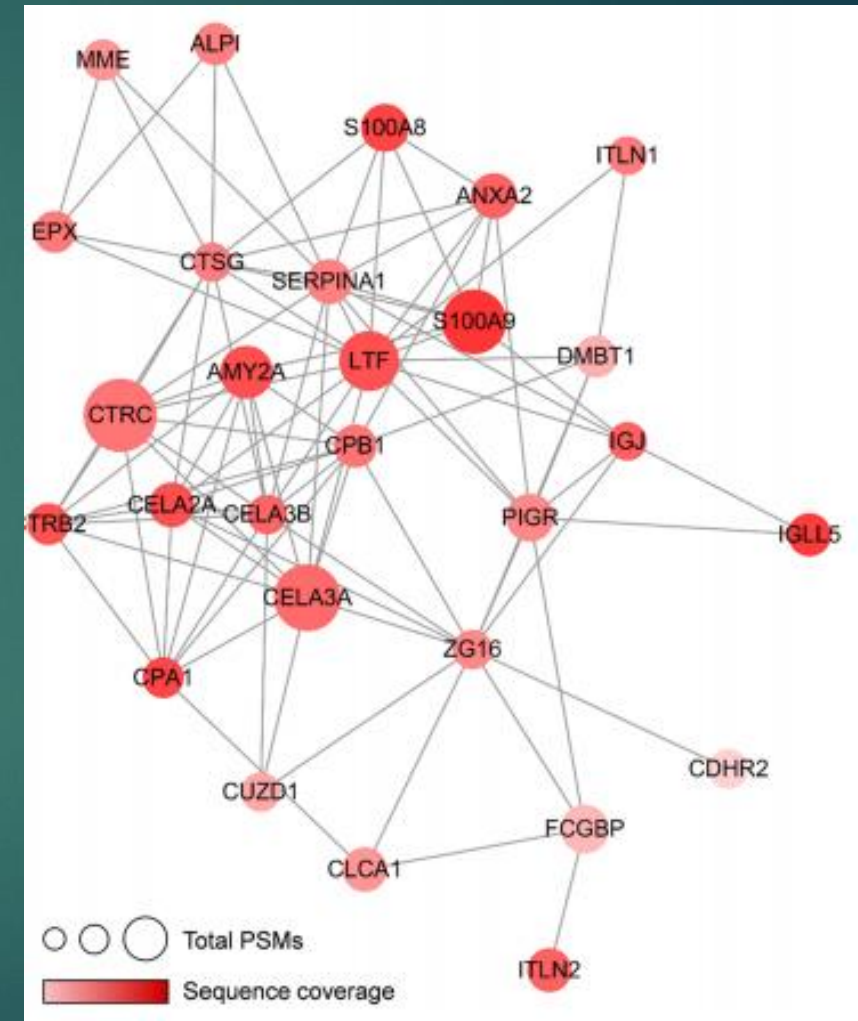



Methodologies: Metaproteomic Approach



Methodologies: Metaproteomic Approach

- ▶ Created human peptide interaction map
 - ▶ Larger circle and darker color indicate greater number of peptides matches
 - ▶ Used STRING to generate





The antimicrobial potential of *Streptomyces* from insect microbiomes

ARTICLE

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OPEN

The antimicrobial potential of *Streptomyces* from insect microbiomes

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Defensive symbioses:

- Microbe produce anti-microbials to protect against opportunistic and specialized pathogens.
- A good source of novel antimicrobials
- Actinobacteria (typically Streptomyces) provide chemical defenses/antimicrobials to combat infectious disease.

Insect-Streptomyces symbiosis

- ▶ Southern pine beetle- Streptomyces: produce the secondary metabolites that protects beetle against its antagonistic fungus
- ▶ Solitary wasps-Streptomyces: produce streptochlorin that provide antibacterial and antifungal chemical protection to their larvae.
- ▶ Wasp 25- Streptomyces: Produce sceliphrolactam that provide antifungal activity.

- 
- ▶ Globally there are over five million insect species that occupy virtually every terrestrial niche
 - ▶ Although insects are the most diverse group in the planet been limited to only a few insect orders, specifically Hymenoptera and Coleoptera.
 - ▶ Insects themselves exhibit complex chemistry that mediates and maintains the diversity of their ecological interactions.

Hypothesis

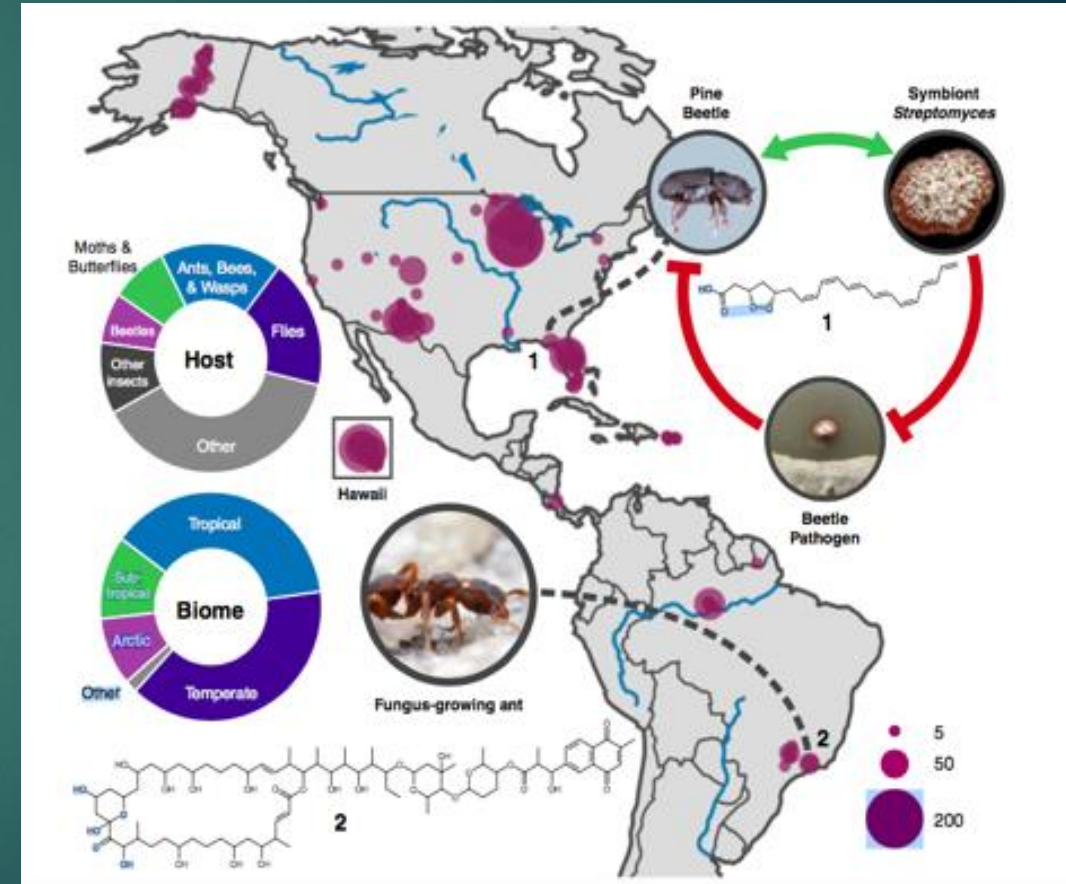
Streptomyces from insect micro biomes represent a promising source of antimicrobials with distinct evolutionary histories from soil Streptomyces, upon which most antimicrobial discovery efforts have focused.


Why Streptomyces?

- (i) is the source of most clinically used antibacterials and antifungals,
- (ii) has established genetic tools to facilitate development⁸, and
- (iii) has been implicated in readily forming associations with diverse insect hosts.

Sampling strategy for Streptomyces from insect microbiomes

- ▶ Streptomyces were isolated from a wide range of insects and geographies (1445 insects; 10,178 strains; dot size, insects sampled)
- ▶ Streptomyces production of the antifungal mycangimycin (1) in the Southern Pine Beetle system is shown at right
- ▶ Cyphomycin (2) is a new antifungal described herein



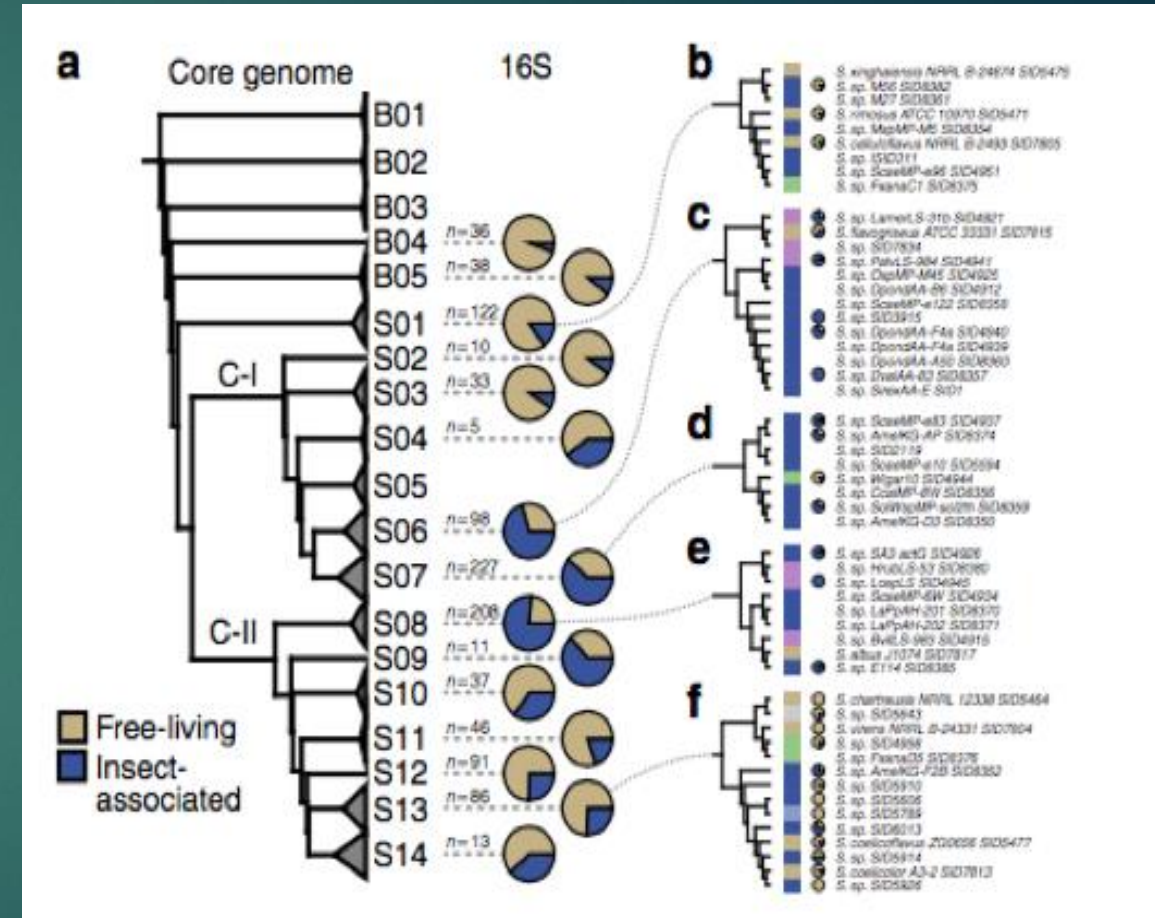
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- ▶ Sampled 2561 insects including 15 taxonomic orders and a wide range of geographies and biomes.
 - ▶ Actinobacteria were isolated from 1445 of 2580 insect micro- biomes (56%) spanning 13 orders, resulting in 10,178 individual isolates, including
 - 2934 from Hymenoptera,
 - 2920 from Diptera
 - 1326 from Lepidoptera
 - 1139 from Coleoptera
 - ▶ 6935 isolates obtained from other sources like soil and plants.
 - ▶ Phylogenetic placement of 536 insect-associated and 571 free-living strains indicates that specific lineages of Streptomyces are enriched for associations with insects.

Streptomyces are commonly found in insect microbiomes

-A genomic phylogeny constructed from core bacterial genes.

-16S sequences mapped to the genomic phylogeny and the distribution of free-living (tan) and insect-associated (blue) strains.

-Detailed mapping of 16S sequences onto the genomic tree is shown for clades S01 (b), S06 (c), S07 (d), S08 (e), and S13 (f).

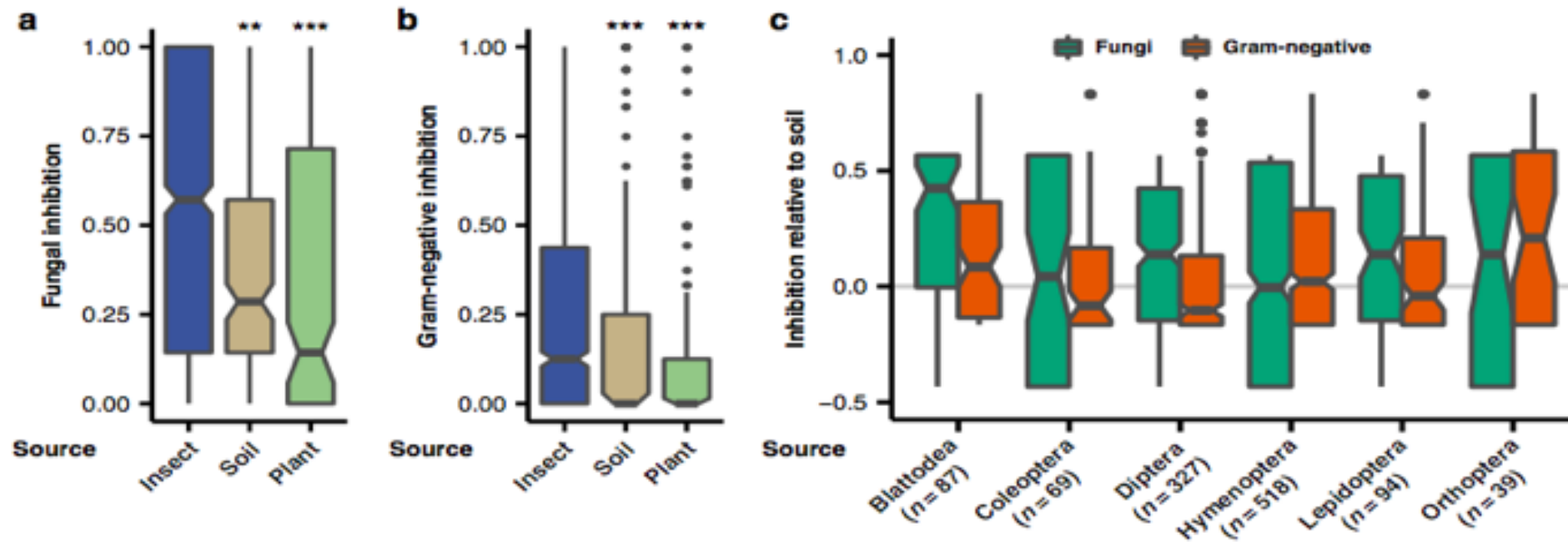


Bioactivity of insect-associated *Streptomyces*

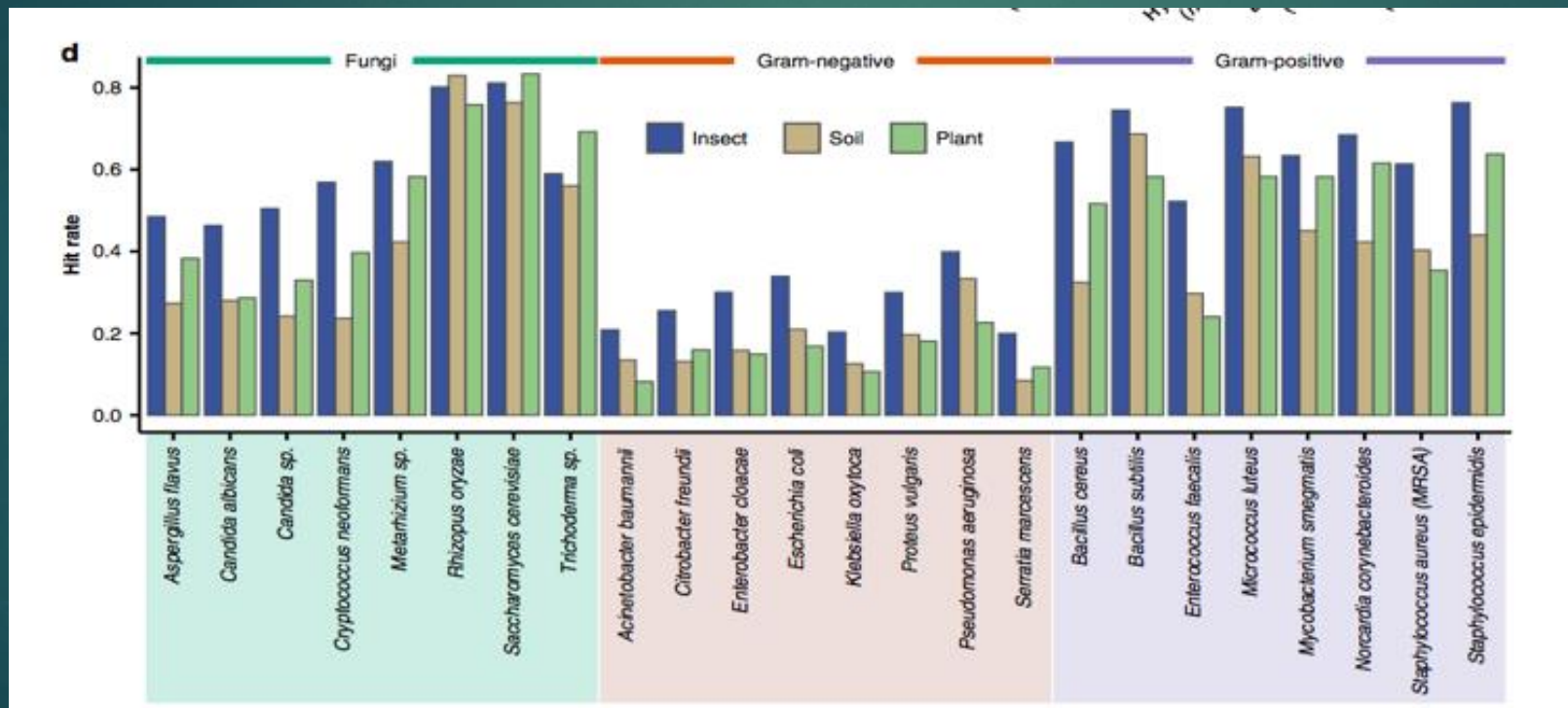
a. Fungal

b. Gram-negative pathogens are significantly more inhibited by insect-associated isolates compared to soil- and plant-sourced *Streptomyces*.

c. Strains vary in antimicrobial bioactivity by insect host orders

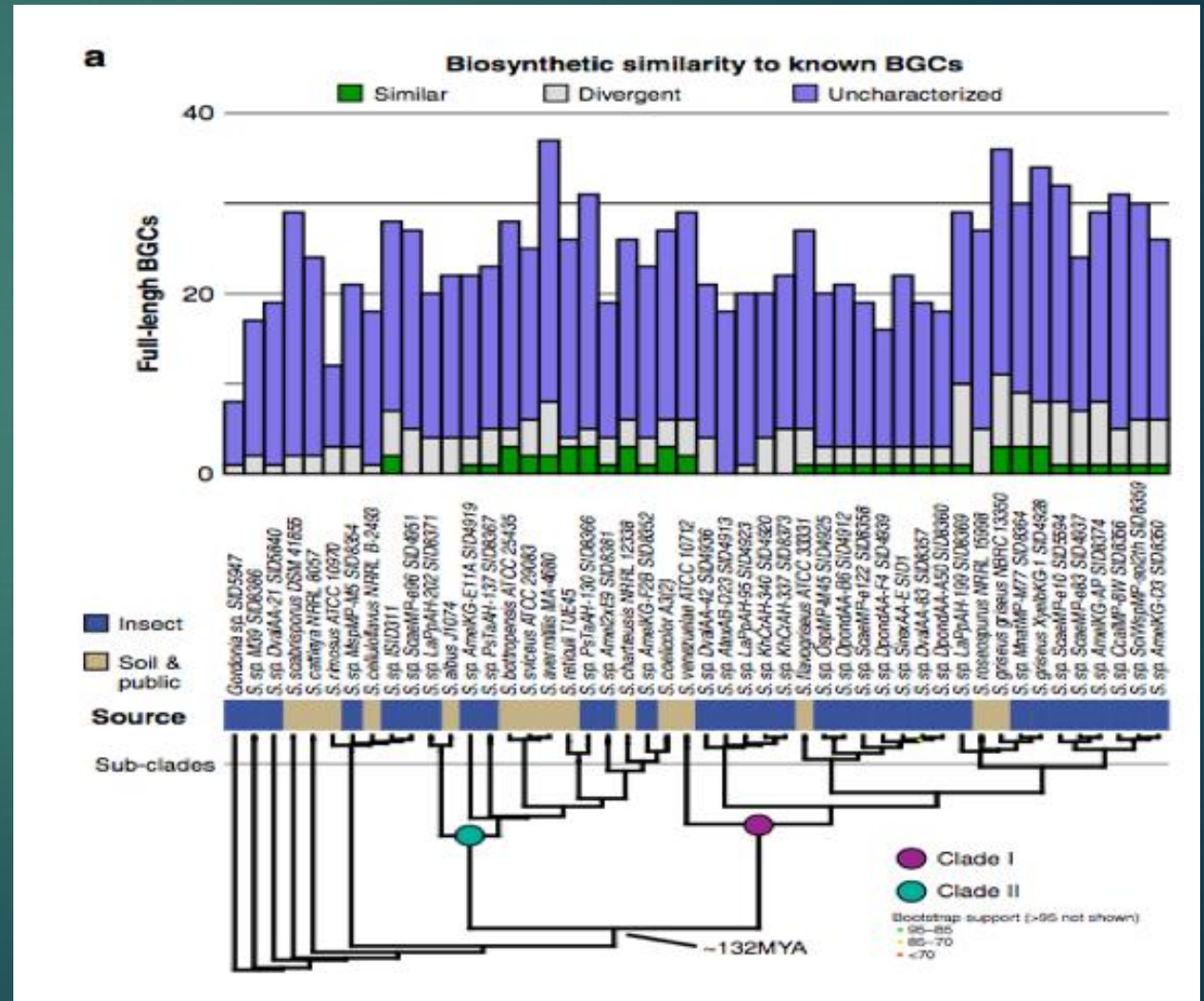


Hit rate for insect, soil, and plant strains against individual pathogens.

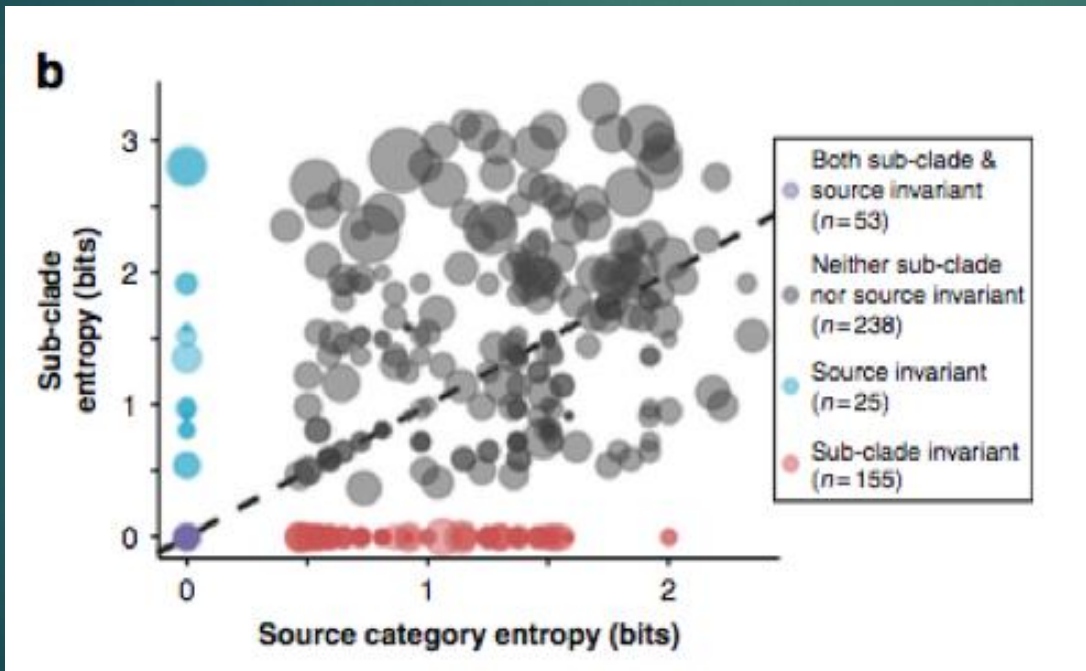


Biosynthetic potential is shaped by ecology and phylogeny

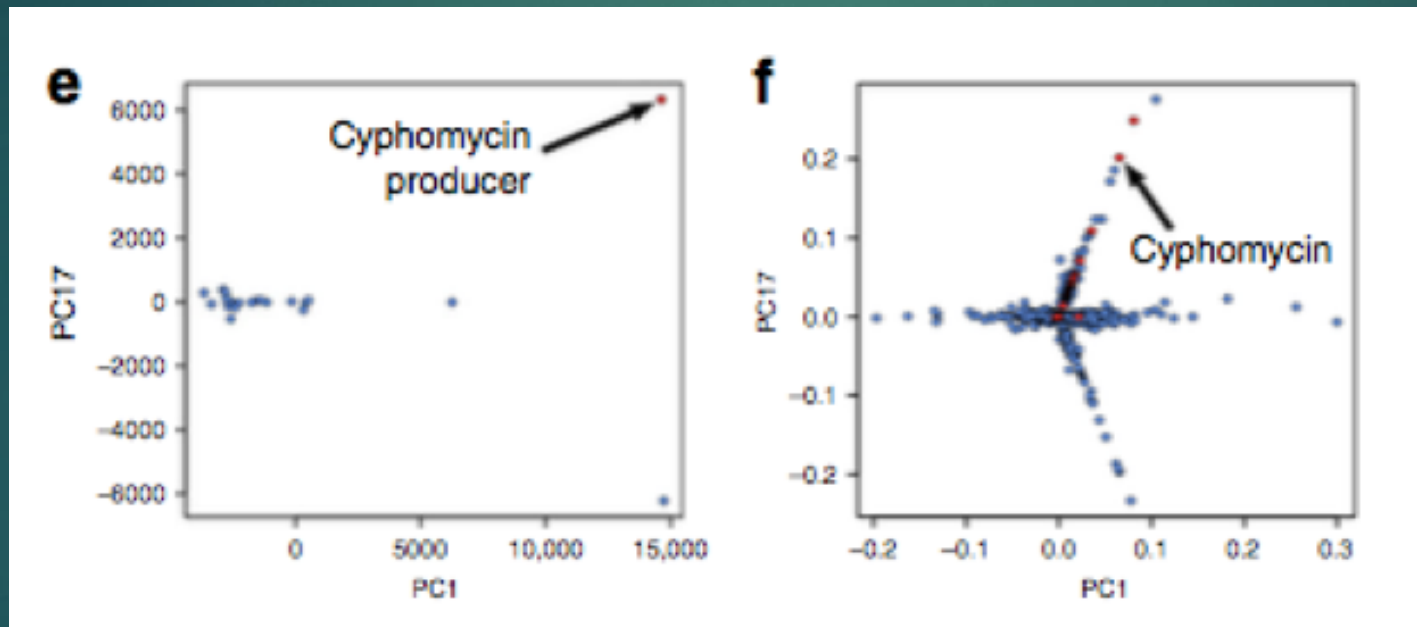
- ▶ A core-genome phylogeny shows evolutionarily distinct lineages of *Streptomyces* associate with insects.
- ▶ BGC similarity to known BGCs highlights the biosynthetic diversity of insect microbiome strains.



Source invariant (blue) and sub-clade invariant (red) BGC families suggest BGC presence is influenced by both source and phylogeny

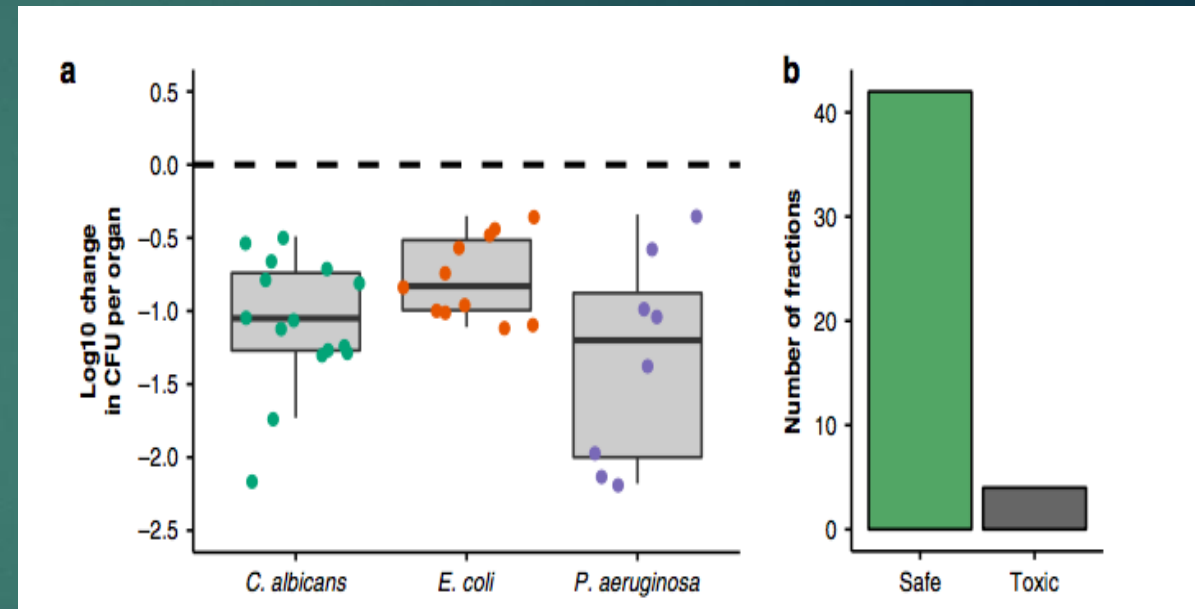


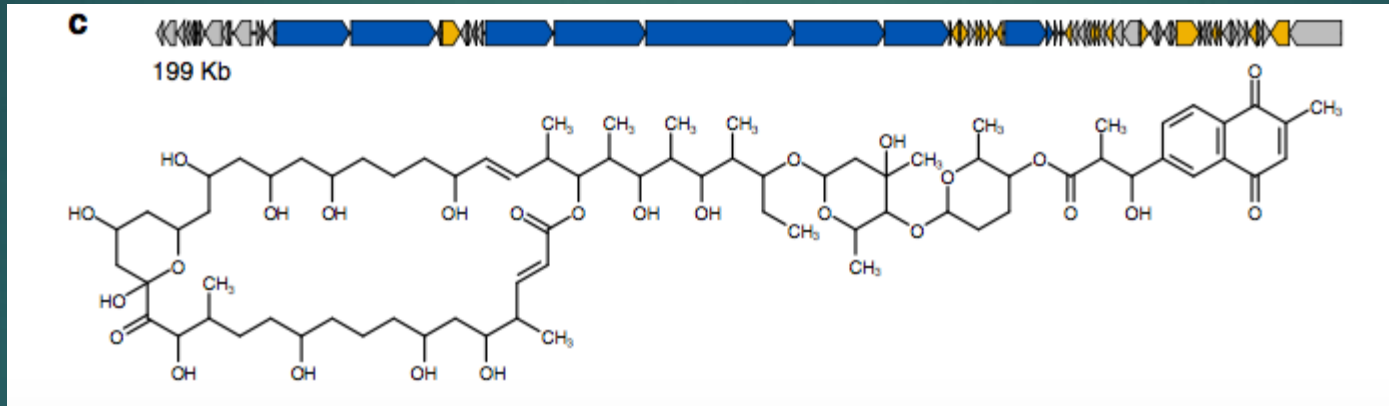
PCA of the metabolomes identified an outlier strain and MFs that contribute to its uniqueness, including cyphomycin.



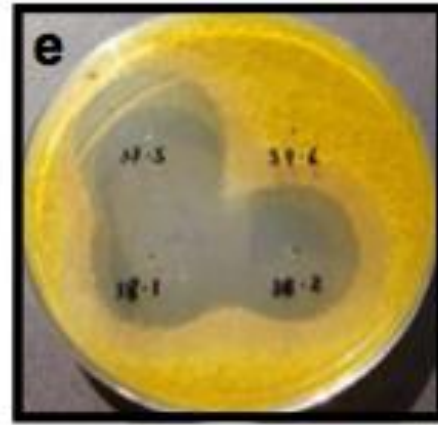
Insect-associated Streptomyces are a source of active antimicrobials

- Fractionated extracts from insect microbiomes are active in multiple murine models of drug-resistant infection.
- Less infective burden is seen in intraperitoneally treated mice after 8 h of infection.
- Each dot represents a unique fraction in one mouse study.
- Most fractions from insect microbiomes show no hemolysis in cell-based assays. Safe indicates no toxicity.

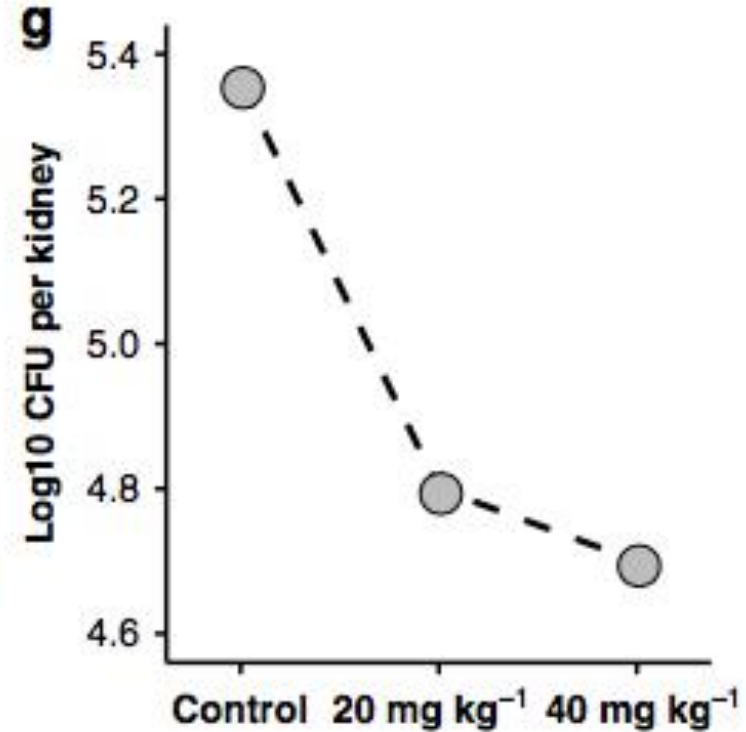




-The antifungal cyphomycin is produced by *Streptomyces* isolated from the fungus-growing ant *Cyphomyrmex* sp.



Escovopsis sp.



f

Strain

Aspergillus fumigatus 11628

Candida glabrata 4720

Candida auris B11211

Drug resistance
phenotype

Triazole (T)

Echinocandin (E)

T, E, Amphotericin B

Cyphomycin
MIC ($\mu\text{g mL}^{-1}$)

0.5

0.5

4

- Cyphomycin-containing fractions show potency against the ant pathogen *Escovopsis* sp.
- Purified cyphomycin exhibits potency against resistant pathogens.
- Mouse candidiasis (*C. albicans*) models showcase reduced infection and a dose-like response to cyphomycin.

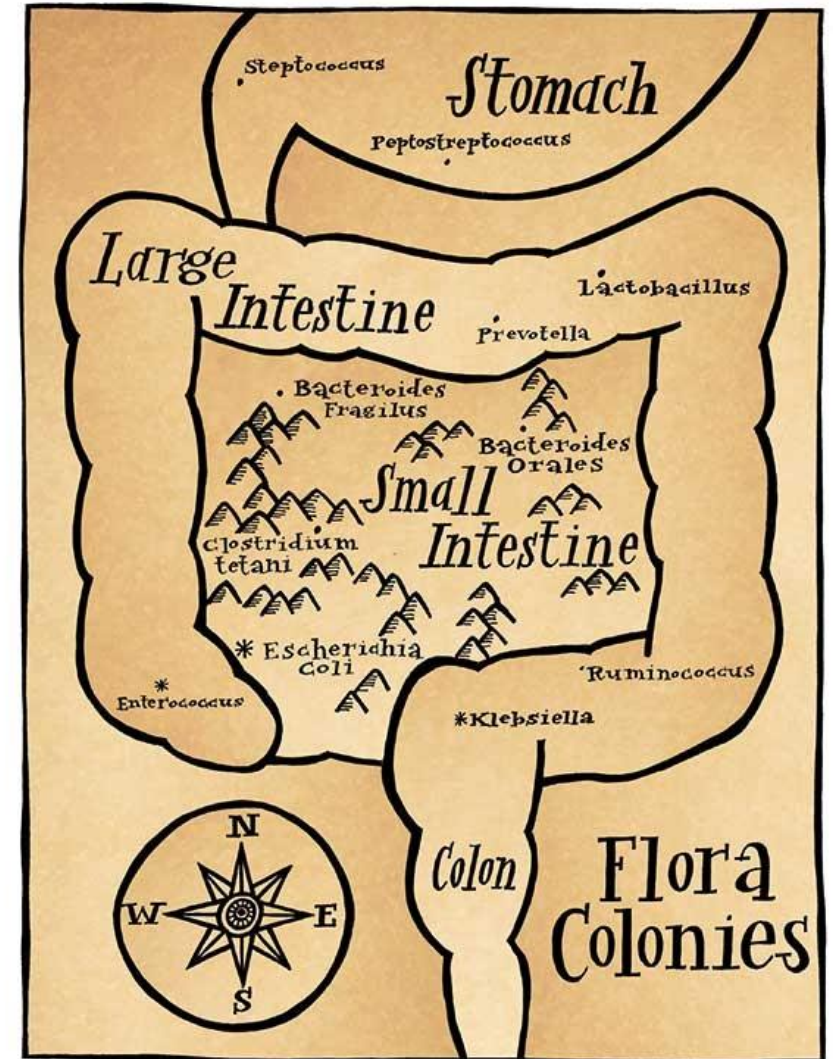
Summary

- ▶ Insect microbiomes present a promising source of novel natural products.
- ▶ application of genomics, metabolomics, and ecologically optimized bioassays facilitates rapid screening of strains to explore their untapped chemical diversity
- ▶ Cyphomycin is an example of new chemistry from this innovative source.

Review

An extensive range of microbiome research applications and methodologies exists. Microbiome research opens the door to personalized medicine in addition to further detailed studies of metabolic pathways and pharmaceutical developments.

Questions?



Methodologies: Microbiome Proof of Method Approach

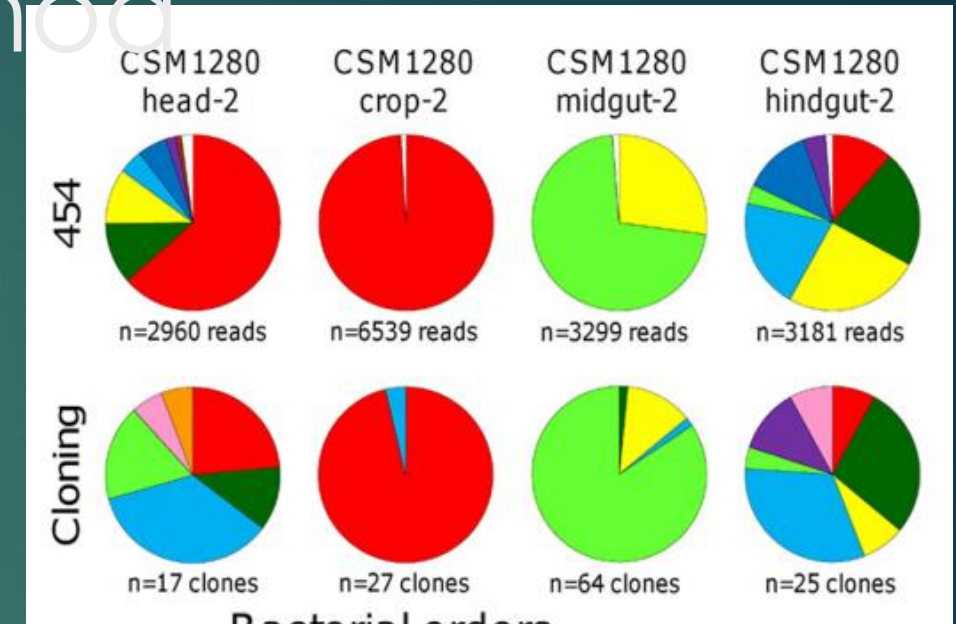


Researchers were looking to compare microbiomes of turtle ants

- **454 pyrosequencing** of rRNA from initial samples
- rRNA PCR amplification to ~200 bases
- Transformation of PCR product into *E. coli*
- **Sanger sequencing** of clones

Methodologies: Microbiome Proof of Method

- ▶ Relatively similar results between two sequencing methods
 - ▶ 454 or Sanger showed greater abundance of certain orders
 - ▶ Possibly due to primer bias (different PCR primers utilized by the two methods)
 - ▶ Less coverage of Sanger sequences
 - ▶ *Not pictured: 454 detected minor operational taxonomic units (OTUs) within orders not detected by Sanger
- ▶ 454 pyrosequencing is cost-efficient method to study various microbiomes of ants



Bacterial orders

