

Extracellular Electron Transport in Human & Environmental Systems

Human Gut Microbiome Communities

Steven Finkel, Ph.D.

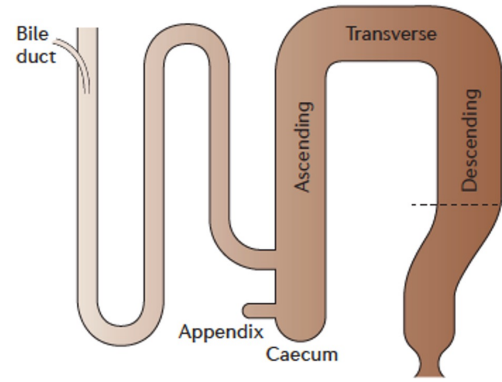
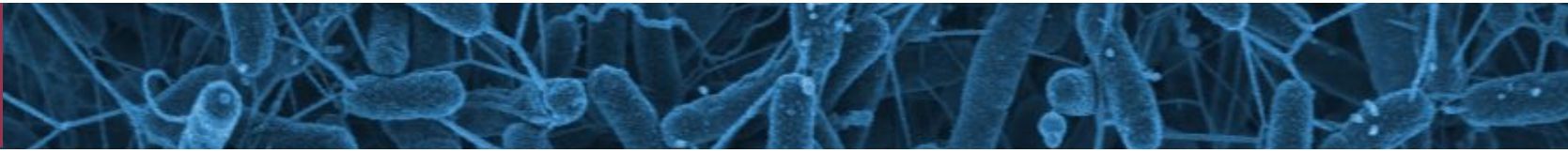
Moh El-Naggar, Ph.D.

David McKemy, Ph.D.

University of Southern California

Research funded by:

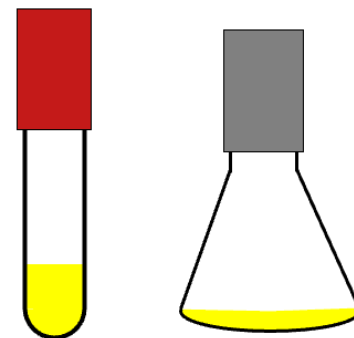
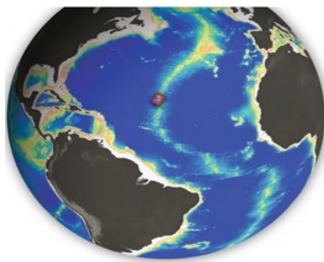




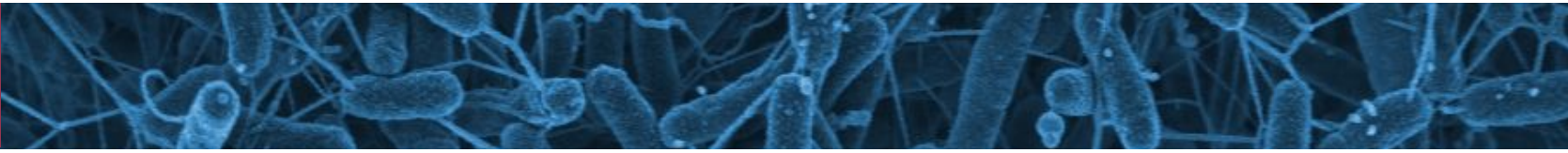
Survival

Adaptation

e⁻



Evolution



"life is nothing but an
e⁻
looking for a place to rest"

Albert Szent-Gyorgyi, 1937 Nobel Prize in Physiology or Medicine

- Brief introduction to Extracellular Electron Transfer (EET)
- Higher resolution identification of microbiome communities & continued identification of EET-Competent bacteria and synergies (or not)
- Identification of mechanism of synergy between *E. coli* and *E. faecalis*
- Developing mouse model system for behavior, mechano-, thermo- and pain perception:
Step 1 – Create “humanized” mice

What is Extracellular Electron Transport?

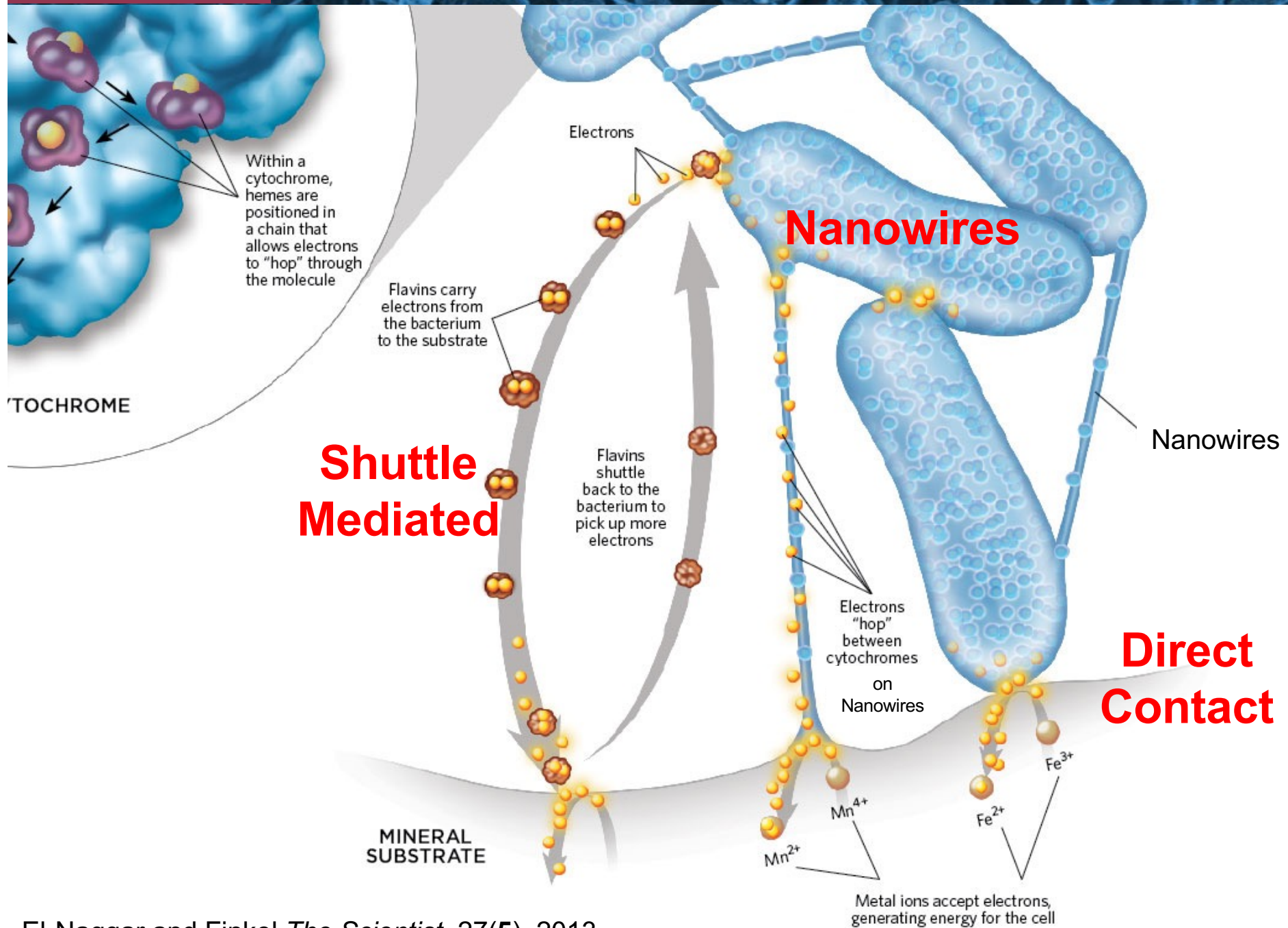
*Another way for bacteria
to “breathe”*

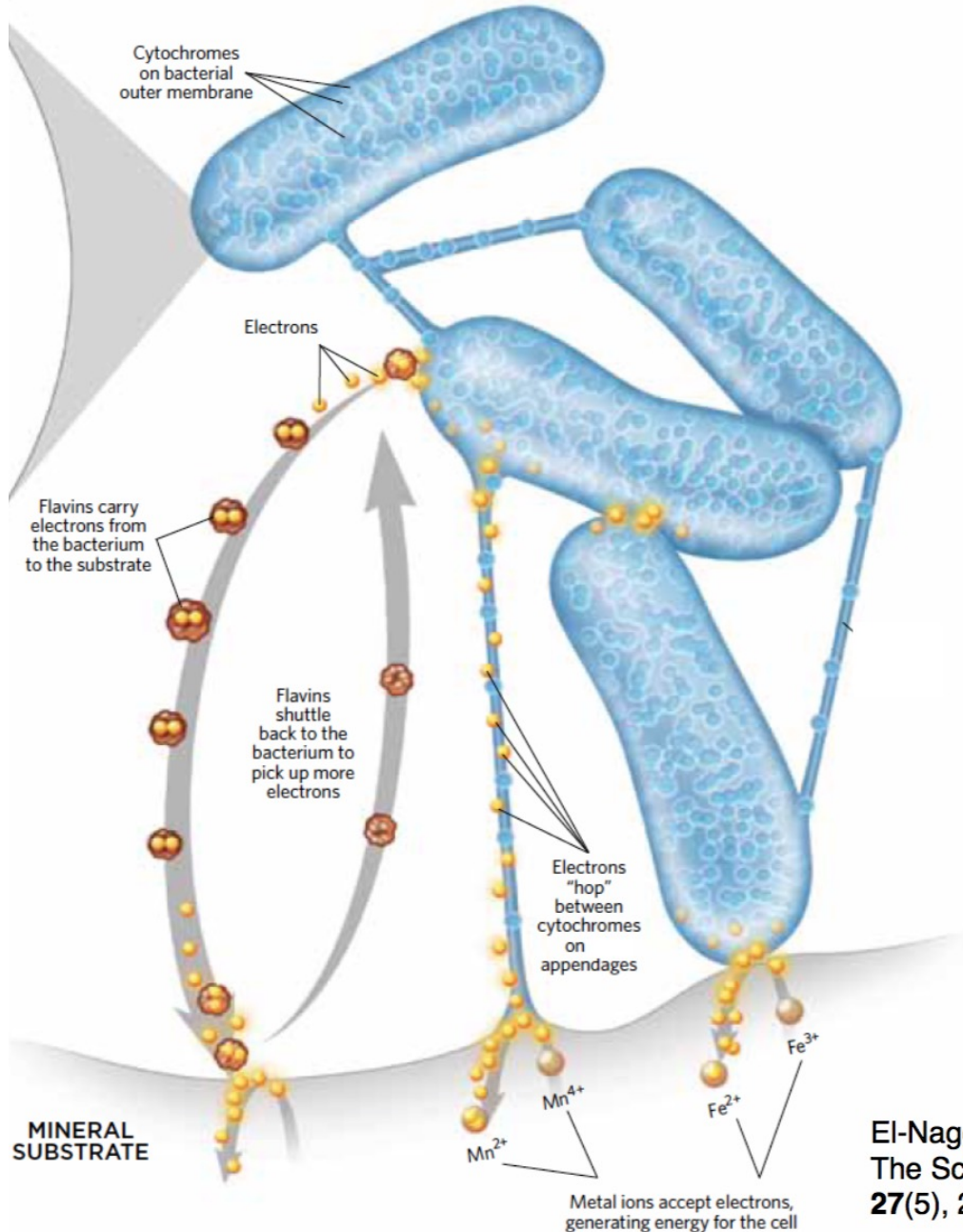
*Novel respiration mechanisms
(and maybe cell-cell and/or cell-host communication)*



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Three Mechanisms of EET





El-Naggar & Finkel,
The Scientist,
27(5), 2013

- Gut
- Oral
- Lung
- Nasopharynx
- Skin/Wounds
- Urogenital

Dominant Gut Microbes

Dominant gut phyla:

Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Verrucomicrobia

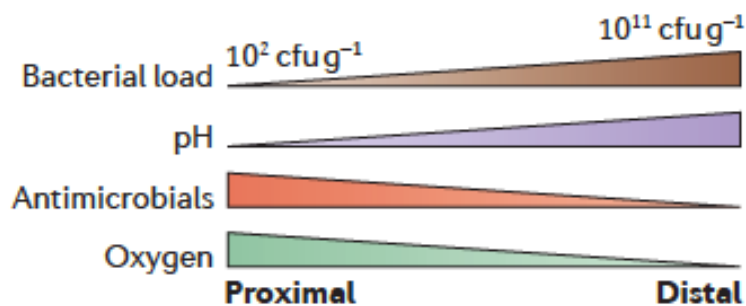
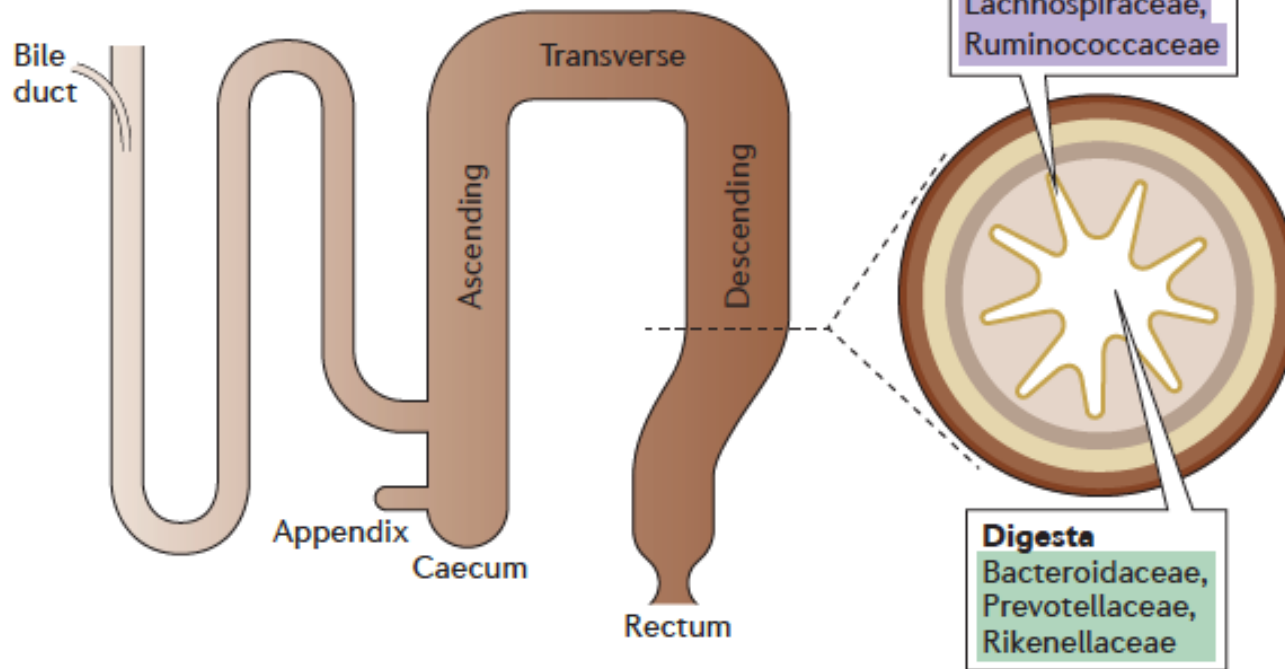
Predominant families in the:

Small intestine

Lactobacillaceae,
Enterobacteriaceae

Colon

Bacteroidaceae, Prevotellaceae,
Rikenellaceae, Lachnospiraceae,
Ruminococcaceae



Dominant Gut Microbes

Dominant gut phyla:

Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Verrucomicrobia

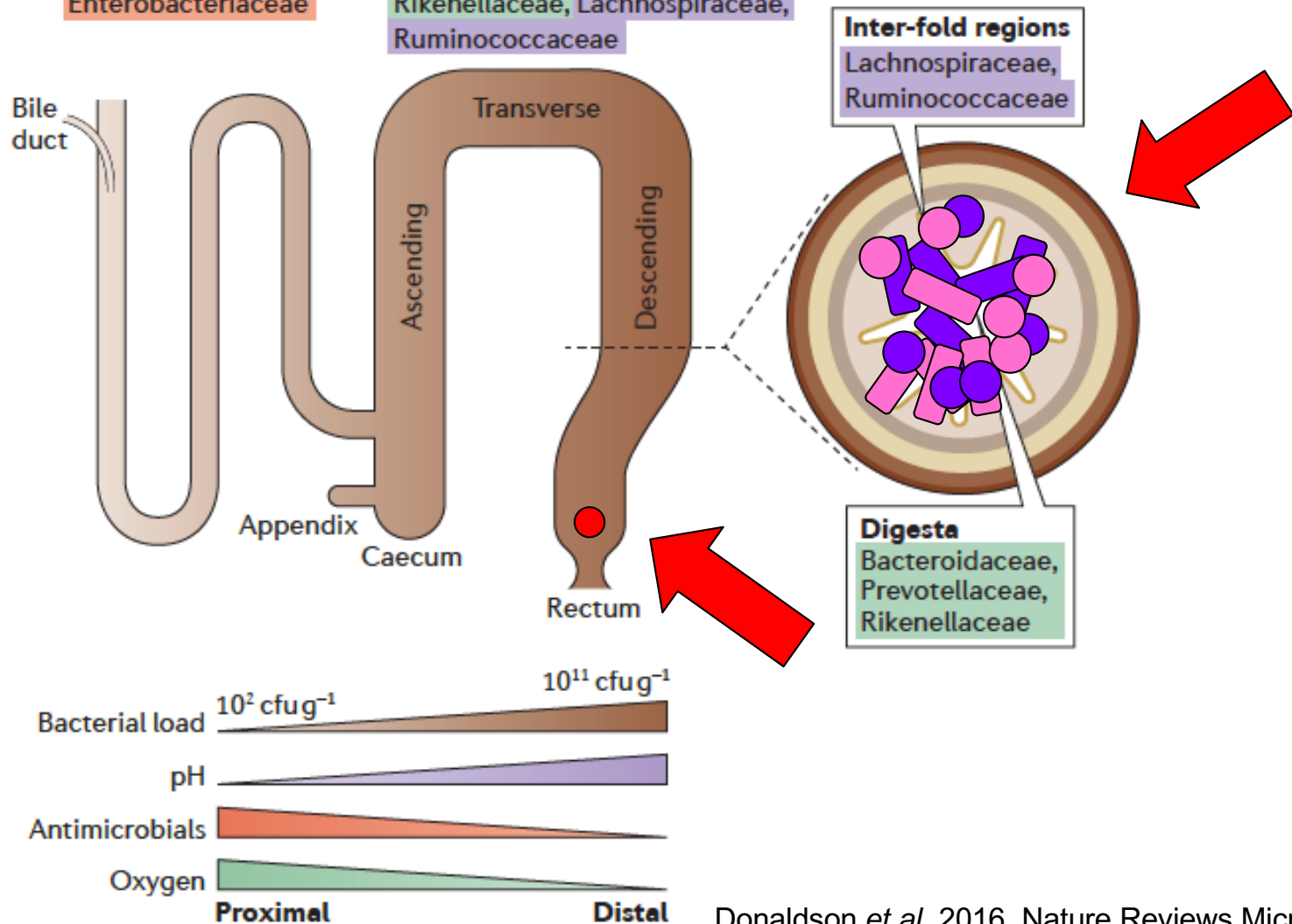
Predominant families in the:

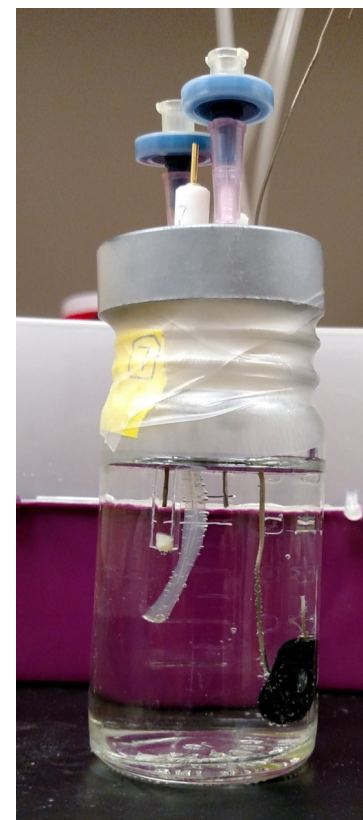
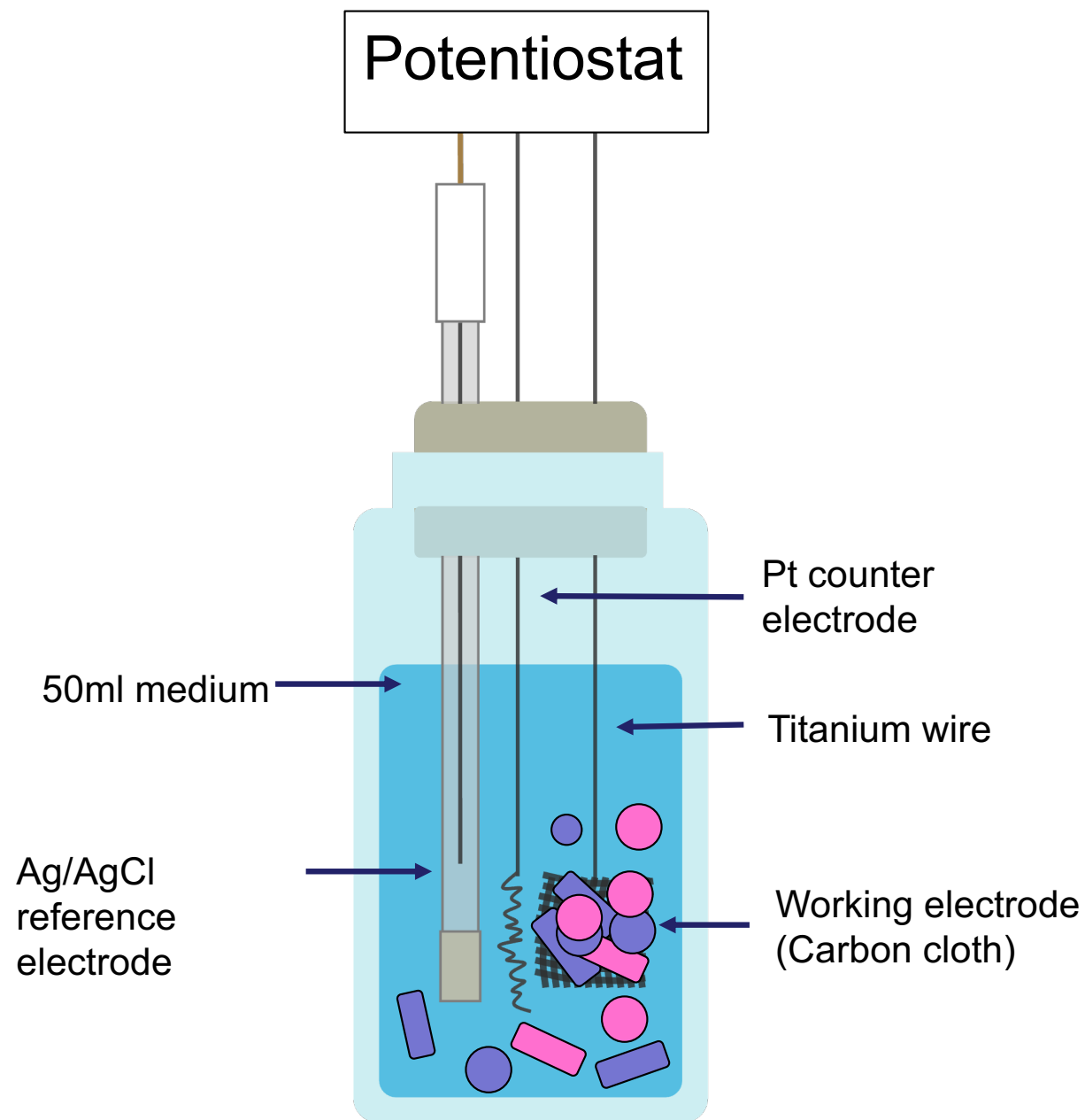
Small intestine

Lactobacillaceae,
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Bacteroidaceae, Prevotellaceae,
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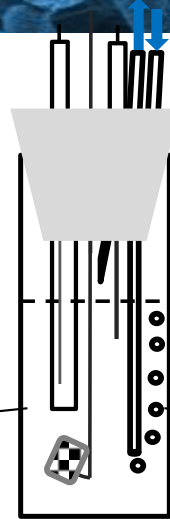
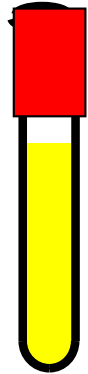




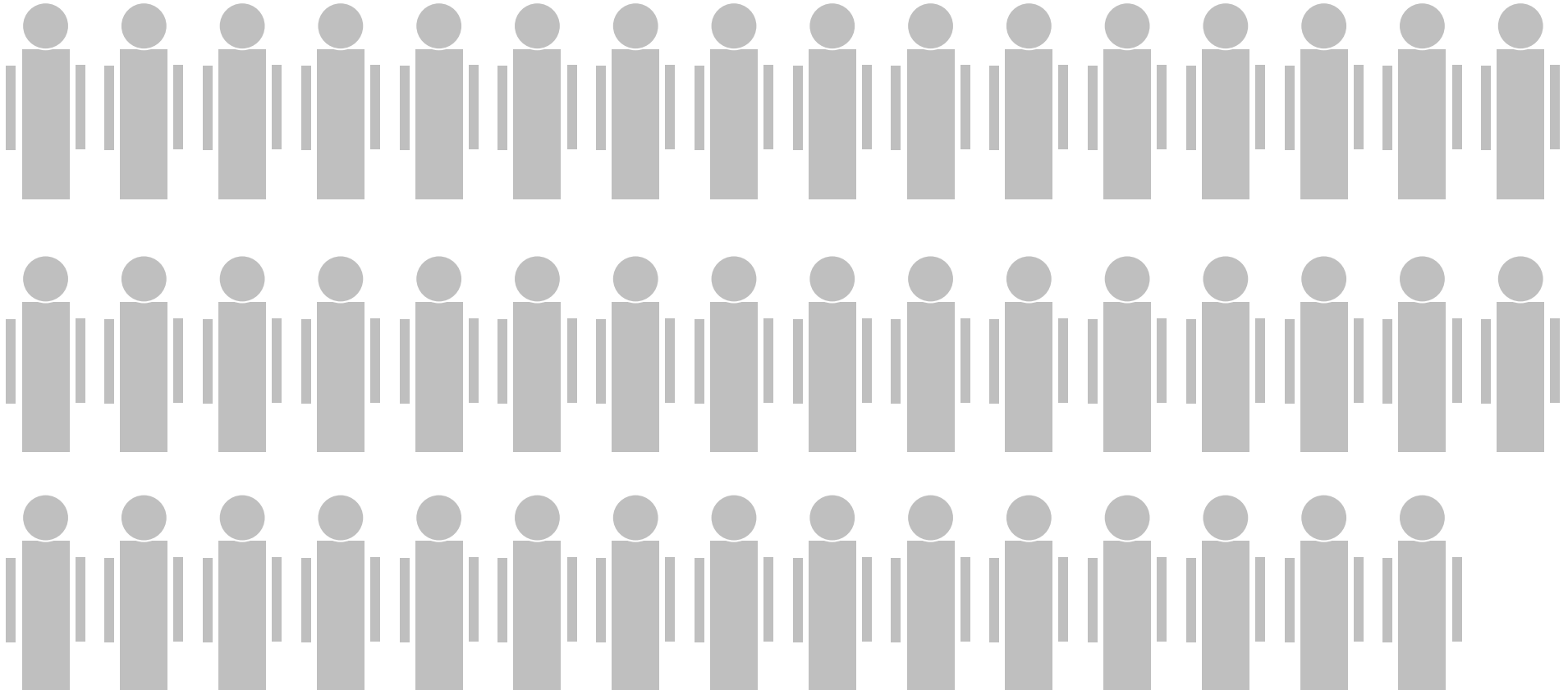


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Identification of Electricigenic Bacteria



**Pooled Fecal Microbiomes
from 47 healthy individuals**

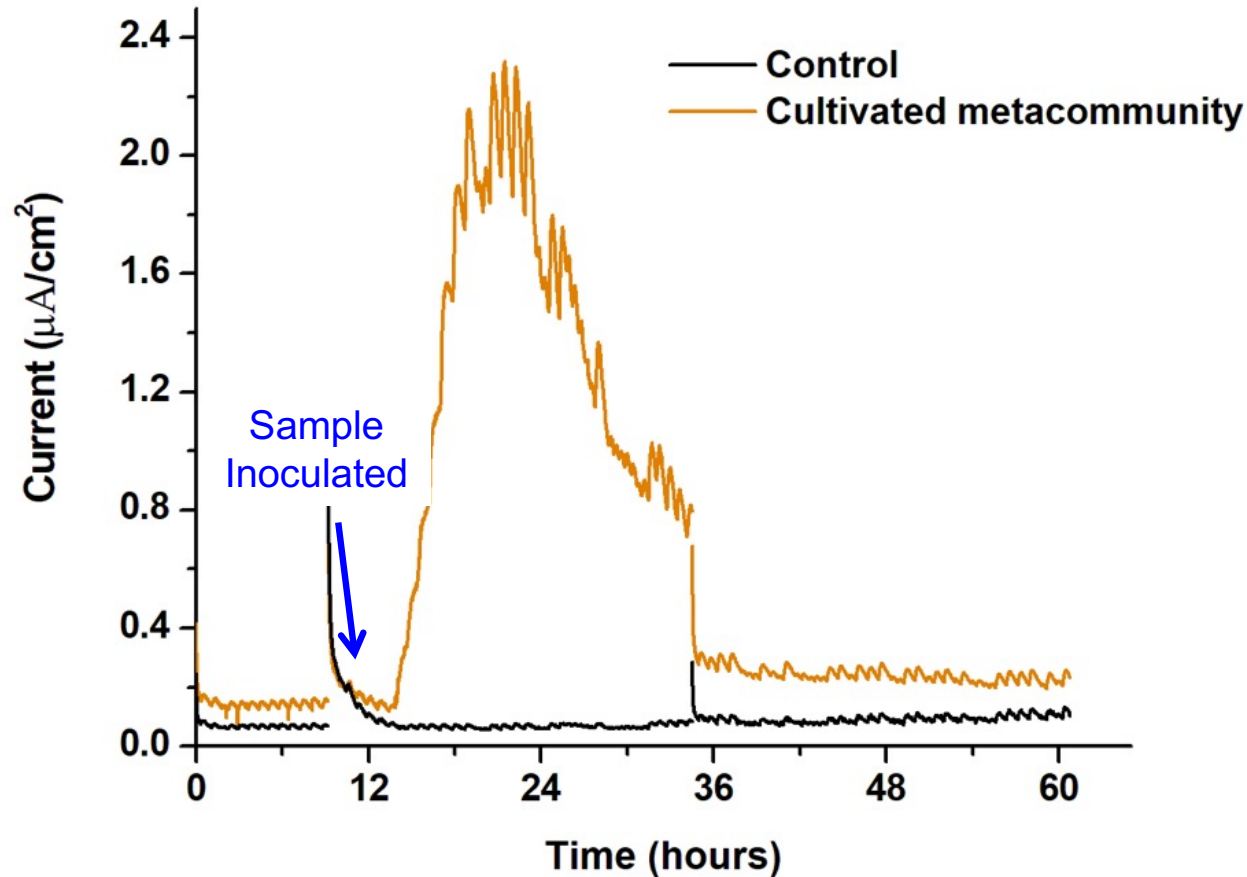
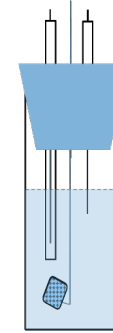


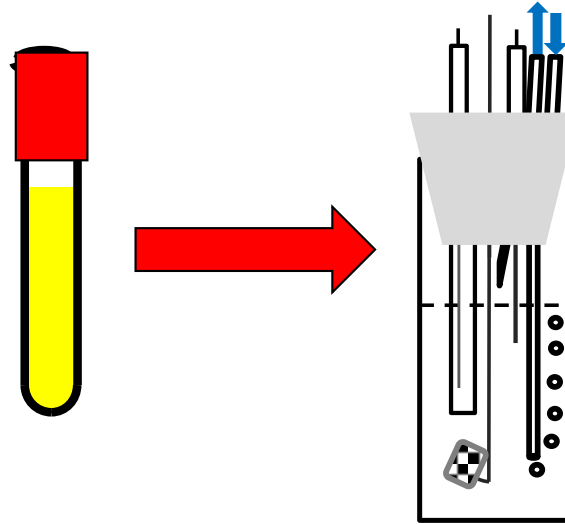


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Identification of Electricigenic Bacteria

“POOLED METACOMMUNITY”





Escherichia coli



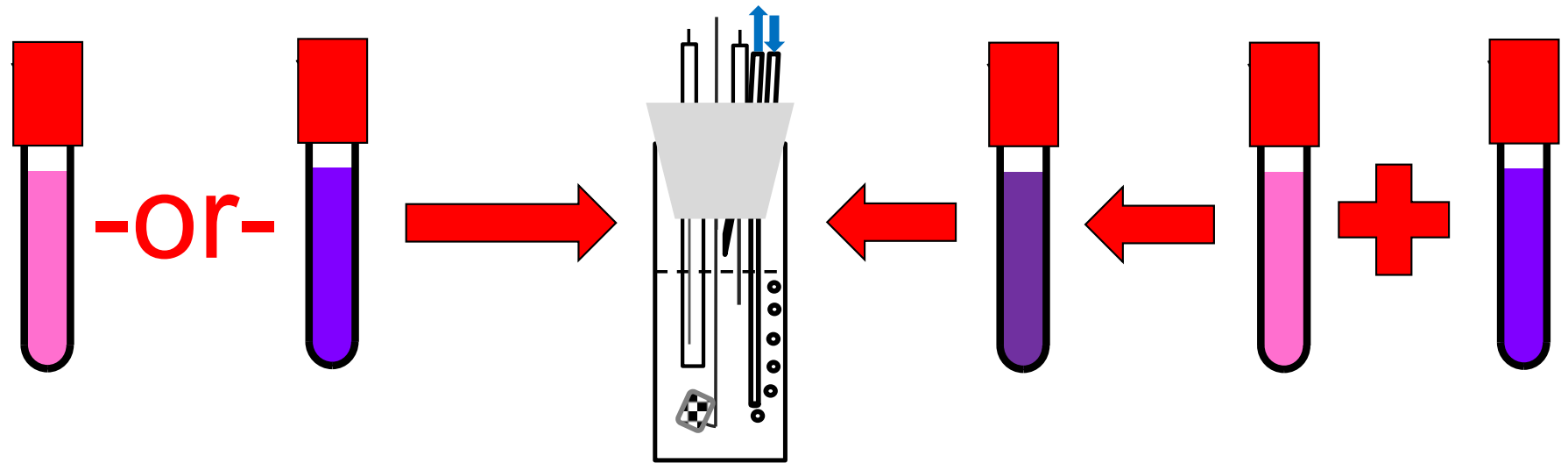
Klebsiella spp.



Enterococcus faecalis



Enterococcus avium



EET production under different growth conditions:

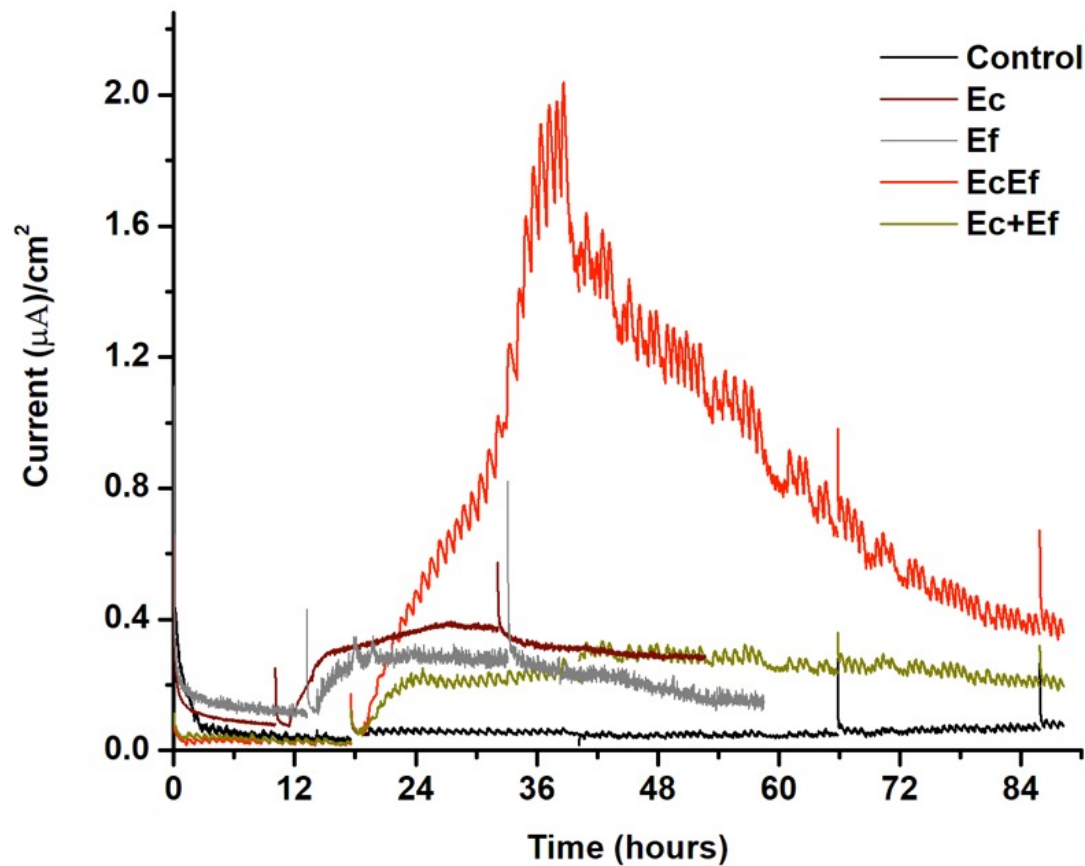
- (1) Each species alone, as **monocultures**
- (2) Initially culture each organism alone, then **mix** (i.e. Ec + Ef)
- (3) **Co-culture** species together & inoculate together (i.e. EcEf)

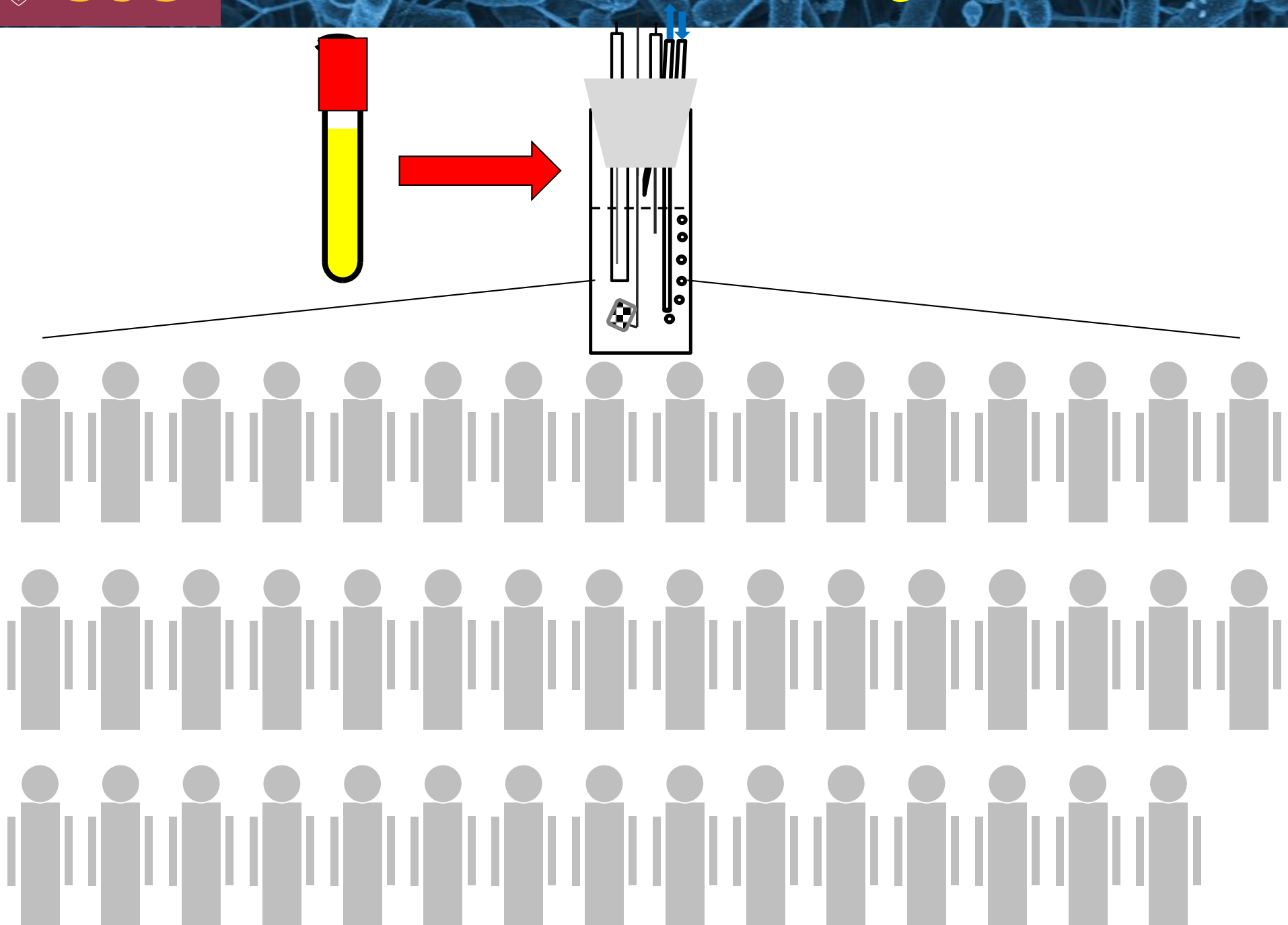


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Multispecies Synergy of EET

Escherichia coli + *Enterococcus faecalis*
Co-outgrown & Co-inoculated

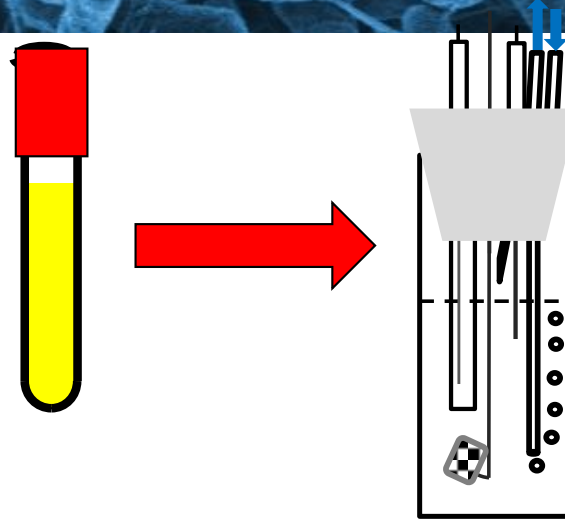






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EET Microbes in Individual “Fresh” Samples



A



B



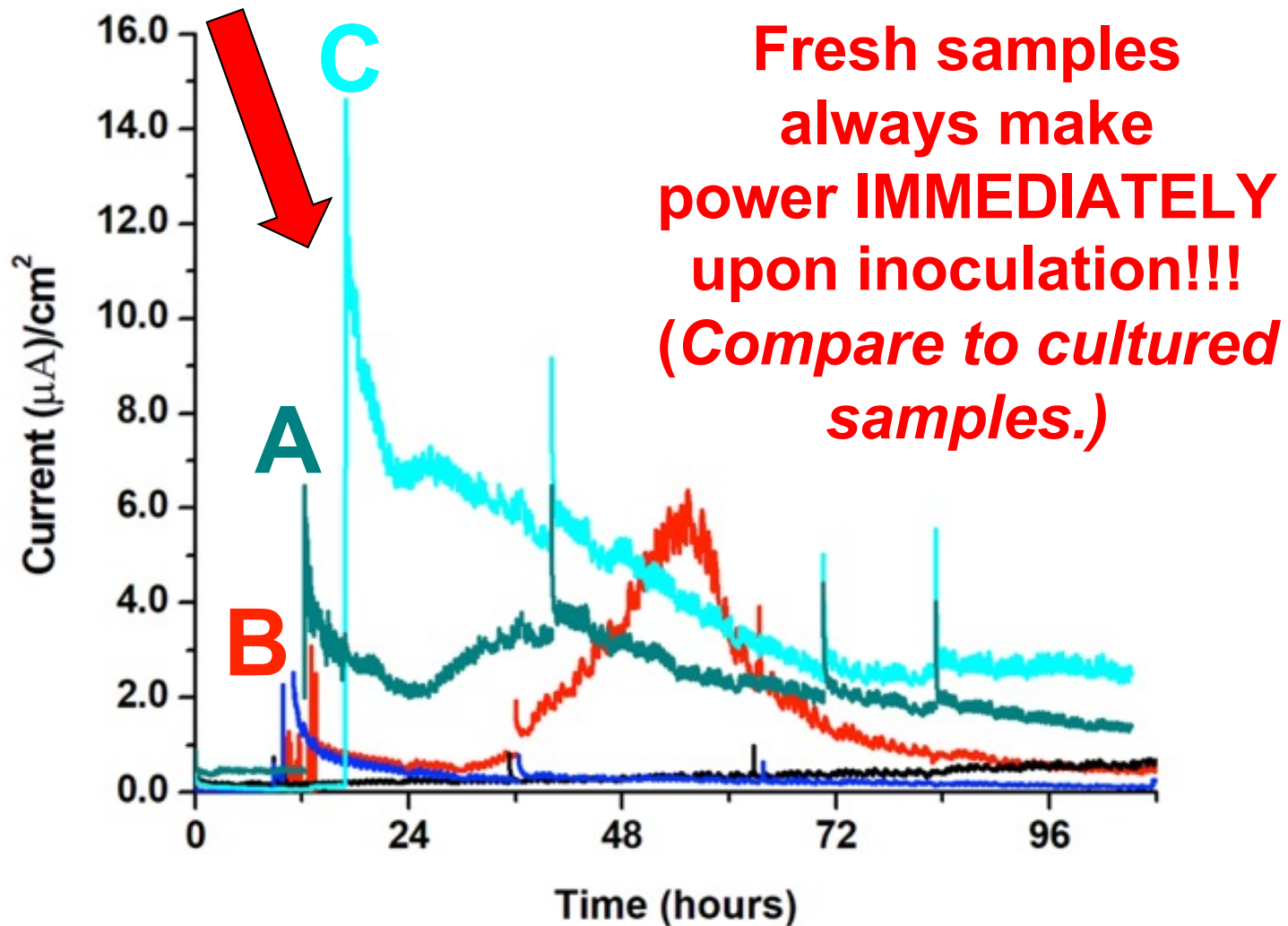
C



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EET Microbes in Individual “Fresh” Samples

Minimally Processed Human Fecal Matter is Electroactive



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- Developing mouse model system for behavior, mechano-, thermo- and pain perception:
Step 1 – Create “humanized” mice



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Identification of Electricigenic Bacteria



A



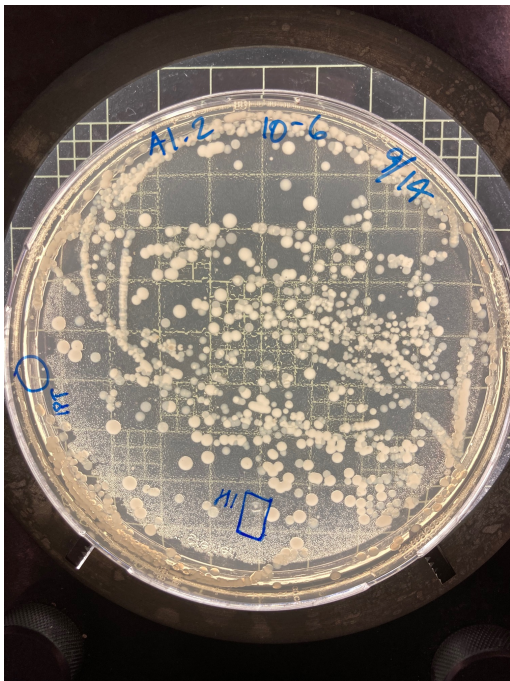
B



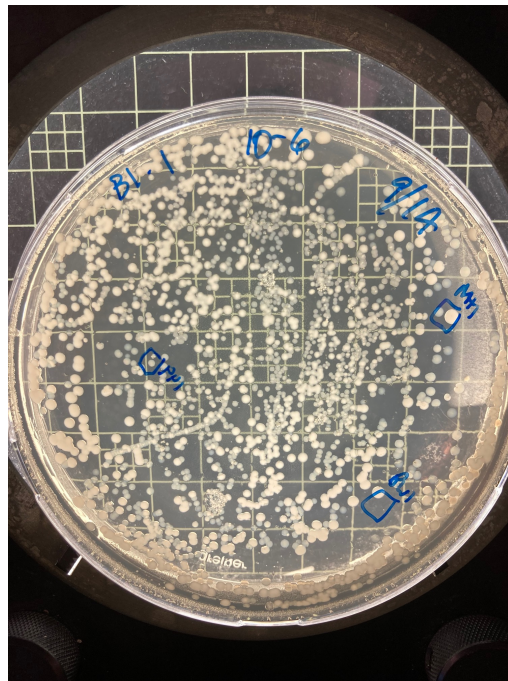
C

What is the community composition of the “electricigenic” microbiota in individuals?

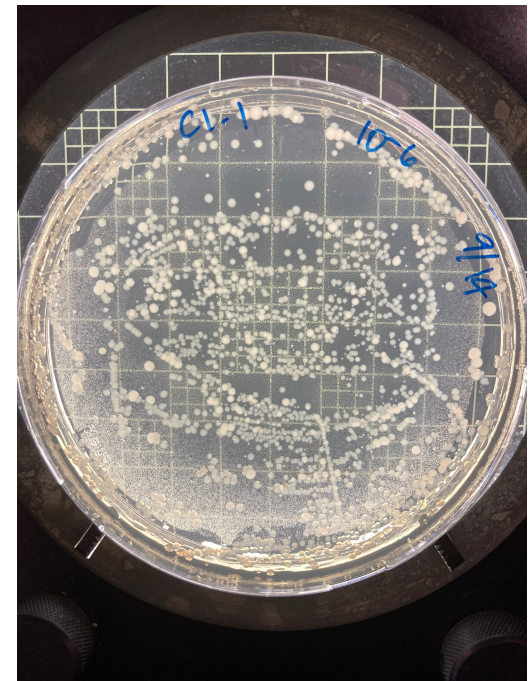
A



B



C

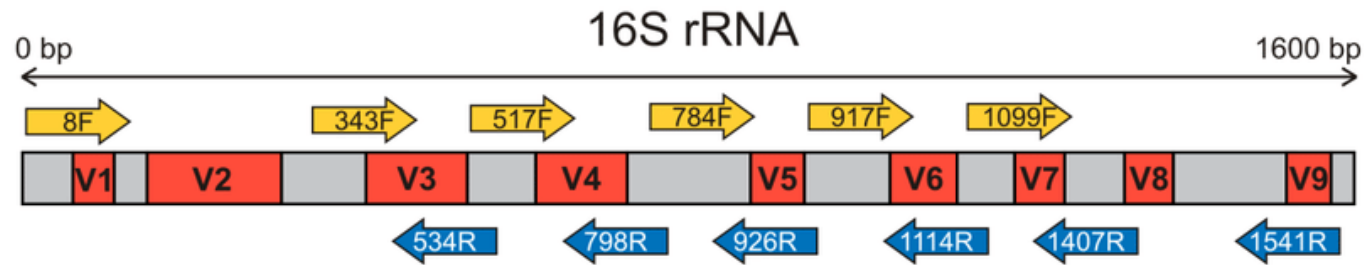




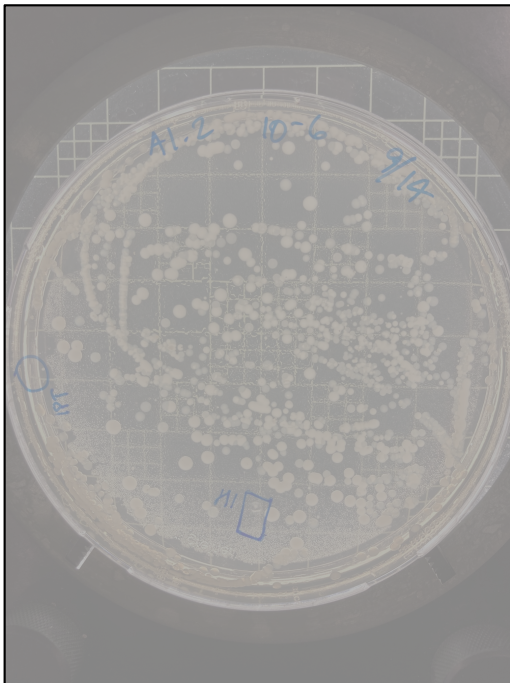
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Identification of Electricigenic Bacteria

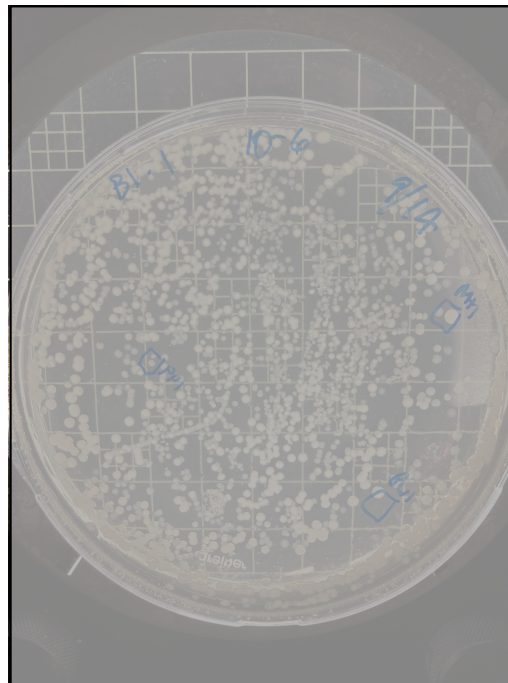
**Isolate individual colonies &
Perform 16S rRNA sequencing to identify species**



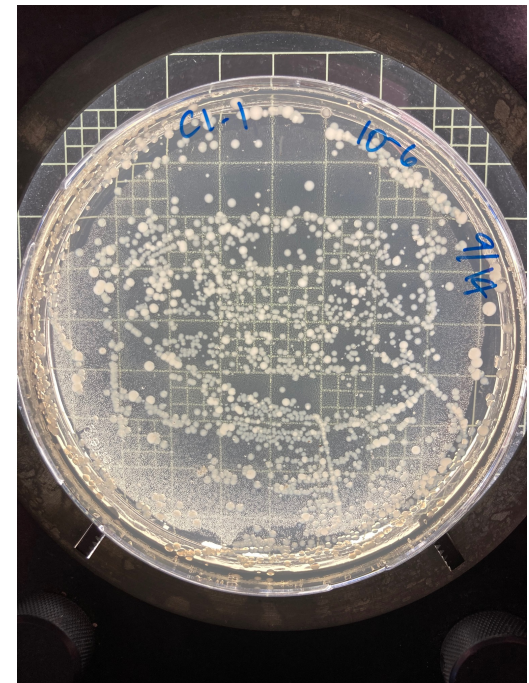
A



B



C



***Klebsiella
pneumoniae
BW2***

***Escherichia
coli
RH1***

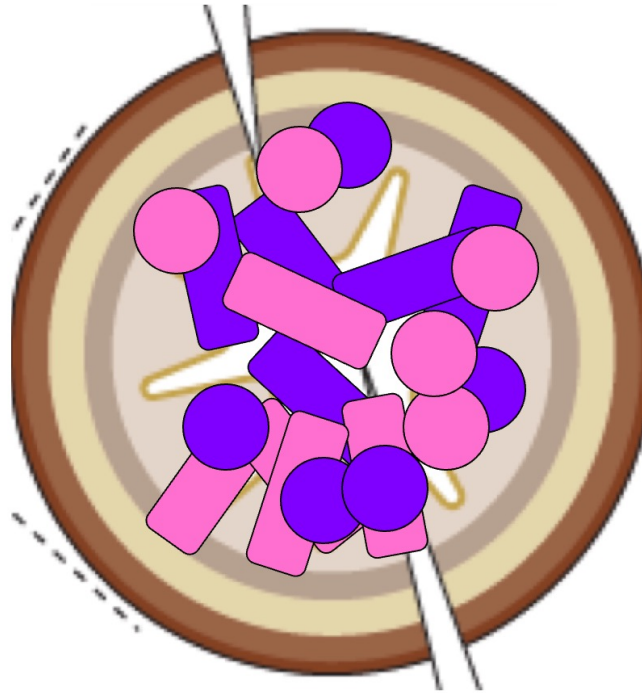
***Citrobacter
amalonaticus***

***Escherichia
coli
BT1***

***Enterococcus
durans***

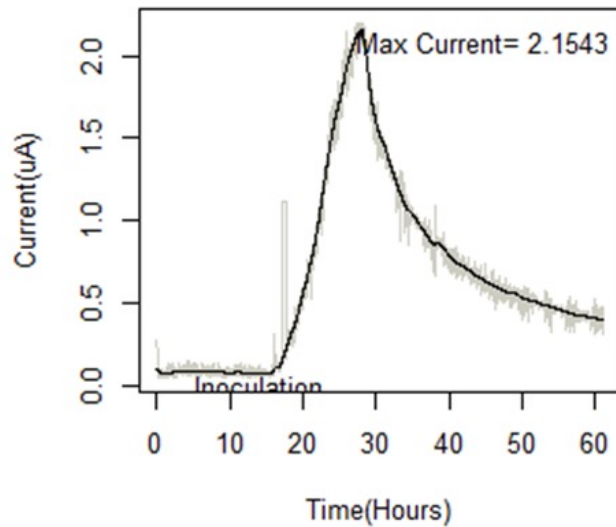
***Enterococcus
faecium***

***Enterococcus
faecalis***

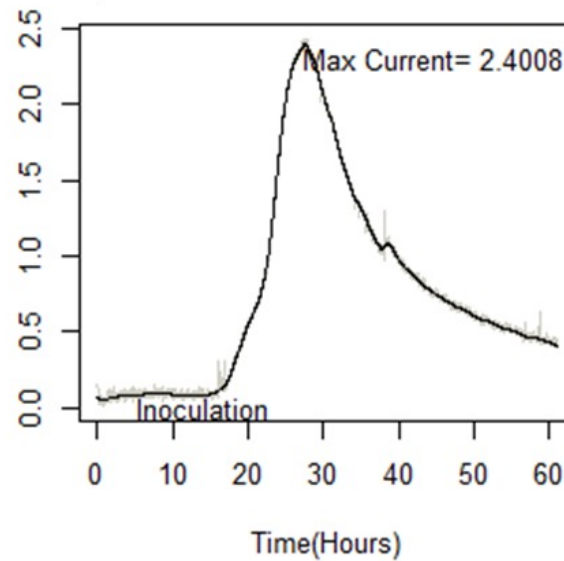


Newly identified organisms produce power

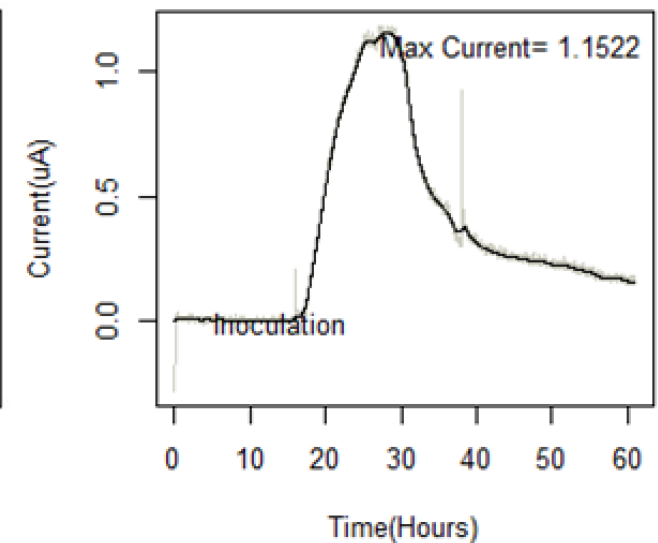
Enterococcus durans



Enterococcus faecium



Citrobacter amalonaticus





***Klebsiella
pneumoniae
BW2***

***Escherichia
coli
RH1***



***Citrobacter
amalonaticus***

***Escherichia
coli
BT1***

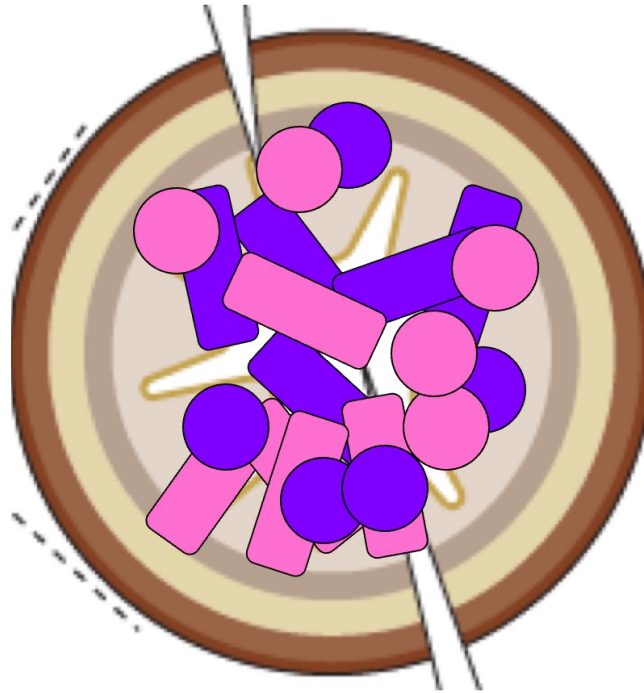


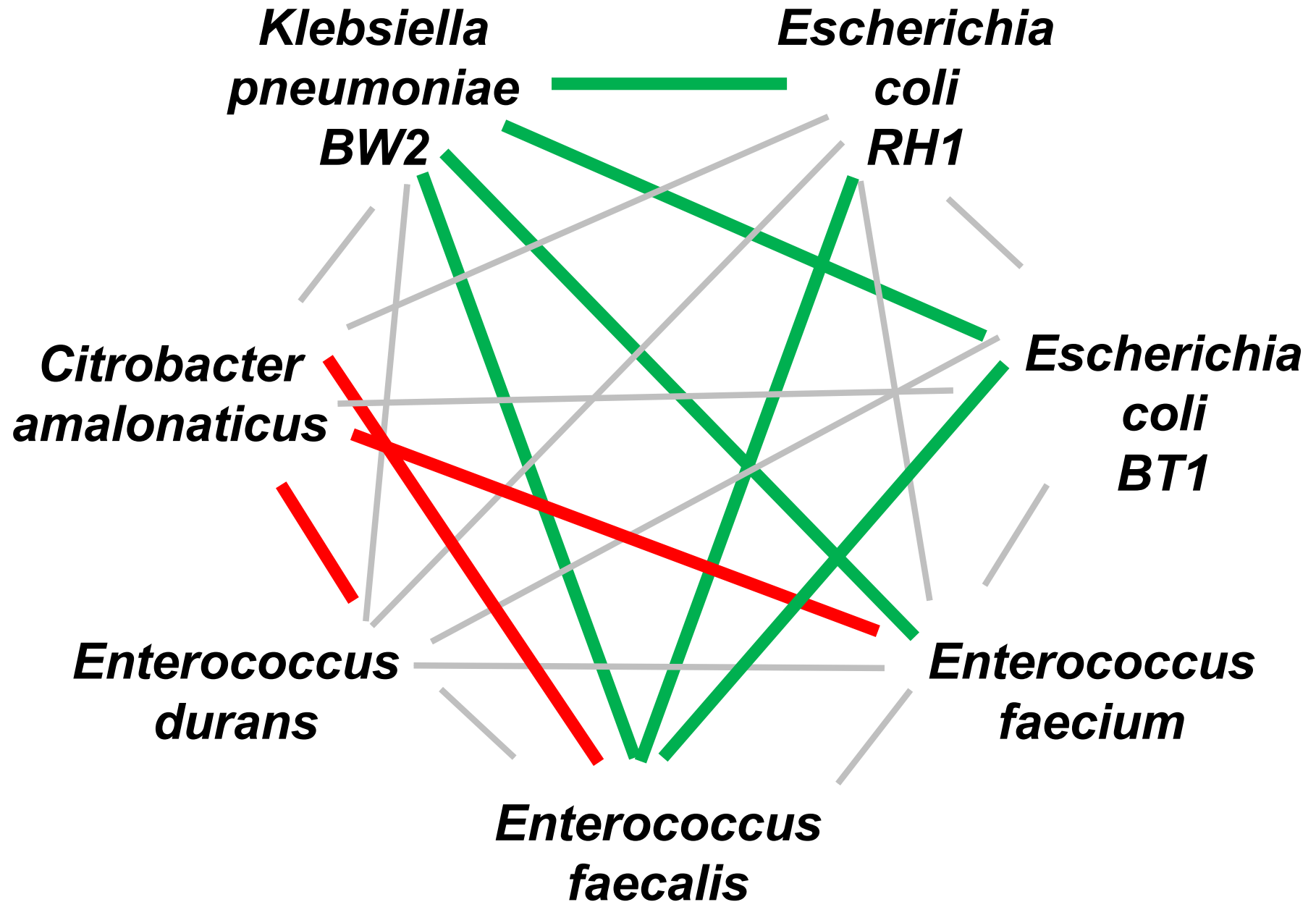
***Enterococcus
durans***

***Enterococcus
faecium***



***Enterococcus
faecalis***



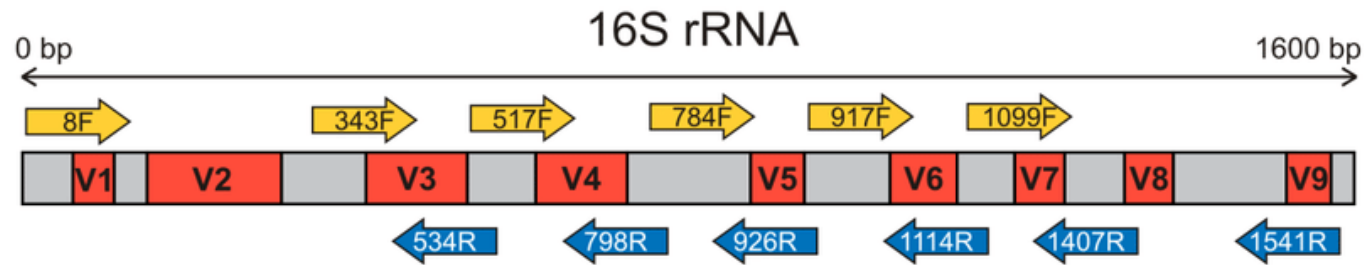




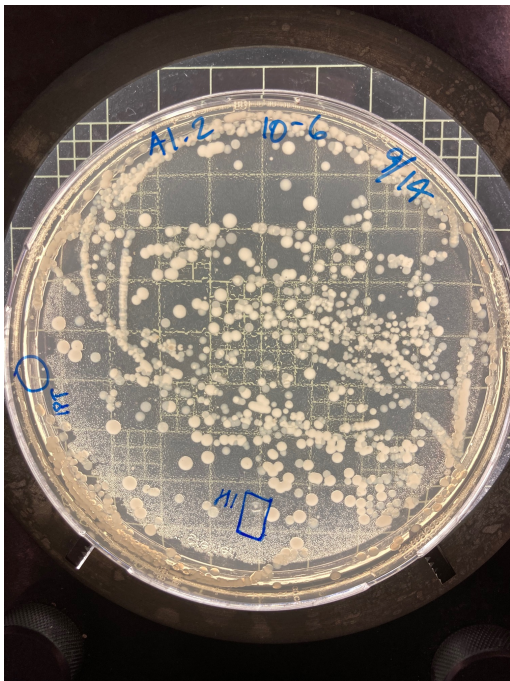
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Identification of Electricigenic Bacteria

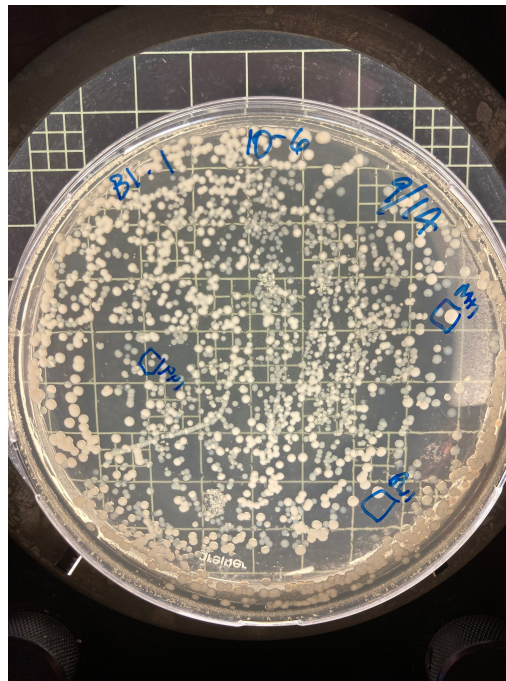
**Isolate individual colonies &
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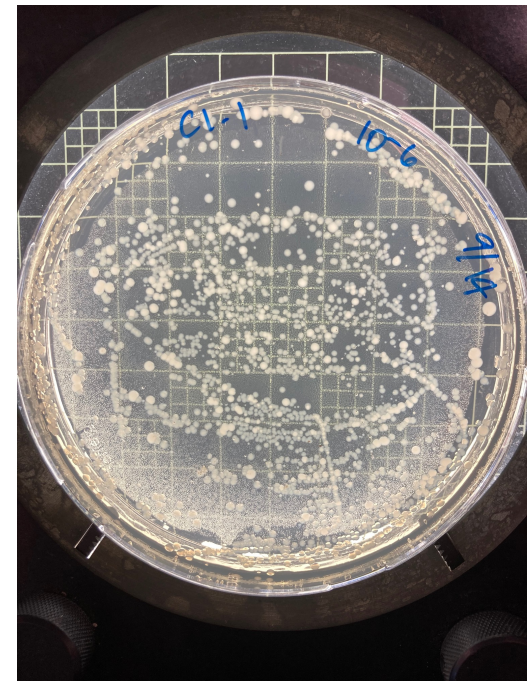
A



B



C





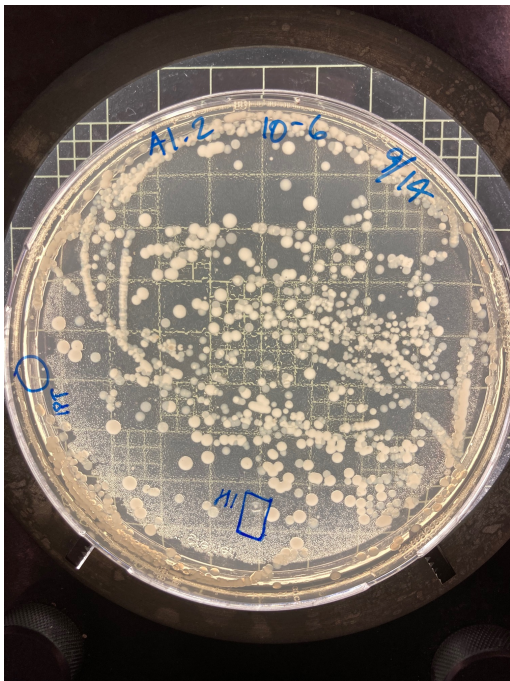
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Identification of Electricigenic Bacteria

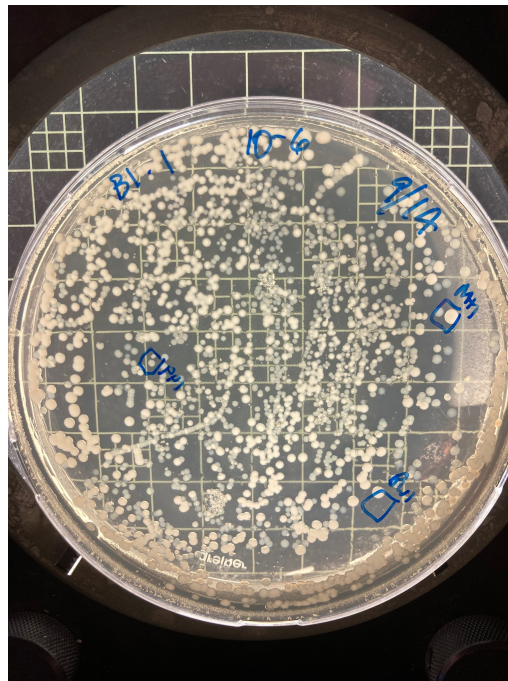
Need to Identify Many More Species in Each Sample



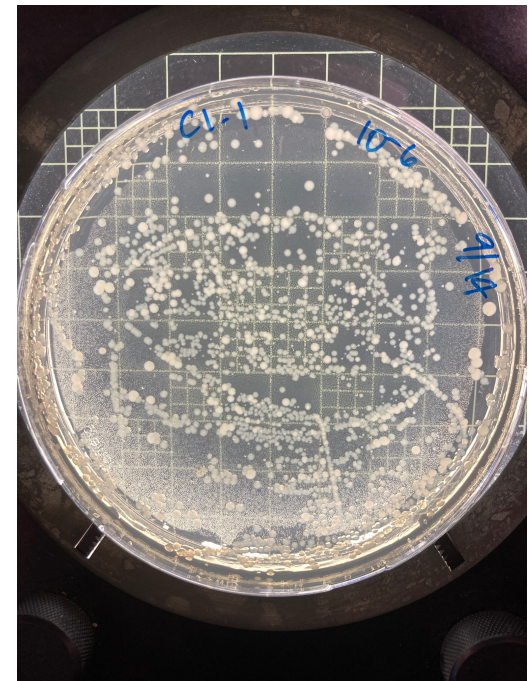
A



B



C





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Identification of Electricigenic Bacteria

Accurate and Sensitive Species Identification Using qPCR Based on Genome Sequences

A

B

C

	%		%		%
Agathobacter rectalis	11.13	Phocaeicola vulgatus	18.26	Faecalibacterium prausnitzii_C	9.41
Phocaeicola vulgatus	10.03	Coprococcus eutactus_A	11.76	Phocaeicola vulgatus	7.12
Bifidobacterium longum	9.57	Faecalibacterium prausnitzii	10.33	Anaerostipes hadrus_A	6.24
Anaerostipes hadrus	9.45	Agathobacter rectalis	8.65	Agathobacter rectalis	5.99
Bifidobacterium pseudocatenulatum	9.45	Collinsella aerofaciens	6.03	Dorea_A longicatena	5.48
Ruminococcus_E bromii_B	8.15	Phocaeicola dorei	5.69	Faecalibacterium prausnitzii	5.17
Faecalibacterium prausnitzii_D	7.10	Faecalibacterium prausnitzii_C	5.14	Ruminococcus_E bromii_B	4.74
Faecalibacterium prausnitzii	5.85	Dorea_A longicatena	3.62	Blautia_A obeum	4.55
Collinsella aerofaciens	4.07	Bariatricus comes	3.32	Anaerobutyricum hallii	4.48
Alistipes putredinis	3.82	Mediterraneibacter lactaris	3.14	Faecalibacterium prausnitzii_D	3.70
Dorea_A longicatena	2.77	Anaerostipes hadrus_A	3.10	Lachnospira eligens_A	3.61
Phocaeicola dorei	2.13	Bacteroides uniformis	2.80	Collinsella aerofaciens	3.25
Porphyromonas_A somerae	2.04	Porphyromonas_A somerae	2.74	Faecalibacterium prausnitzii_I	3.17
Anaerobutyricum hallii	1.63	Anaerostipes hadrus	2.30	Alistipes putredinis	3.13
Faecalibacterium prausnitzii_C	1.59	Faecalibacterium prausnitzii_G	1.97	Bariatricus comes	2.91
Blautia_A obeum	1.47	Faecalibacterium prausnitzii_I	1.95	Anaerostipes hadrus	2.58
Dorea formicigenerans	1.34	Anaerobutyricum hallii	1.27	Roseburia inulinivorans	2.55
Faecalicatena torques	1.23	Blautia_A obeum	1.12	Barnesiella intestinihominis	2.45
Bacteroides ovatus	1.06	Coprococcus_A catus	1.02	Porphyromonas_A somerae	2.42
Barnesiella intestinihominis	0.97	Dorea formicigenerans	0.98	Bifidobacterium adolescentis	2.20
Eubacterium_I ramulus_A	0.63	Faecalicatena torques	0.85	Bacteroides uniformis	2.16
Bilophila wadsworthia	0.59	Lachnospira eligens_A	0.82	Faecalicatena torques	2.10
Streptococcus salivarius	0.54	Faecalibacterium prausnitzii_D	0.79	Phocaeicola dorei	2.02
Bacteroides uniformis	0.47	Eubacterium_I ramulus_A	0.53	Dorea formicigenerans	2.00
Dorea_A longicatena_B	0.44	Roseburia inulinivorans	0.43	Faecalibacterium prausnitzii_G	1.09
Roseburia inulinivorans	0.38	Bacteroides thetaiotaomicron	0.37	Bilophila wadsworthia	0.93
Ruminococcus_B gnavus	0.31	Dorea_A longicatena_B	0.34	Coprococcus_A catus	0.57
Coprococcus_A catus	0.30	Bacteroides ovatus	0.22	Bacteroides ovatus	0.56
Parabacteroides distasonis	0.26	Porphyromonas uenonis	0.18	Methanobrevibacter_A smithii	0.48
Faecalibacterium prausnitzii_G	0.25	Bacteroides xylanisolvens	0.17	Bacteroides thetaiotaomicron	0.36

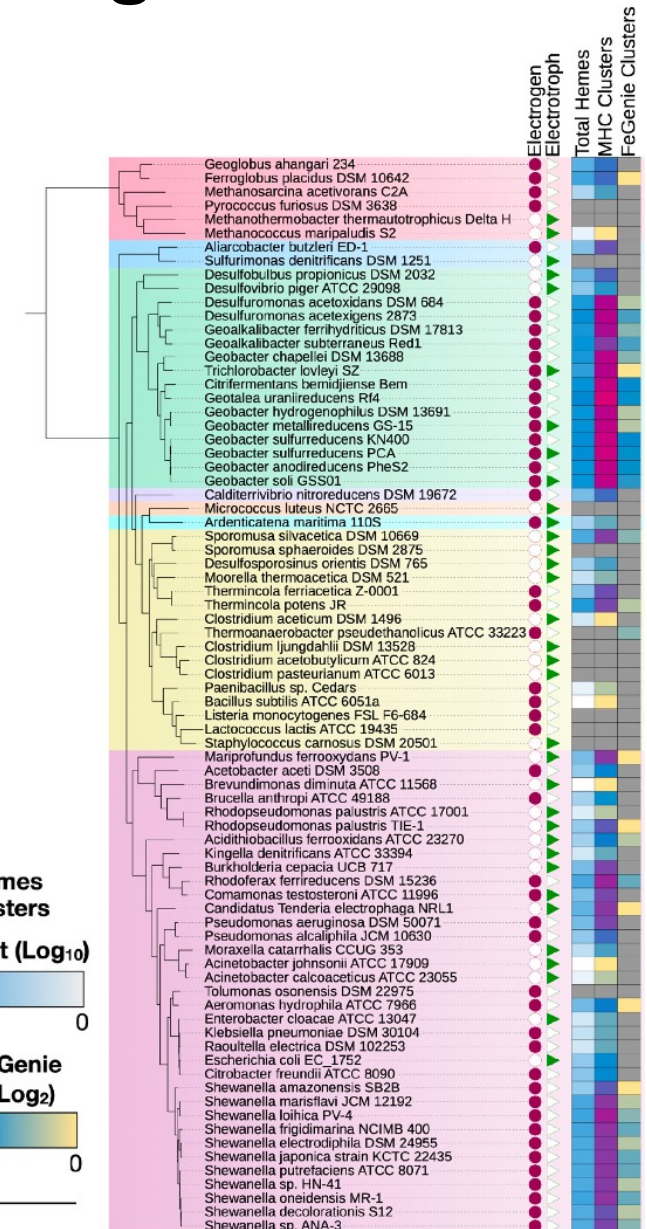
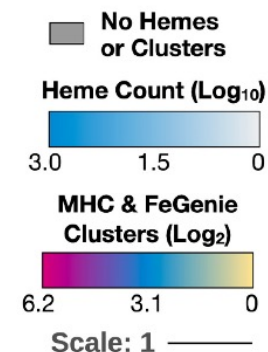
Sensitive
to
~0.01%

Identify Potential EET-Enabling Genes

Large-scale prediction of outer-membrane multiheme cytochromes uncovers hidden diversity of electroactive bacteria and underlying pathways

Arkadiy I. Garber^{1*}, Kenneth H. Nealson² and Nancy Merino^{3*}

¹Biodesign Center for Mechanisms of Evolution, School of Life Sciences, Arizona State University, Tempe, AZ, United States, ²Department of Earth Sciences, University of Southern California, Los Angeles, CA, United States, ³Biosciences & Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA, United States

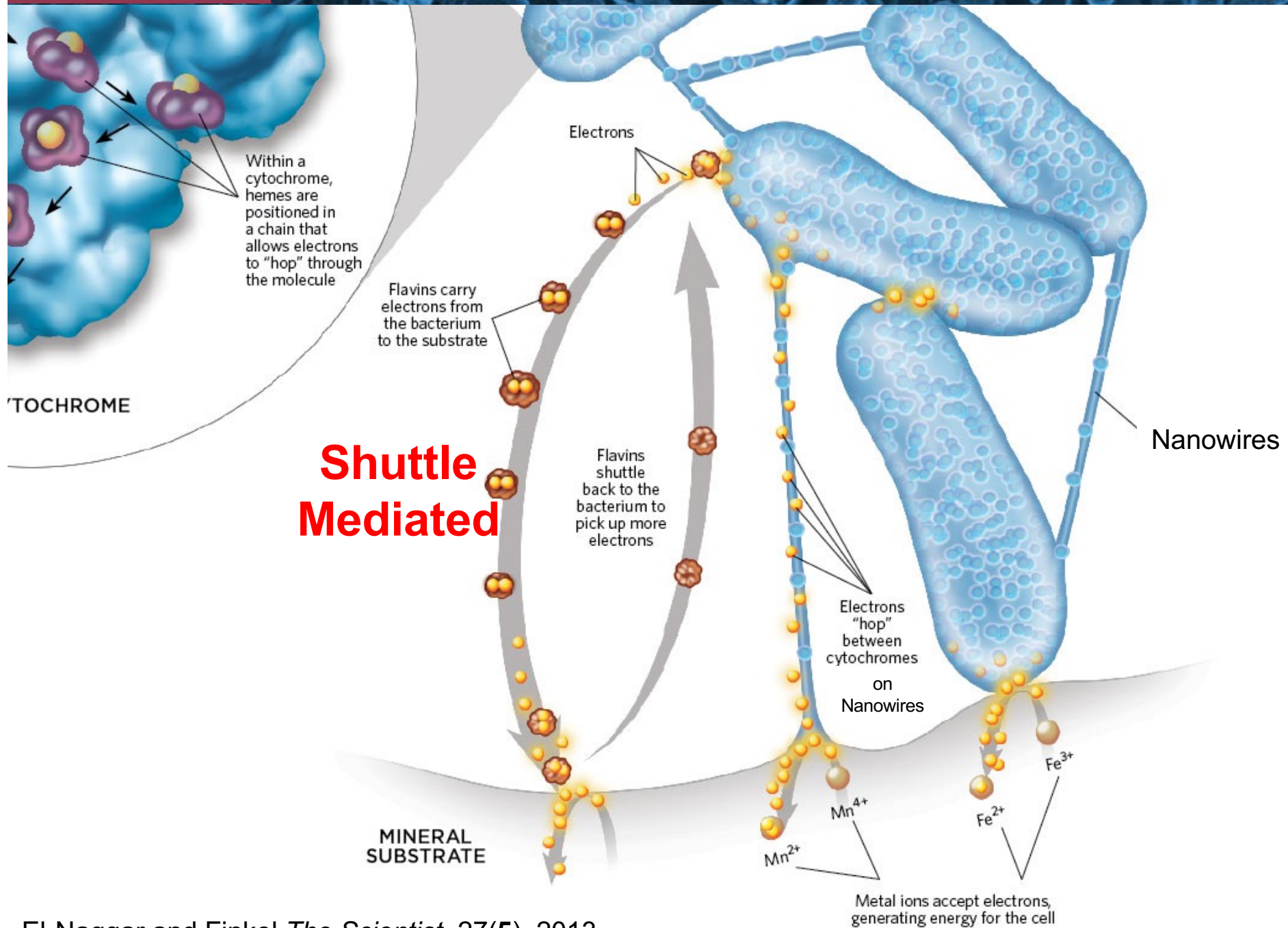


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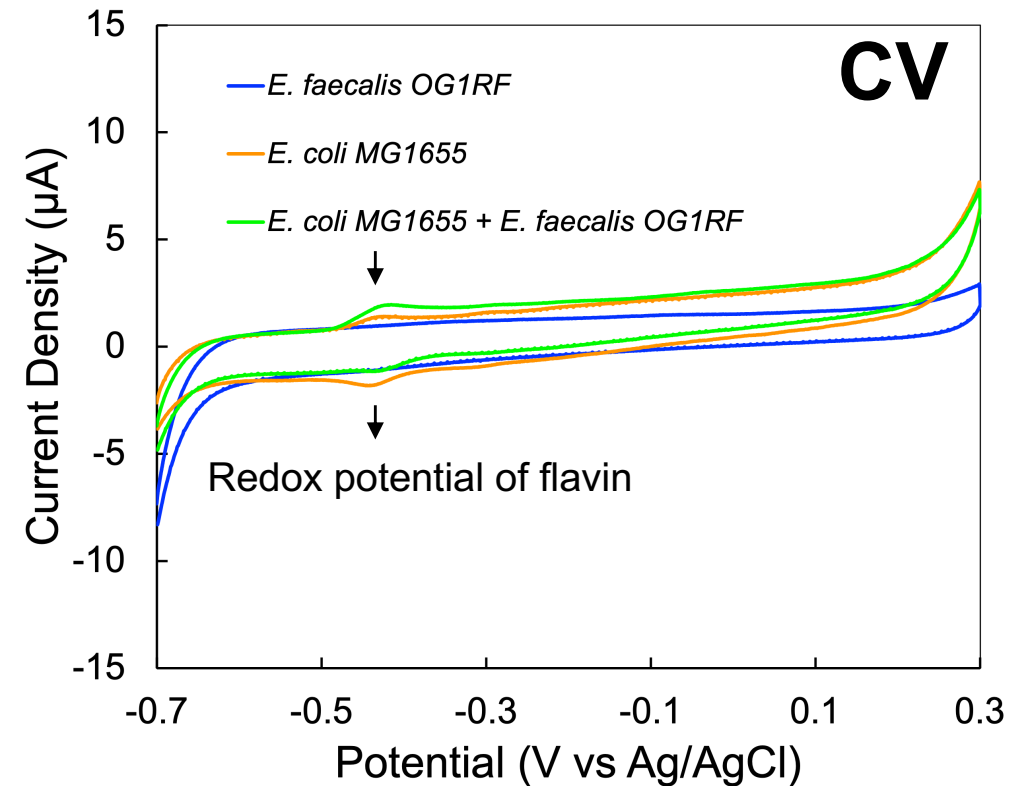
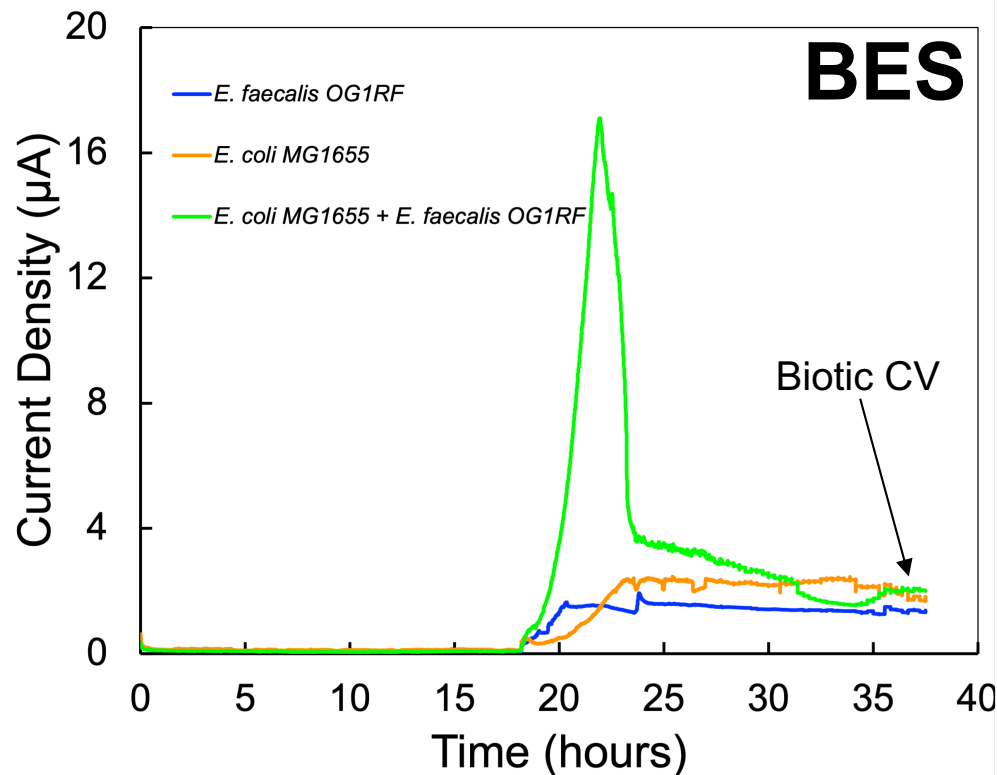


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Mechanism of Synergy

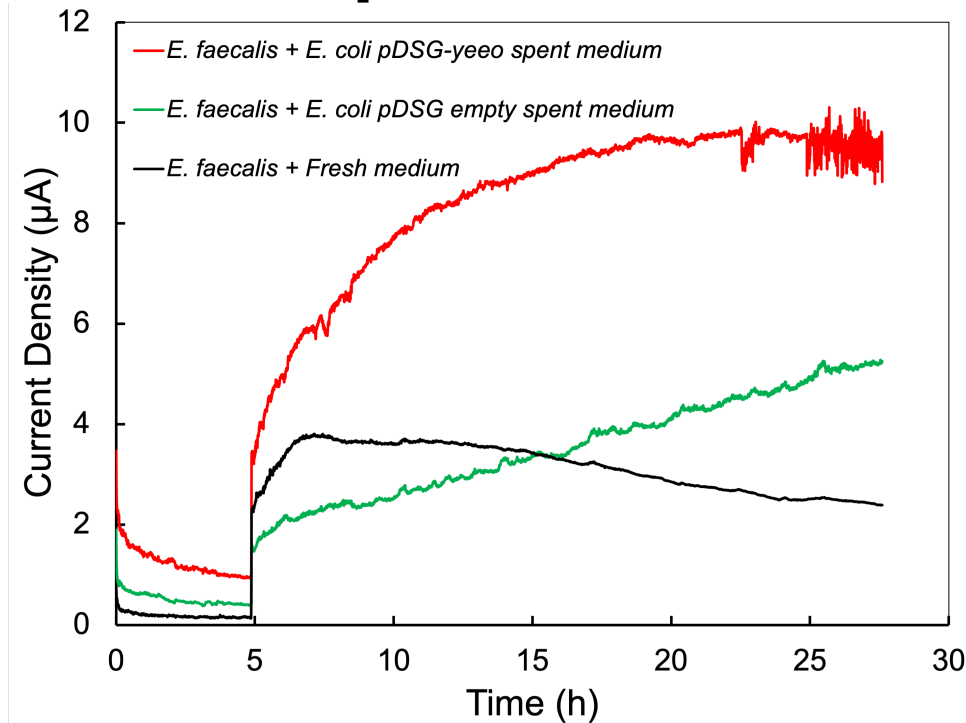


Flavin Detected Under Conditions of EET Synergy

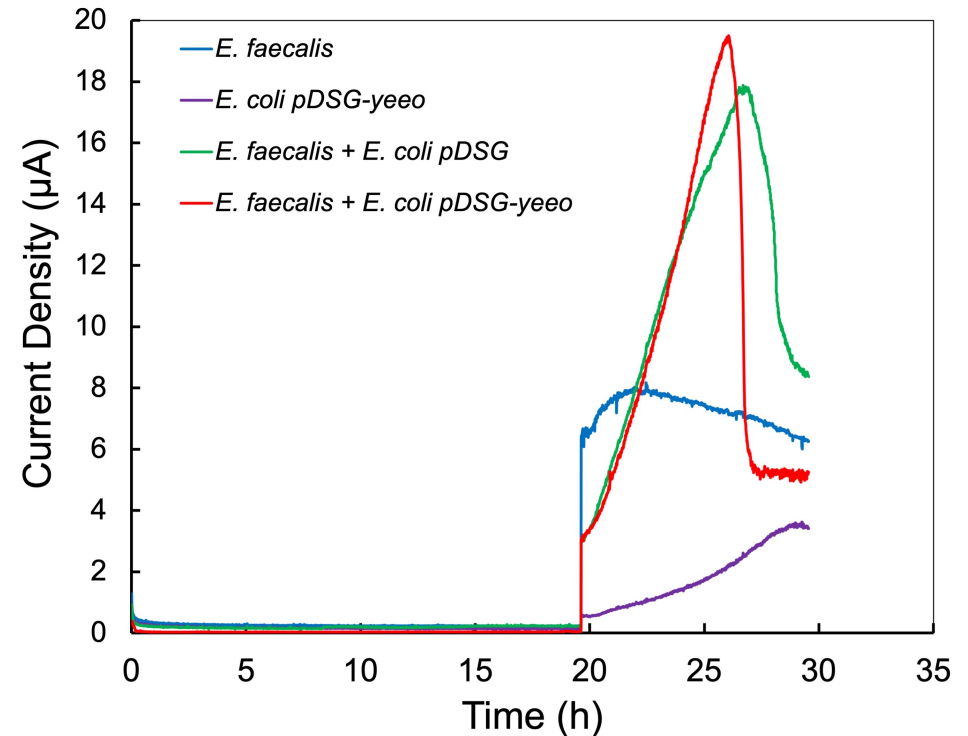


Overexpression of *E. coli* Flavin Transporter (*yeeO*) Increases Current Production

In Spent Medium

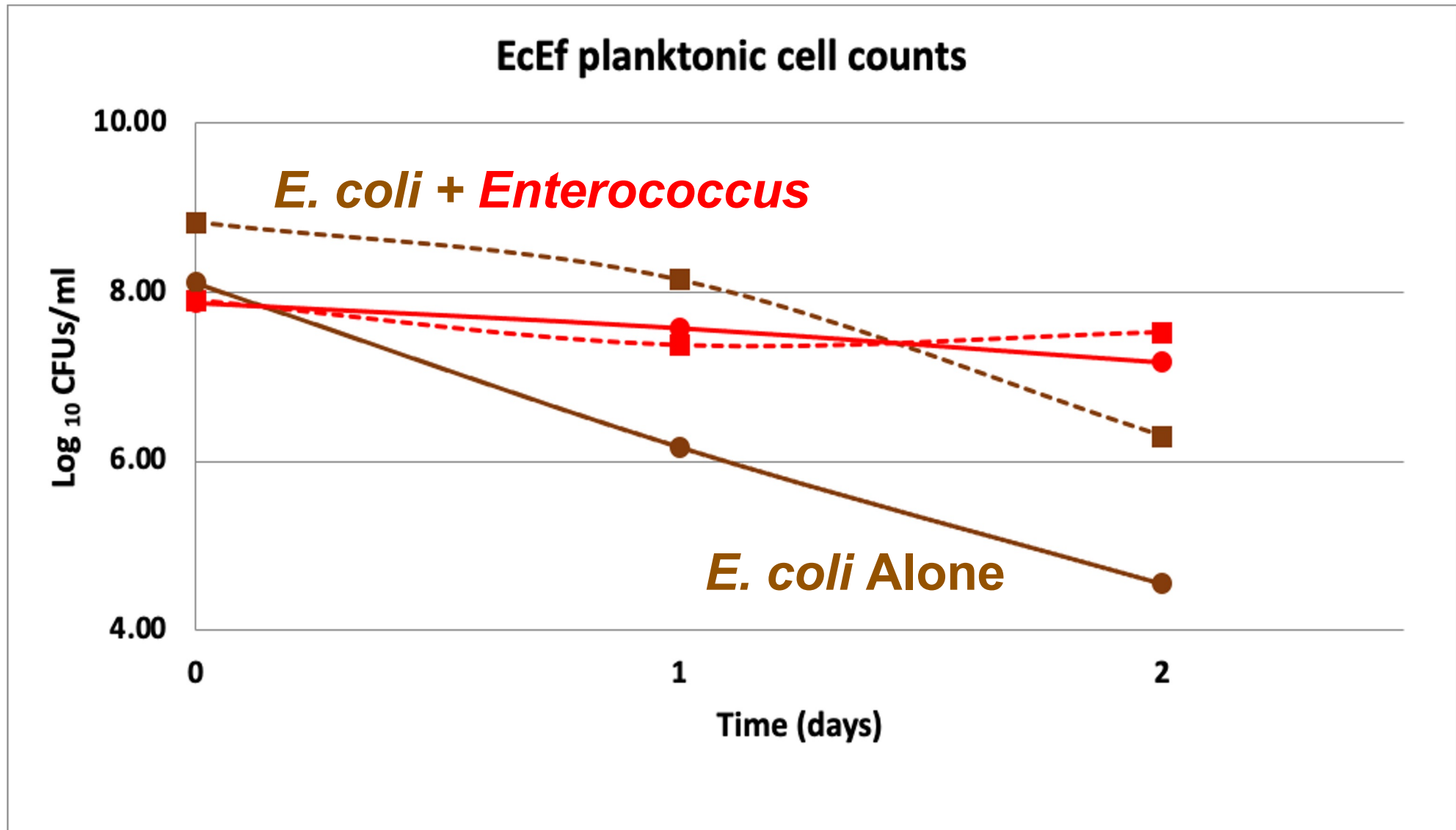


In vivo



*So E. coli is providing Enterococcus with something to “breathe”...
...Does E. coli get something out of it?*

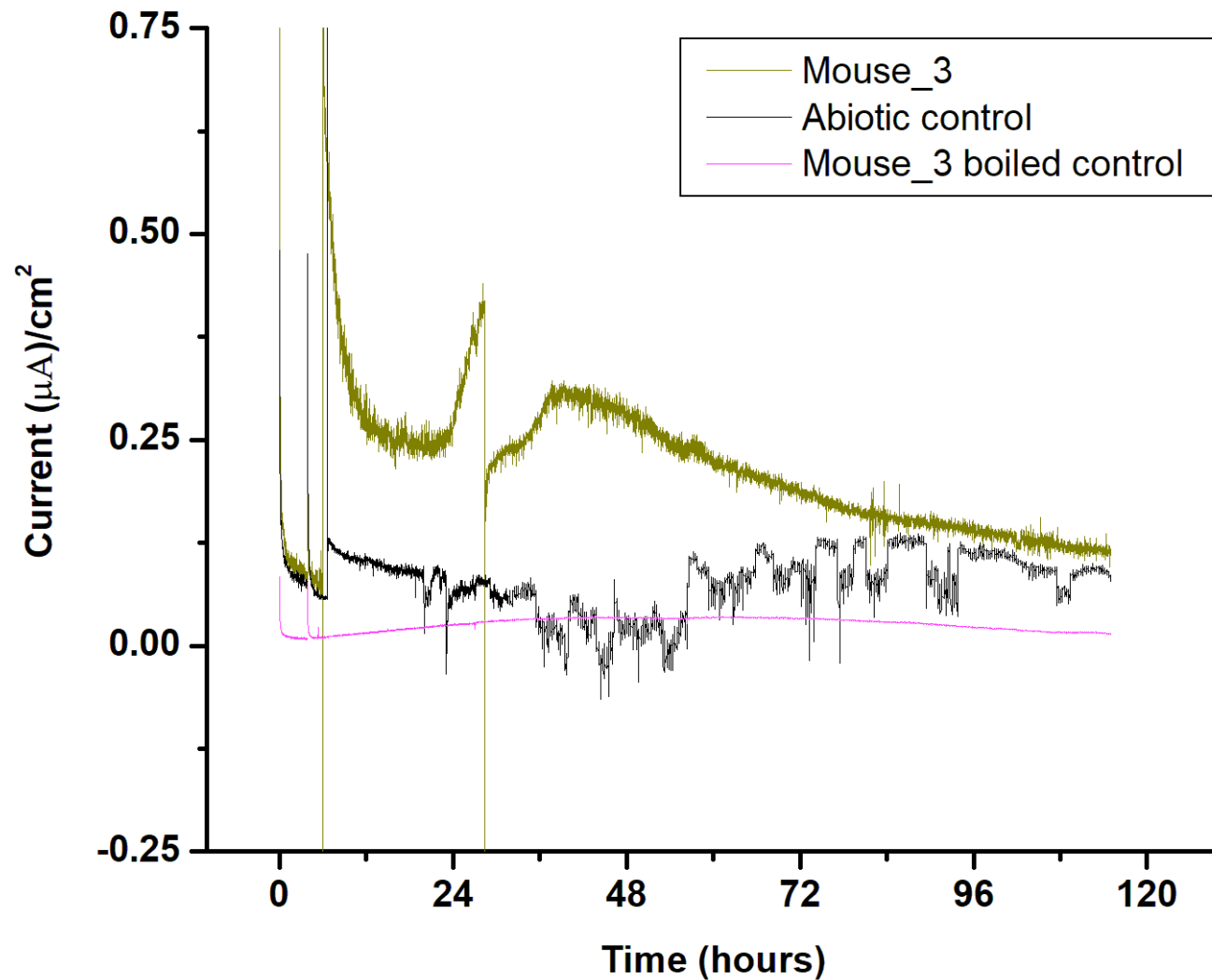
E. coli Yield & Survival Enhanced in the Presence of *Enterococcus*



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Step 1 – Create “humanized” mice



EET occurs in the Mouse Gut Microbiota



Preparation of a “Humanized” Mouse Gut Microbiome Model

Since mice are not raised gnotobiotically, we must first remove the native microbiome before replacement with human gut microbiota or specific species.

Antibiotics are introduced orally over 14 days:

ampicillin (1mg/mL)

neomycin (1mg/mL)

vancomycin (0.5mg/mL)

metronidazole (1mg/mL)

Preparation of a “Humanized” Mouse Gut Microbiome Model

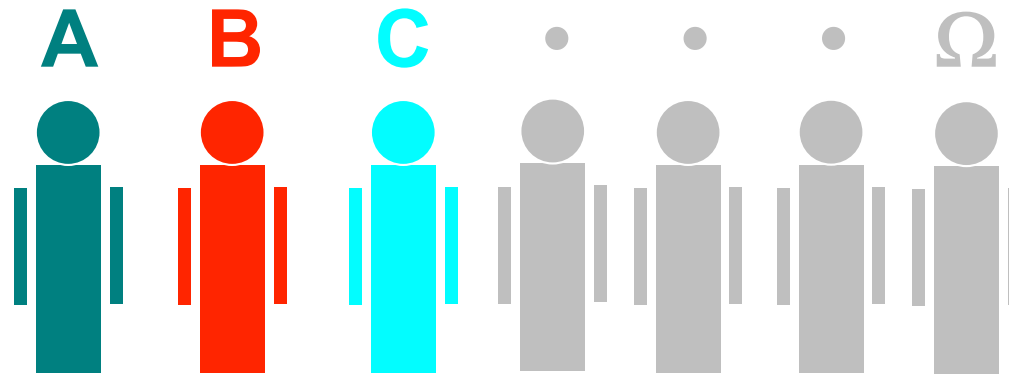


Mouse gut microbiota
reduced up to
1000-fold

“Humanized” Mouse Sensorimotor, Behavior & Nociception

- Mechanical sensitivity
- Temperature sensitivity
- Strength and coordination assessment
- Novel Object Recognition (NOR) assay
- Maze assays
- Open Field (OF) test

- Continue to Fully Characterize Gut Microbes Capable of EET and Develop Proxy Microcosms



- Identify Mechanisms of Gut EET at the Biophysical, Biochemical & Genetic Levels
- Continue Development of a "Humanized" Mouse Model System to Determine the Role of Gut EET on Animal Behavior, Mechano/Thermo Sensation & Nociception



Acknowledgments

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Nicolaus Jakowec ***Andrew Janeiro*** ***Zlatas Serebnitskiy*** ***Melissa Finegan***

Moh El-Naggar, Ph.D. ***Fengjie Zhou*** *Amruta Karbelkar, Ph.D.*

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David McKemy, Ph.D. ***Chenyu Yang*** ***Matt Dean, Ph.D.*** ***Caleb Ghione***

Branchpoint Biosciences ***Ben Tully, Ph.D.*** ***Christopher Corzett, Ph.D.***





Genetic, Biophysical, and Behavioral Characterization of Microbial Extracellular Electron Transport in Human and Animal Systems

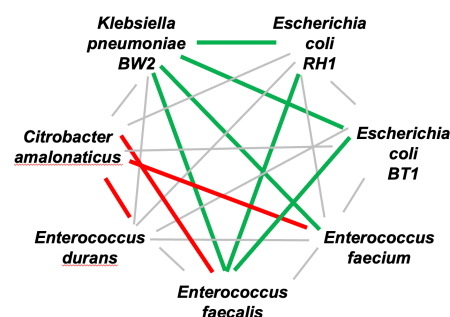


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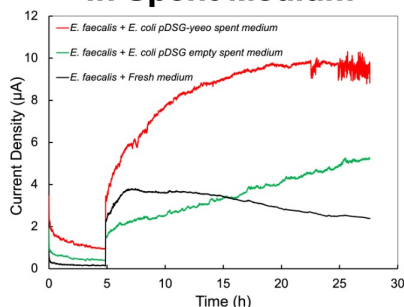


Objectives

- (1) Understand the electrochemical behavior of bacteria;
- (2) Develop electrode-based cultivation;
- (3) Identify human and environmental microbes performing Extracellular Electron Transport.



In Spent Medium



Technical Approaches

- (1) Culture of microbes in BioElectrochemical Systems, derived from Microbial Fuel Cell reactors;
- (2) Real-time analysis EET;
- (3) Probe of bacteria-electrode interactions using chemical and biophysical techniques

Uniqueness

- Labs with complementary expertise in microbial physiology & ecology, molecular genetics & genomics, and biophysics & biochemistry.
- Informs understanding of “gut” health, as well as roles of microbial community members in many environments.

Accomplishments

- High resolution characterization of gut microbiome communities.
- Have identified a mechanism of synergy between *E. coli* and *E. faecalis*, including the release of flavins by *E. coli* for *Enterococcus* EET.
- Identified more pairs of synergizing gut EET microbes
- Developing “humanized” mouse model
- 2023–2024: 6 articles published or in preparation.
- 2023–2024: 5 invited or keynote presentations by the Co-I's. 11 conference talks or posters by team's students and postdoctoral researchers.

DoD Benefit

- Better understanding of EET of microbes associated with humans can improve human performance and overall health.
- Synthetic biology applications for the development of DoD-relevant novel materials.

Collaborators

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