



Unique R&D pathways from agricultural microbiology to human subjects research: Development of biofertilizers and the human gut microbiome

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Outline

- ❑ **Part-I: Agricultural microbiology:** Development of a drought-tolerant inoculant (biofertilizer).
- ❑ **Part-II: Rhizosphere soil microbiomes:** Understanding of changes in microbial communities for the soybean high-yield state (Nebraska).
- ❑ **Part-III: Human gut microbiomes:** Effects of fermented soybean products (FSPs) on human gut health.



Agricultural microbiology: dealing with plant-associated microbes

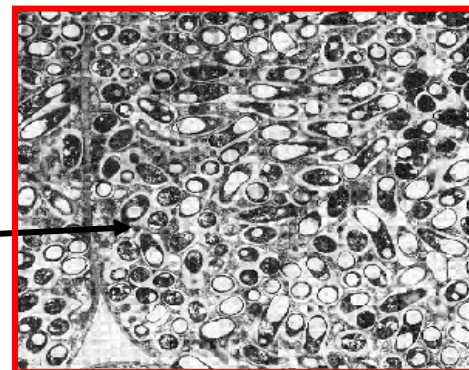
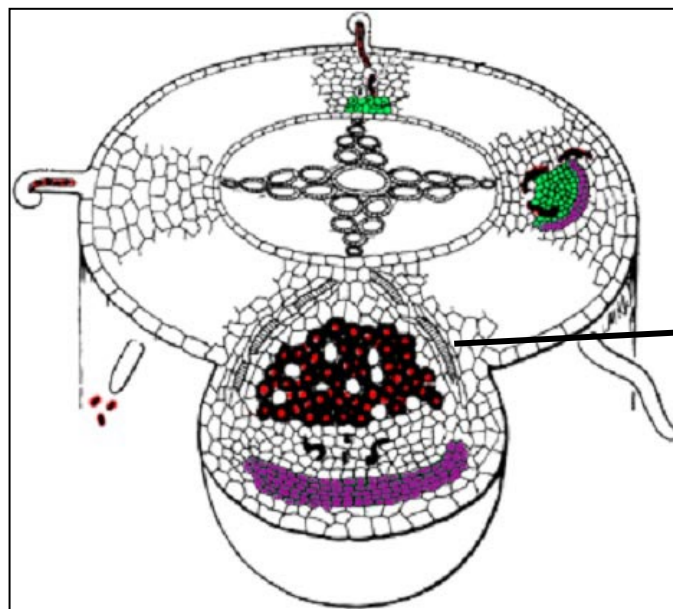
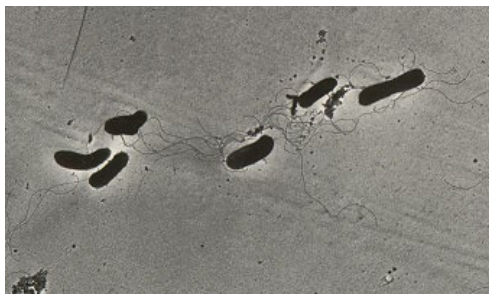
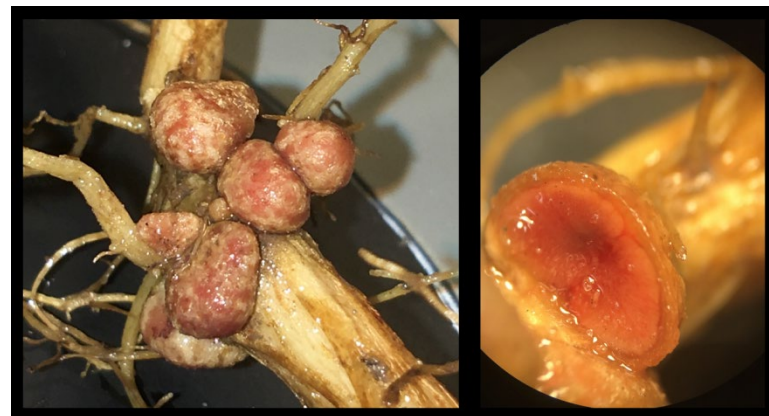
Part-I

- ❑ Many agricultural microbiologists focus on plant disease, which is more likely in the field of plant pathology.
- ❑ A few microbiologists study on beneficial microbes in plants.
- ❑ As a beneficial microbe, rhizobia can fix atmospheric nitrogen into ammonia, so called “nitrogen fixation”.
- ❑ Rhizobia can establish the symbiotic relationship with legume plants such as soybeans, chickpeas, alfalfa, etc.



Soybean symbiont *Bradyrhizobium japonicum* as an inoculant (biofertilizer)

- *Bradyrhizobium japonicum*
 - Nitrogen (N)-fixing microorganism
 - Nodule formation on soybean roots
 - Microbial inoculant (biofertilizer)



$N_2 \rightarrow NH_3$
nitrogenase



Importance of *B. japonicum* for biological nitrogen fixation (BNF) in soybean

Part-I



Not inoculated



Inoculated





Why are N-fixing biofertilizers important?

Because of problems associated with chemical nitrogen fertilizers

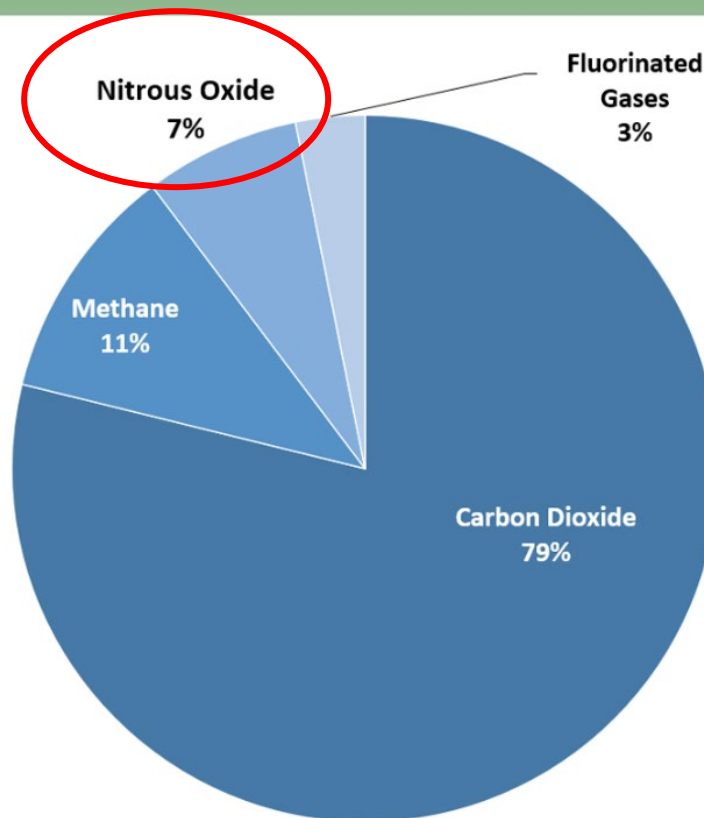
- ☐ Economical problems: high price of chemical nitrogen fertilizers.
- ☐ Environmental problems: greenhouse gas (GHG) emission.
- ☐ Ecological problems: eutrophication.



Environmental problems: overview of greenhouse (GHG) gases

Part-I

Overview of U.S. Greenhouse Gas Emissions in 2020



In agriculture, synthetic nitrogen fertilizers have been a key factor in the increased crop yields. However, they are also significant source of greenhouse gas (GHG) emissions from global agricultural production, resulting in significant emissions of

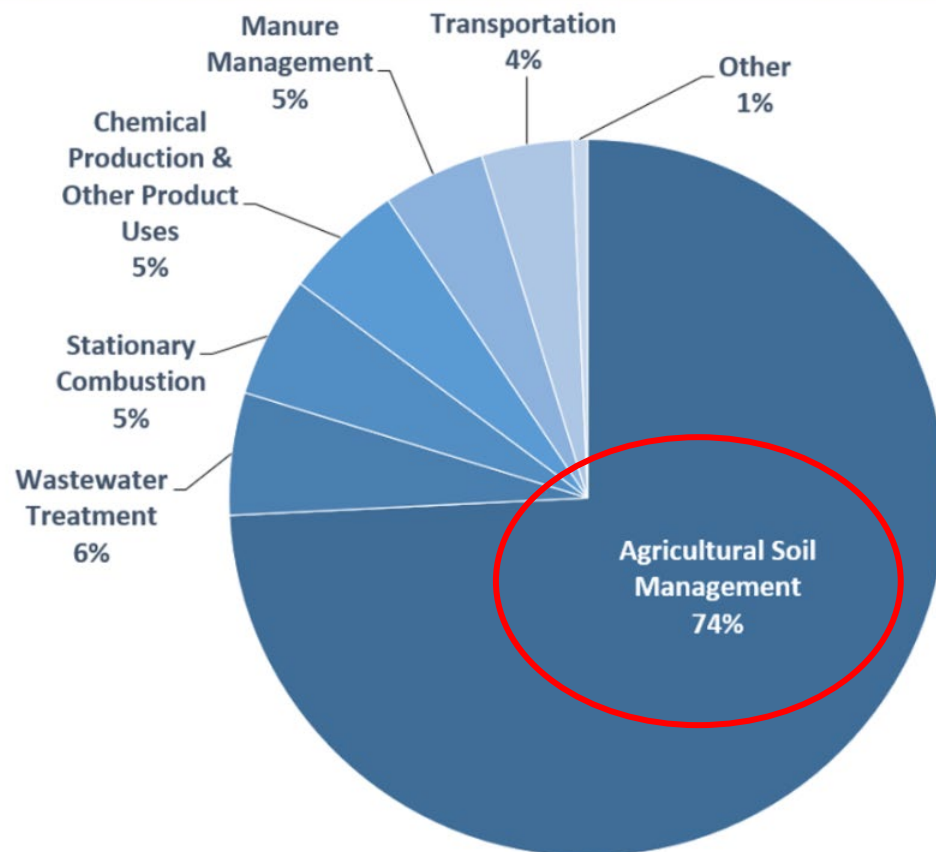
nitrous oxide (N_2O), a GHG with approximately 300 times the global warming potential of carbon dioxide (CO_2).



Environmental problems: Nitrous oxide (N_2O) emissions

Part-I

2020 U.S. Nitrous Oxide Emissions, By Source



- Major source: synthetic nitrogen fertilizers.

- Through the denitrification process which is typically anaerobic respiration by soil microorganisms.



Chemical nitrogen fertilizers-associated problems

Part-I

Hypoxia: “low oxygen”
due to excess nutrients
in water.

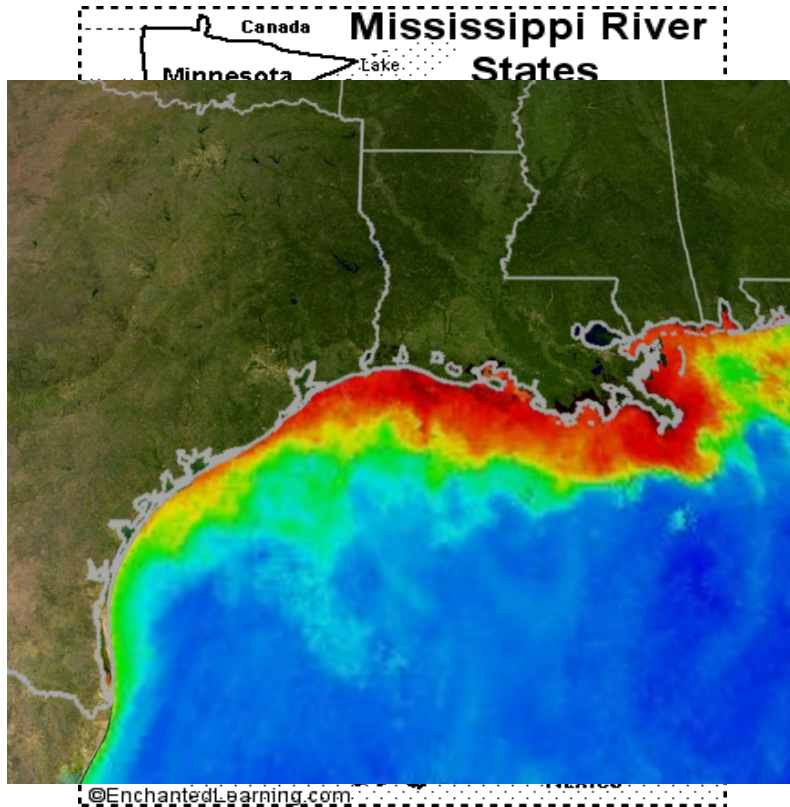




Chemical nitrogen fertilizers-associated problems

Part-I

Hypoxia: “low oxygen”
due to excess nutrients
in water.



The Gulf of Mexico

The hypoxic zone is thought to be caused primarily by excess nitrogen delivered from the Mississippi River.



Chemical nitrogen fertilizers-associated problems: algal bloom

Part-I



CNN, 2013



National Geographic, 2013



Business Insider, 2013



National Geographic, 2013



Are N-fixing biofertilizers perfect to solve all problems?

Biofertilizers are live microorganisms. Therefore.....

- ☐ Standardization issue.
- ☐ Competition with indigenous microorganisms.
- ☐ Shelf-life issue.
- ☐ Environmental stresses (e.g., drought).



Why are we interested in drought and drought-tolerant inoculants (biofertilizers)?

- ☐ Drought is the most severe stress that affects soybean production in the U.S.
- ☐ Soybean breeders have developed drought-tolerant (slow-wilting) cultivars.
- ☐ Little effort has been made to develop a drought-tolerant inoculant for the synergistic effect on soybean yield under drought.



Isolation of drought-tolerant rhizobia in Texas

Goal: To isolate drought-tolerant rhizobial strains and test them in their capacity to increase soybean production in drought-prone areas.

Strategy: By using a molecular marker system, drought-tolerant strains were selected and used to inoculate soybean plants.



Molecular marker system used to evaluate desiccation-tolerant capability

From transcriptomics studies on *B. japonicum*, we identified 4 marker genes to evaluate desiccation/drought tolerance.

Gene	Marker ID	Function
bll0322	otsA	probable trehalose-6-phosphate synthase
bll0323	otsB	probable trehalose-phosphatase
blr6767	treS	trehalose synthase
blr2455	aceA	isocitrate lyase



Sampling sites in TX





Sampling of soybean nodules to screen more drought-tolerant rhizobia

Part-I





The three strains showing the most desiccation/drought tolerance

In the desiccation condition (24% RH), all showed better survival than the control strain (*Bradyrhizobium japonicum* USDA110).

Strain	% Survival (4 h)	% Survival (72 h)
USDA110 (control)	94.00 ± 0.16%	80.00 ± 0.98%
TXCP-01	96.61 ± 0.01%	98.55 ± 0.27%
TXVA-1107-3003	96.72 ± 0.40%	87.1 ± 0.61%
TXEA-1109-2000	100.0 ± 0.27%	93.9 ± 0.63%



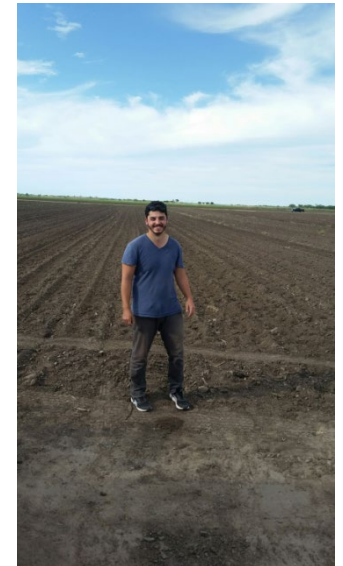
Field studies at Rio Farms located in Monte Alto, TX in 2016





Field test set-up in Rio Farms, TX

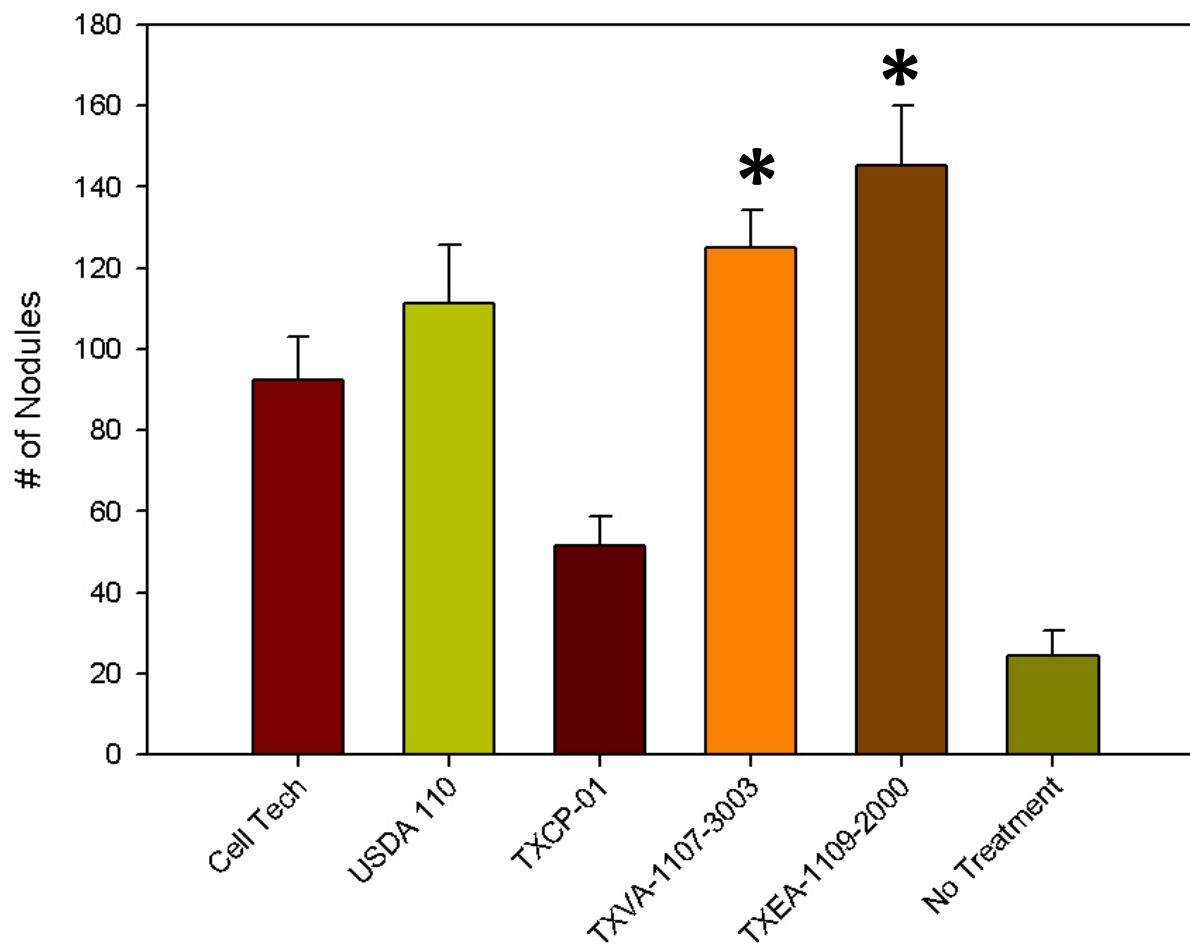
- ❑ 3 plots (one for each seed variety)
- ❑ 24 sub-plots/plot (8 strains with 3 biological replicates)
- ❑ 2 planted rows/sub-plot; 20 holes/row; 3 seeds/hole
=360 seeds/strain/plot; =2,880 seeds/plot; =8,640 seeds total
- ❑ Each seed variety was pre-treated by adding inoculant (10^9 cells /ml) and mixing until seeds were adequately covered.





Nodule # / Plant

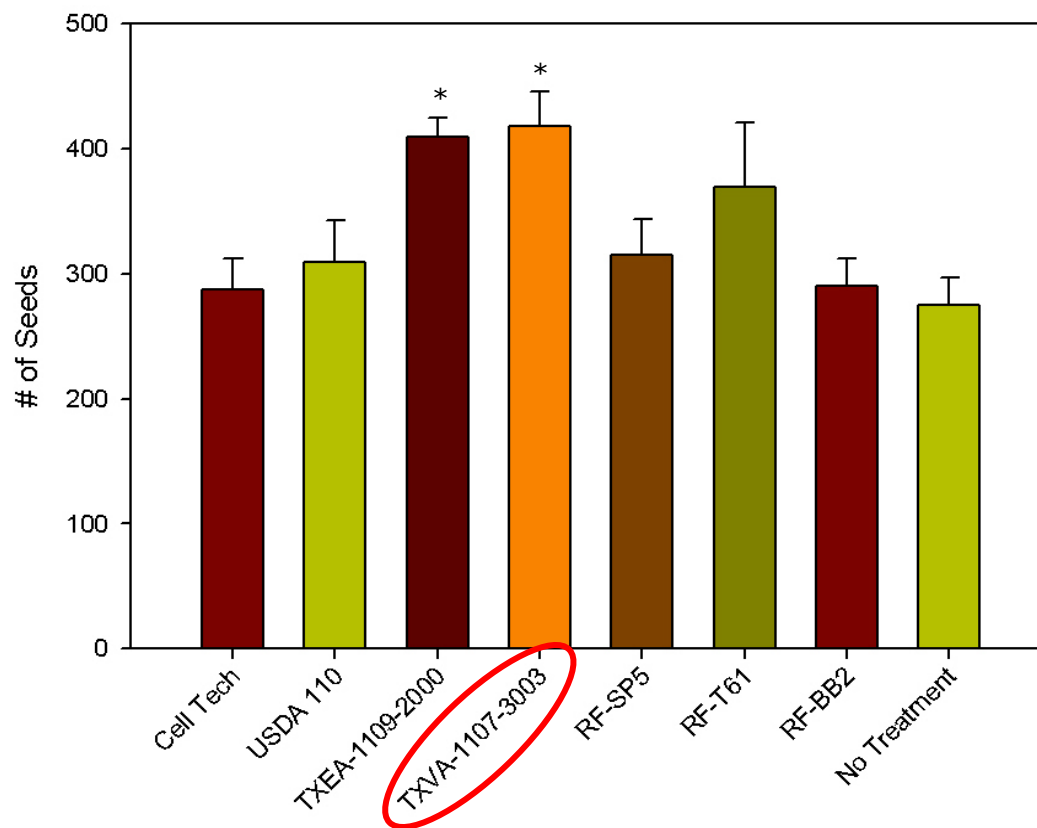
Avg Nodule # / Plant





Seeds # / Plant

Average# of Seeds/Plant (51410555)





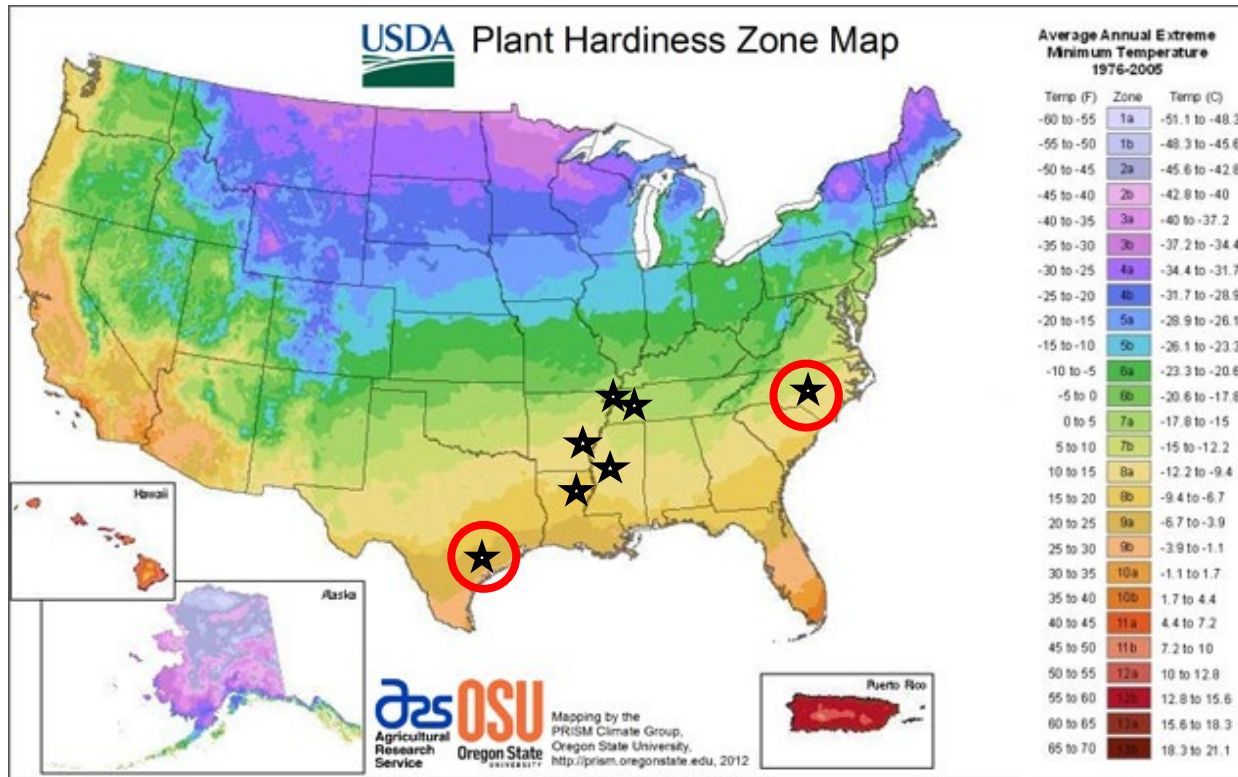
A novel drought-tolerant rhizobium was isolated and tested in Texas

■ **TXVA-1107-3003**

- It was isolated in Victoria county, TX.
- It showed the high desiccation/heat resistance.
- Desiccation-related genes were highly expressed in the TXVA strain compared to the type strain USDA110.
- It formed more nodules on soybean roots compared to the commercial inoculant (i.e., Cell-Tech).
- Previously, small-plot experiments demonstrated that TXVA enhanced soybean nodulation and growth.



2019 - 2021 field trials

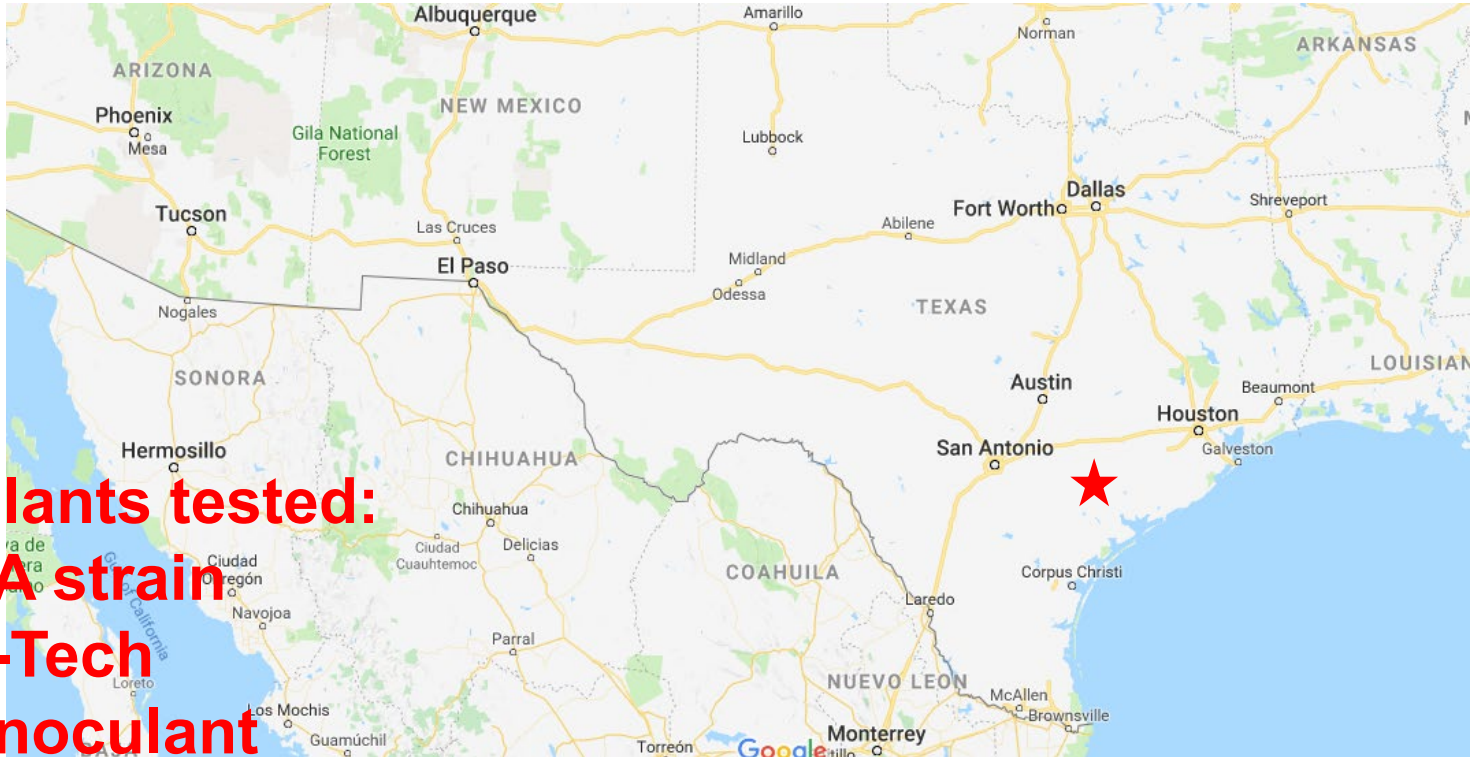


Inoculants tested:

- TXVA
- Cell-Tech (commercial inoculant)
- No inoculant



Field study in Yoakum, TX (2019)



Inoculants tested:

- TXVA strain
- Cell-Tech
- No inoculant

Soybean cultivar planted:

- S52RS86 (MG 5E)



Soybean yield in Yoakum, TX (2019)

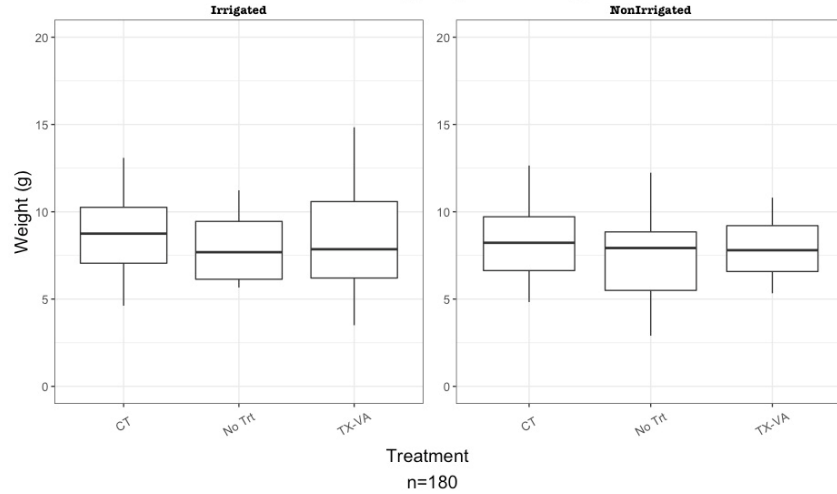
- Planting date: Apr. 2
- Sampling date: May 22
- Harvesting date: Sep. 6

<i>Final Yield (Bushels/acre)</i>	Yoakum, TX	
	Irrigated	Non-irrigated
TXVA	27.0	22.0
No Treatment	26.7	19.4
Cell-Tech	26.8	18.9

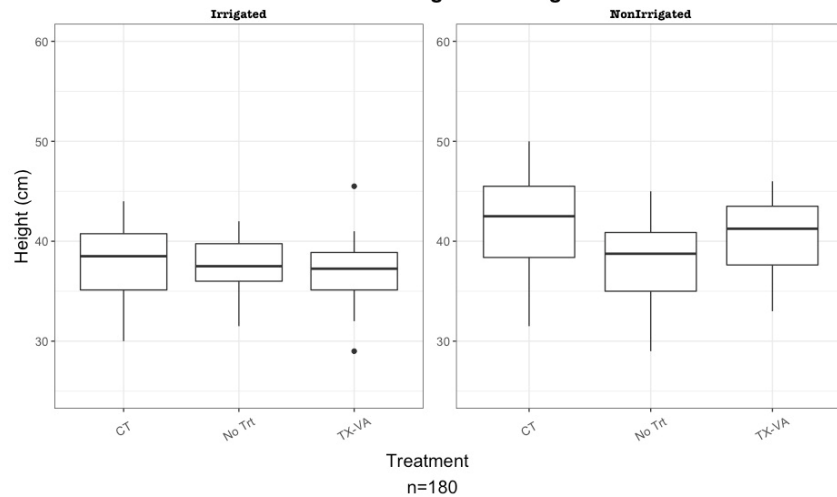


Soybean height and dry weight in Yoakum, TX

Yoakum TX Avg. Dry Plant Weight

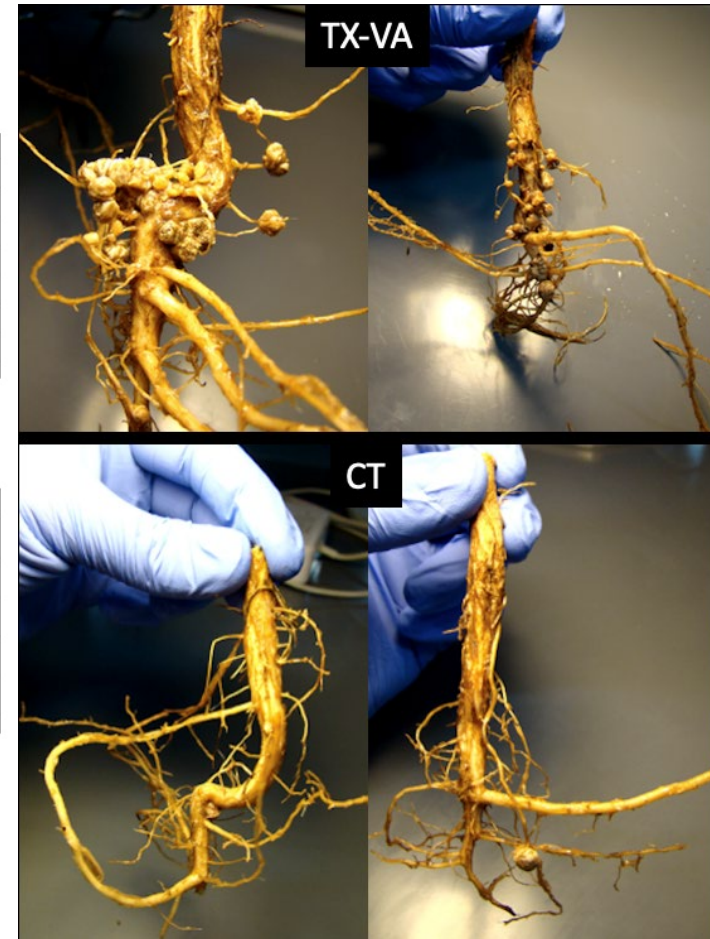
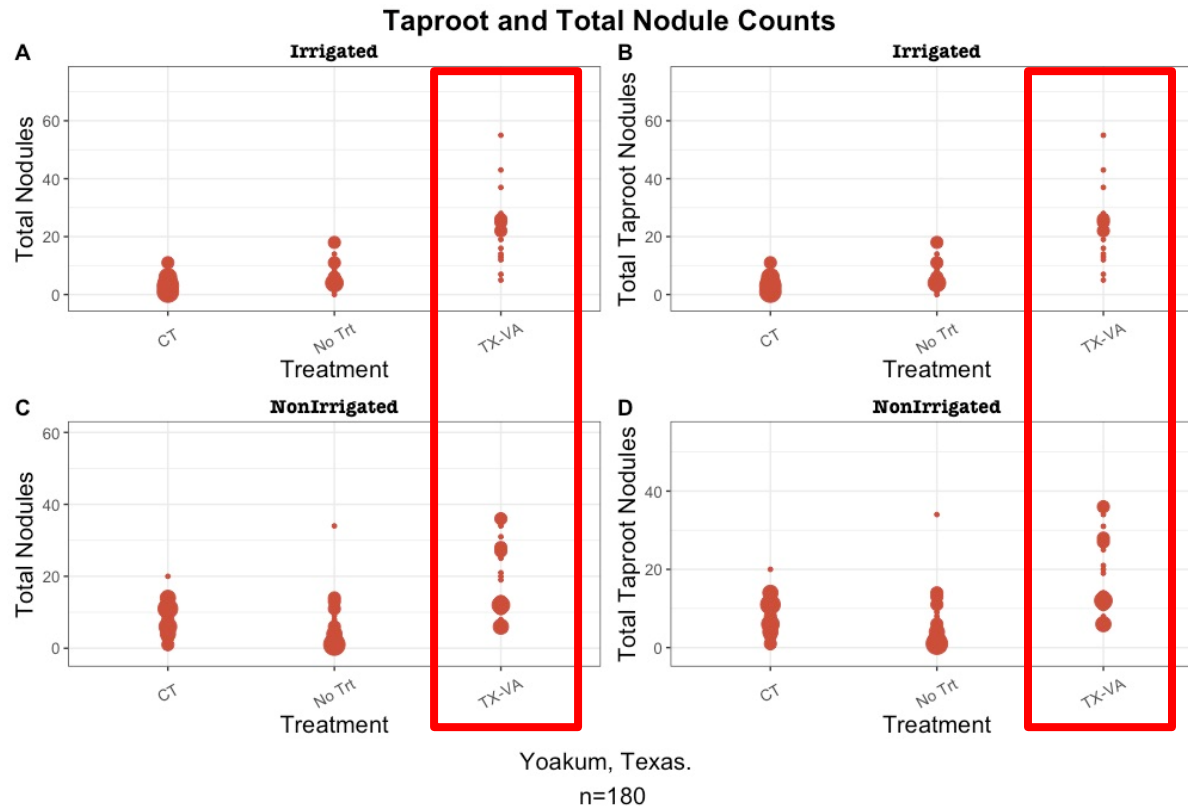


Yoakum TX Avg. Plant Height





Nodulation in Yoakum, TX





Nodule pictures



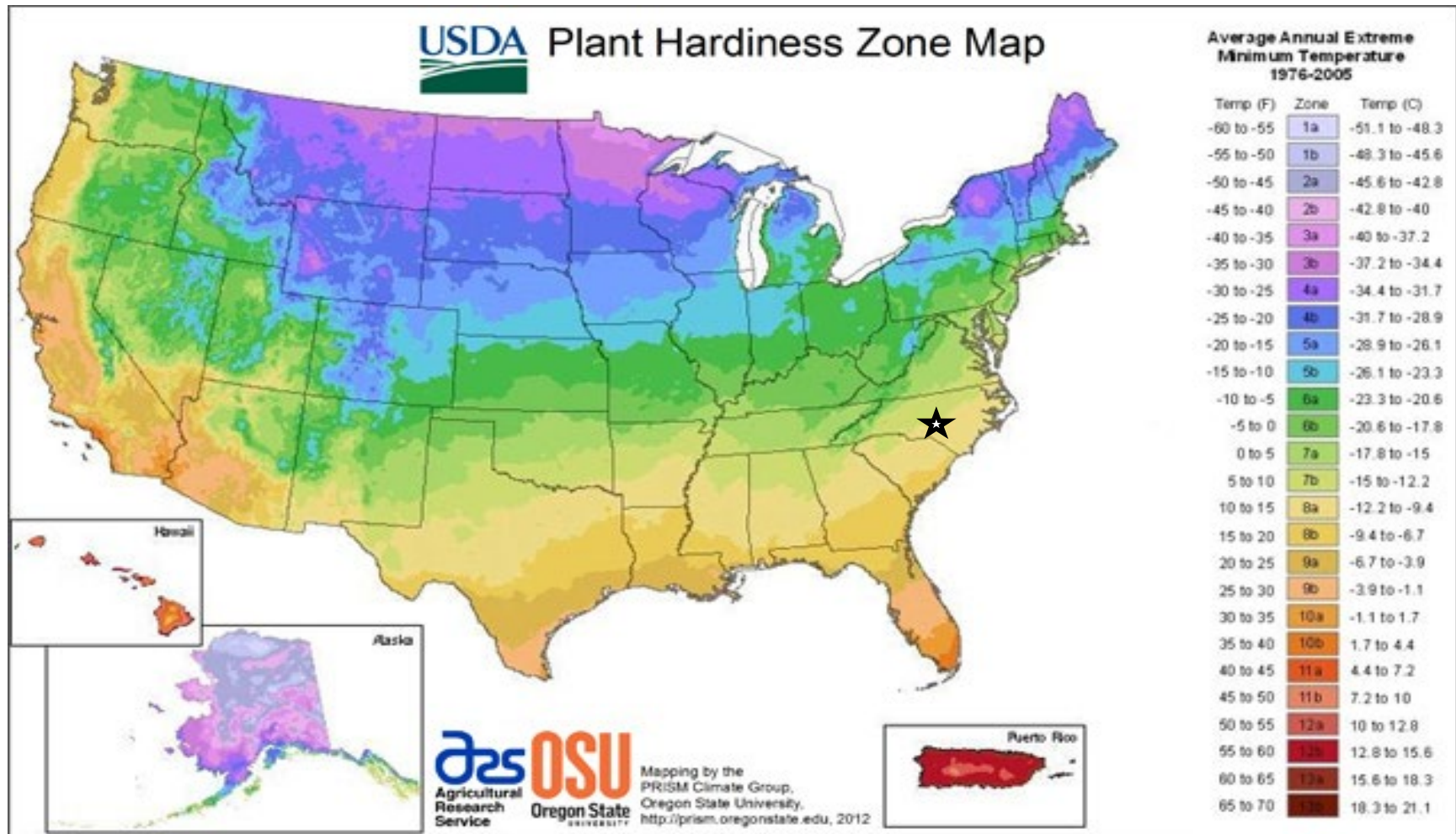
Not inoculated



Inoculated



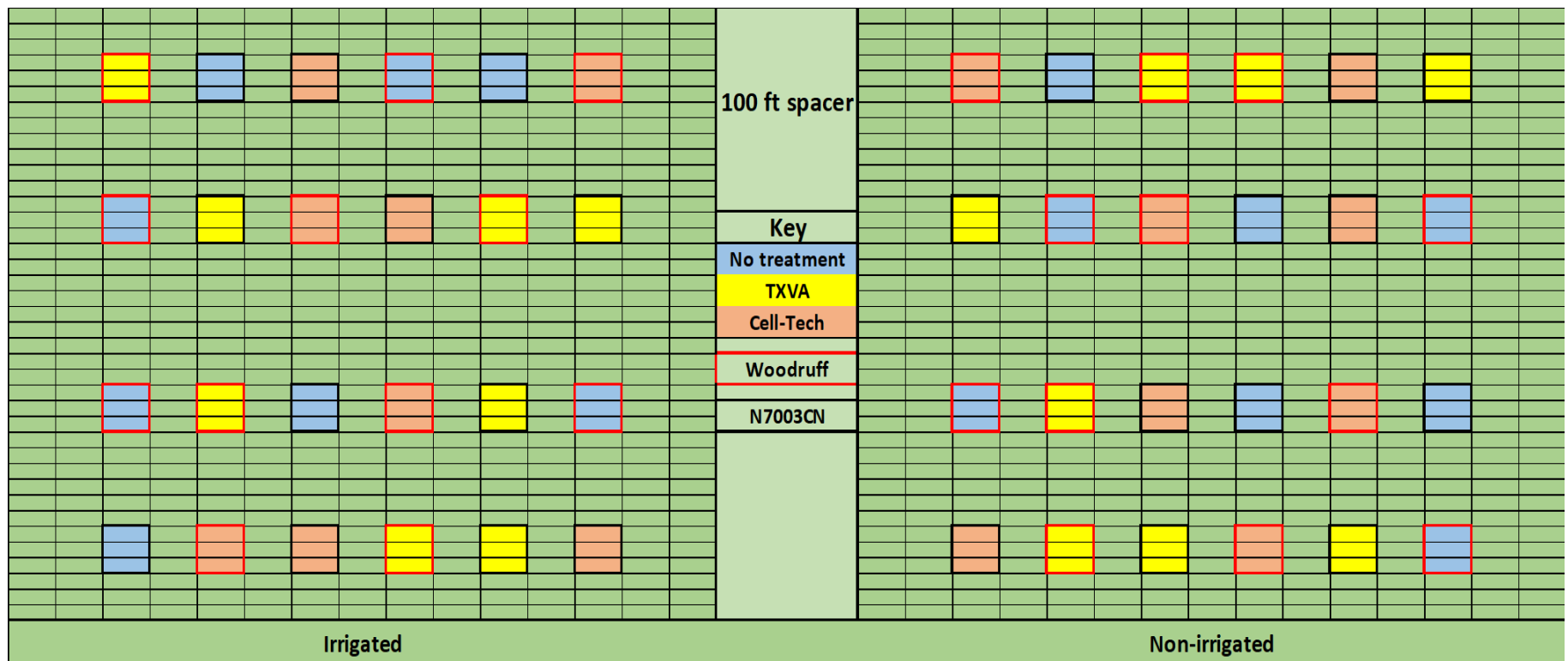
Field study in Sandhills, NC (2019 and 2020)





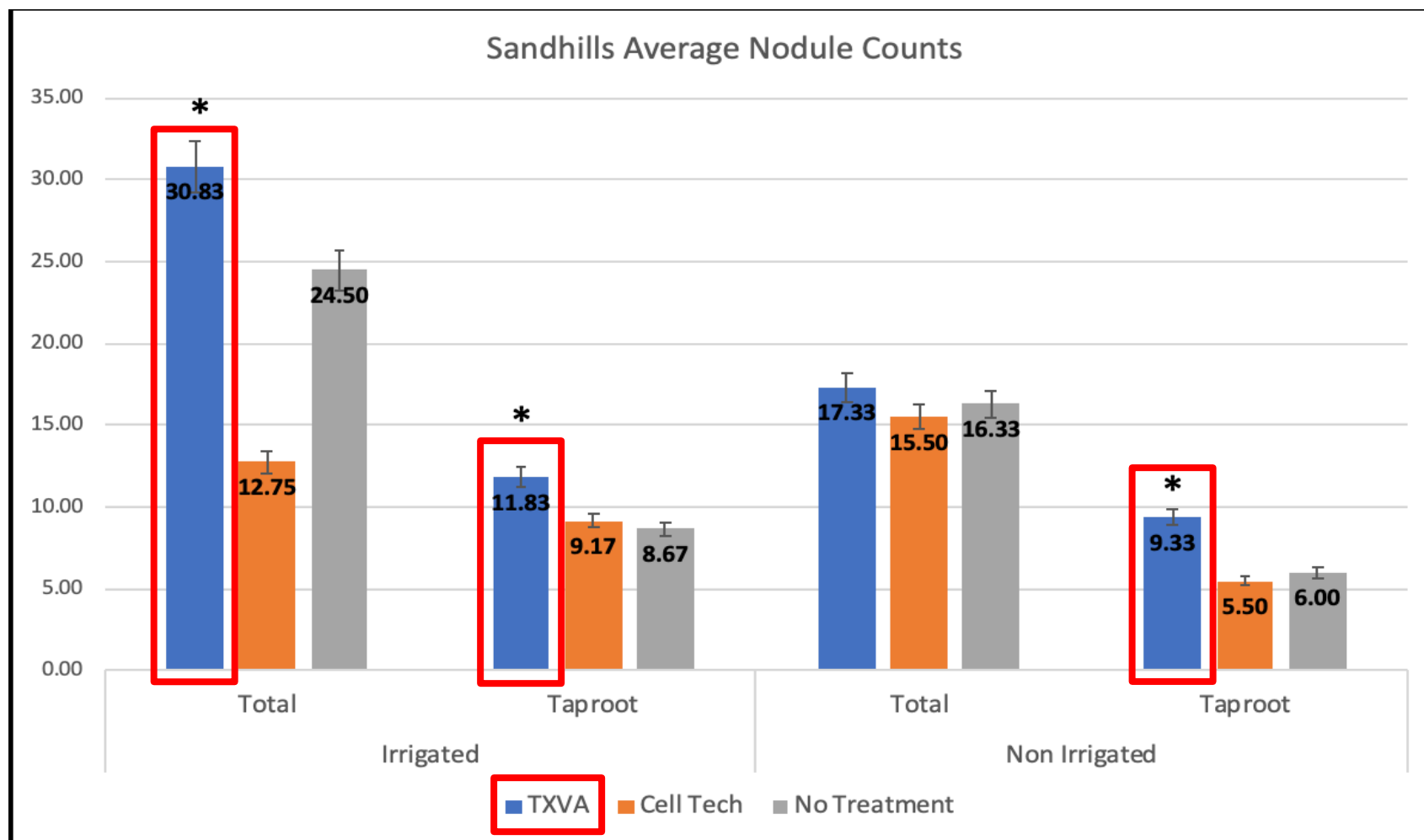
RCB design in Sandhills, NC

- The irrigated and non-irrigated portions were separated by a 100-foot space. Each plot consisted of 3 rows and was bordered on all sides to prevent interference among treatments.





Nodulation in Sandhills, NC





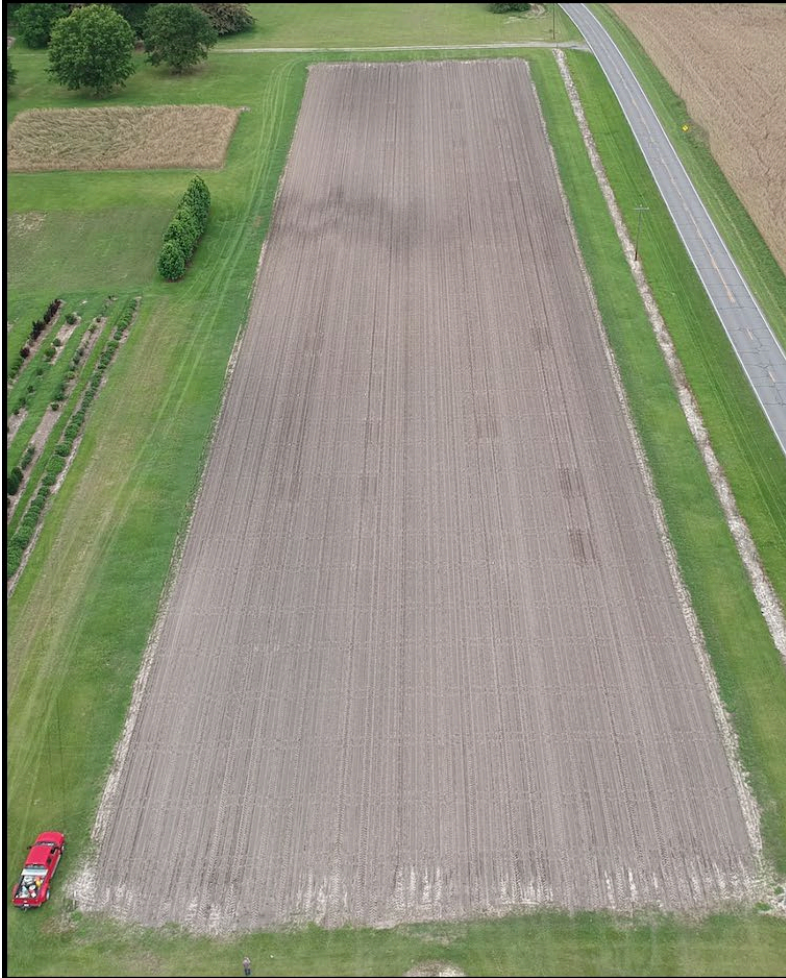
Soybean yield in Sandhills, NC (2019)

- Planting date: Jun. 7
- Sampling date: Aug. 20
- Harvesting date: Nov. 13
- Cultivar: N7003CN (MG VII)

<i>Final Yield (Bushels/acre)</i>	N7003CN	
	Irrigated	Non-irrigated
TXVA	34.0	13.7
No Treatment	30.4	10.6
Cell-Tech	32.5	12.0



Soybean planting in Sandhills, NC (2020)





A drone video of soybean planting in Sandhills, NC

Part-I





Soybean yield in Sandhills, NC (2020)

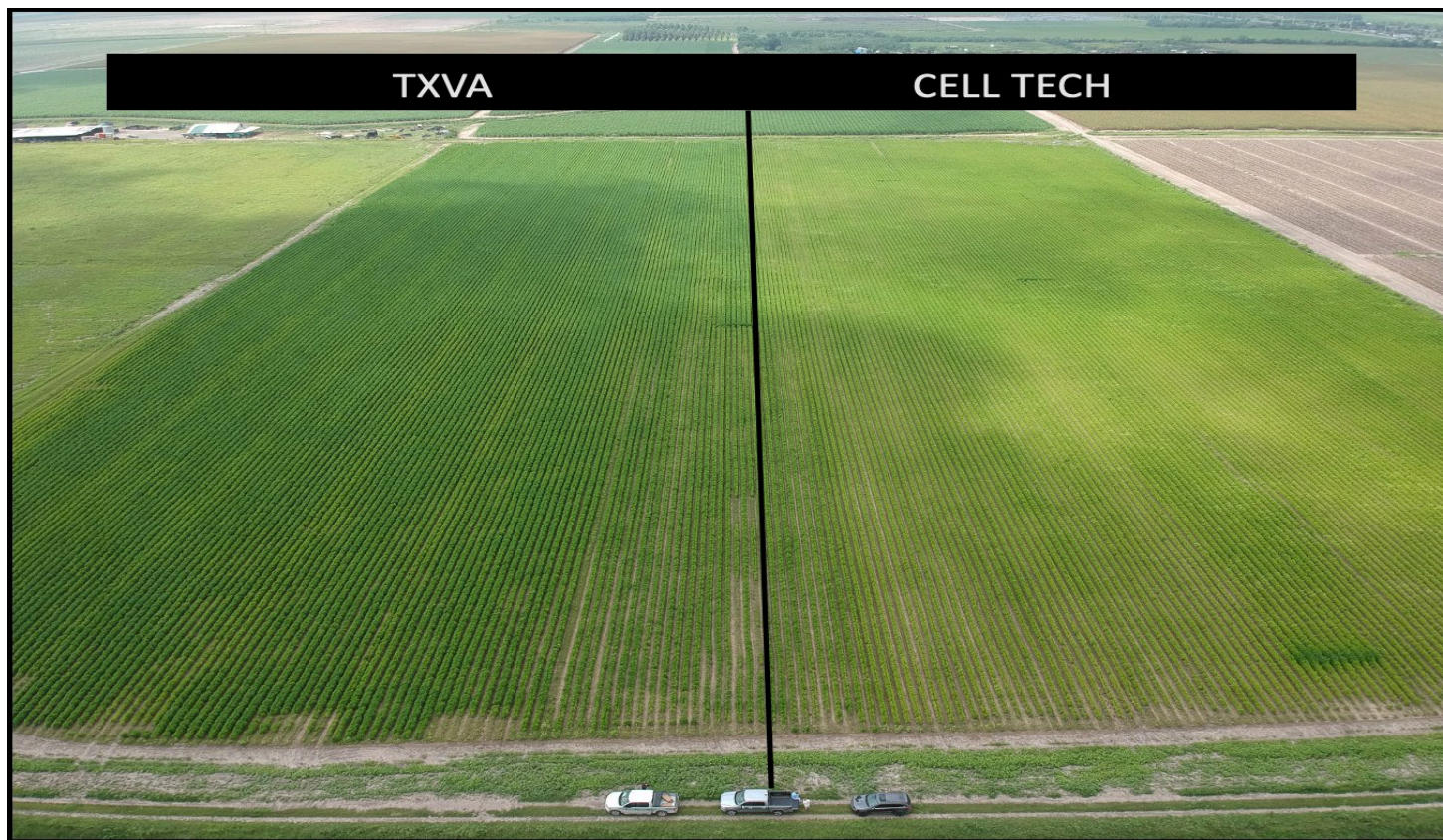
- Planting date: Jun. 17
- Sampling date: Aug. 18
- Harvesting date: Dec. 2
- Cultivar: Woodruff (slow-wilting, MG VII)

<i>Final Yield (Bushels/acre)</i>	Woodruff (slow-wilting)	
	Irrigated	Non-irrigated
TXVA	42.7	41.9
No Treatment	44.2	37.2
Cell-Tech	42.3	41.0



Commercial-sized plot testing in South Texas (Rio Grande Valley)

Vanderpool farm



Vanderpool farm – 30 acre split plot.
Soil had no history of soybean production.



Commercial-sized plot testing in South Texas (Rio Grande Valley)

Part-I

Soybean Yield (bushels/acre)

Location	Johnson farm (24 acre)	Fike farm (33 acre)
TX-VA	46	64
Cell-Tech	42	62

- * Planting date: 2/28 and 3/6 for Johnson and Fike farms
- * Harvest date: 7/20 and 7/21 for Johnson and Fike farms



Genome sequence of TXVA is available



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Resource Announcements

GENOME SEQUENCES

August 2022 Volume 11 Issue 8 e00467-22
<https://doi.org/10.1128/mra.00467-22>

Draft Genome Sequences of Two Desiccation-Tolerant Strains, *Bradyrhizobium japonicum* TXVA and TXEA, Isolated from the Root Nodules of Soybean Grown in Texas

Christian Peterson, Sarbjeet Niraula ^{*}, Dylan Parks, Woo-Suk Chang 

Department of Biology, University of Texas at Arlington, Arlington, Texas, USA

ABSTRACT Two *Bradyrhizobium japonicum* strains, TXVA and TXEA, were isolated for their desiccation tolerance and symbiotic performance with soybean as biofertilizers. Their genomes were sequenced and annotated using the Department of Energy Joint Genome Institute annotation pipeline. Sequencing yielded chromosomes of 9,193,770 and 9,339,455 bp for TXVA and TXEA, respectively.



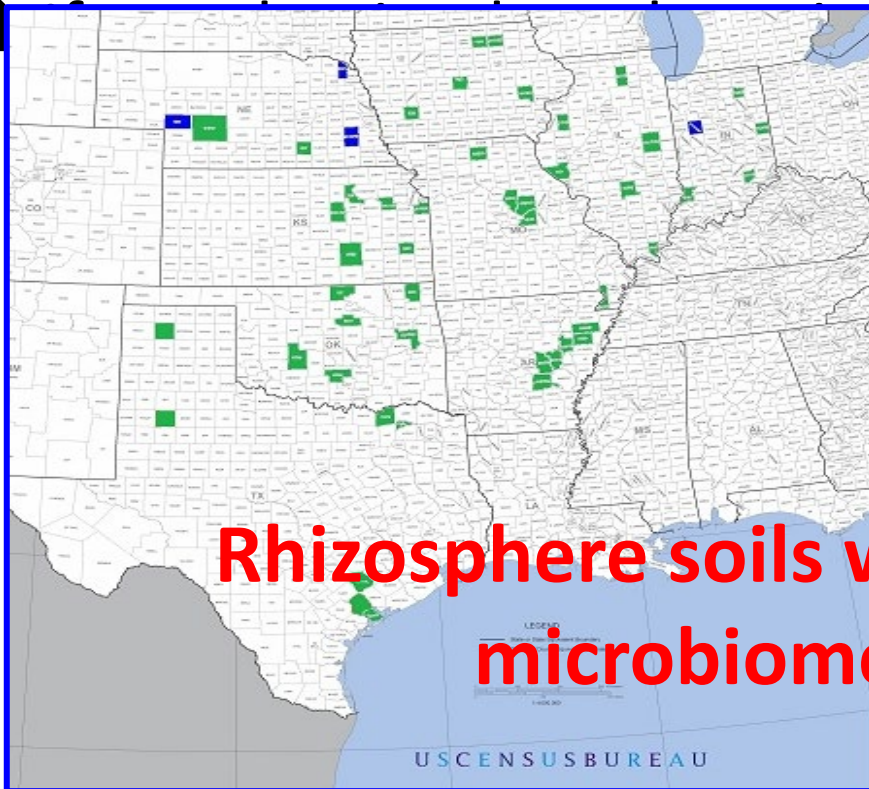
Switch to Part-II

- ❑ **Part-I: Agricultural microbiology:** Development of a drought-tolerant inoculant (biofertilizer).
- ❑ **Part-II: Rhizosphere soil microbiomes:** Understanding of changes in microbial communities for the soybean high-yield state (Nebraska).
- ❑ **Part-III: Human gut microbiomes:** Effects of fermented soybean products (FSPs) on human gut health.



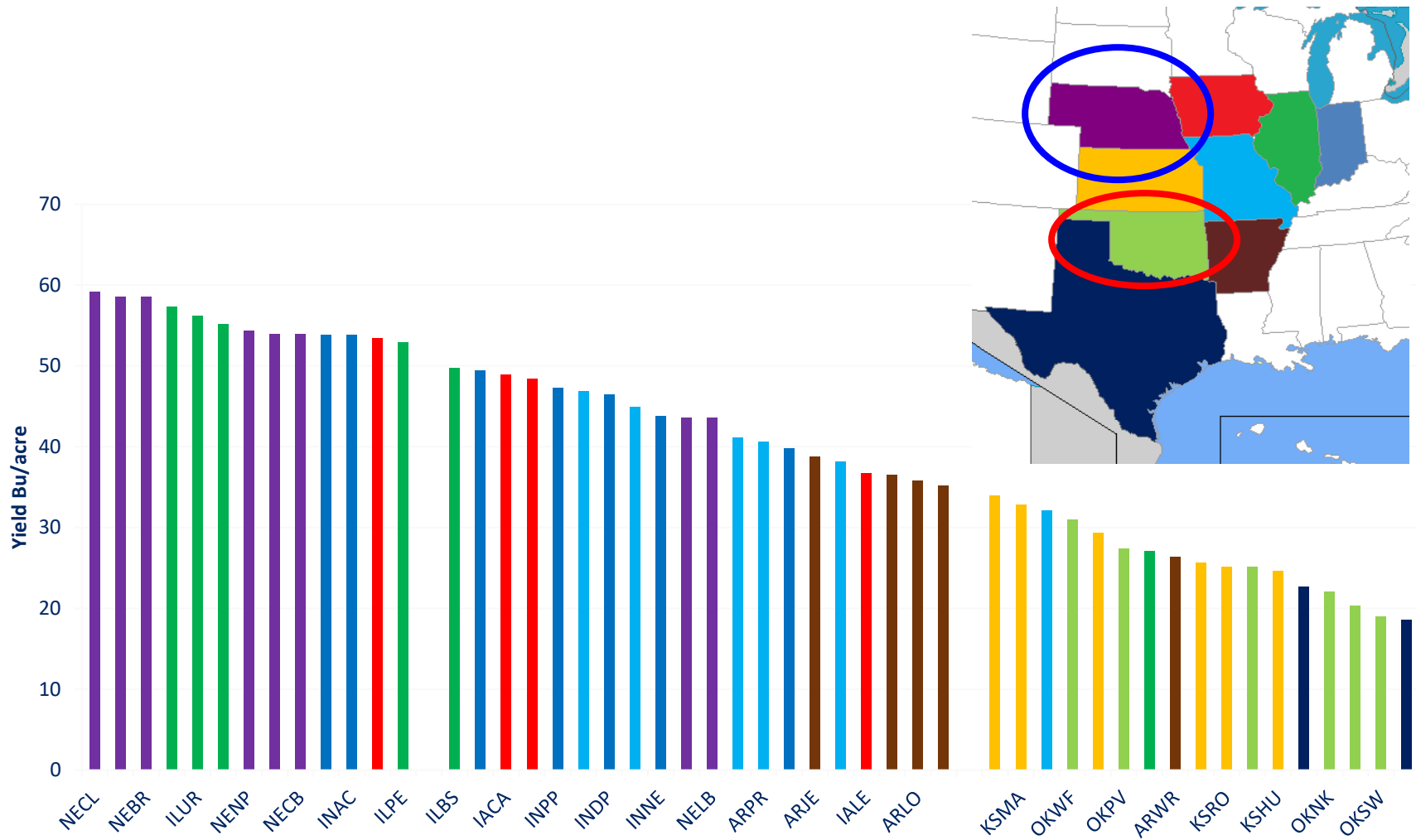
Why studying microbiome in soybean rhizospheres?

- ☐ Are there different microbial communities in different soybean fields?
- ☐ If so, do microbial communities affect soybean yield?
- ☐ Do microbial communities vary at the regional level?





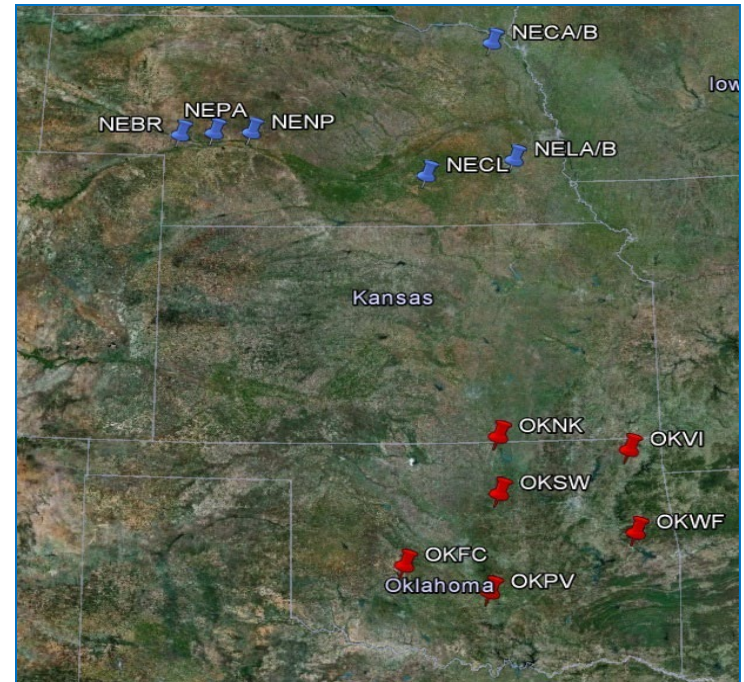
Soybean yield from sampling sites





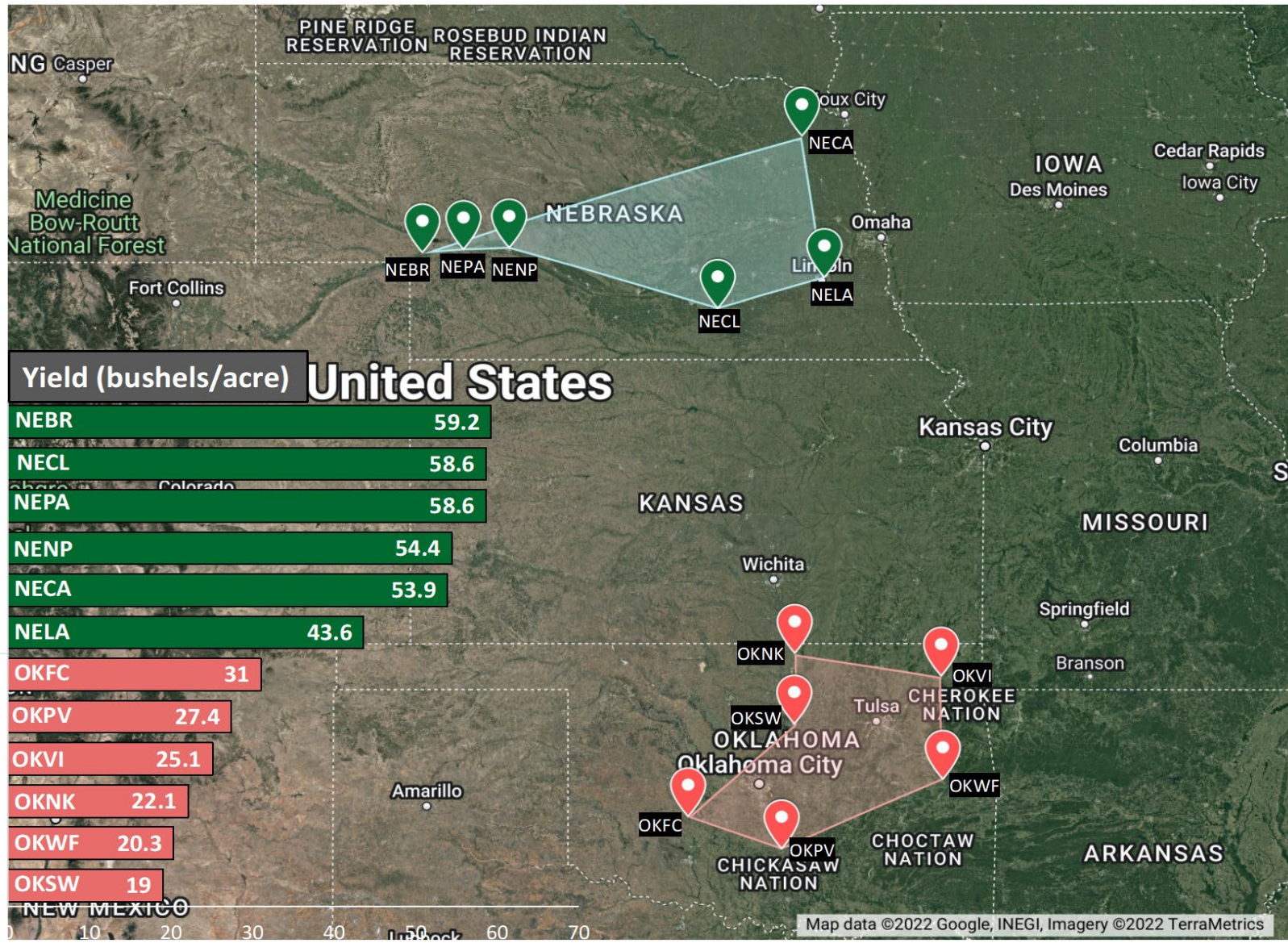
Nebraska (NE) vs. Oklahoma (OK)

- ☐ Compare the physico-chemical properties of NE and OK.
- ☐ Characterize microbial communities in soybean rhizospheres between NE and OK.
- ☐ Study the correlation of microbial community with physico-chemical properties.



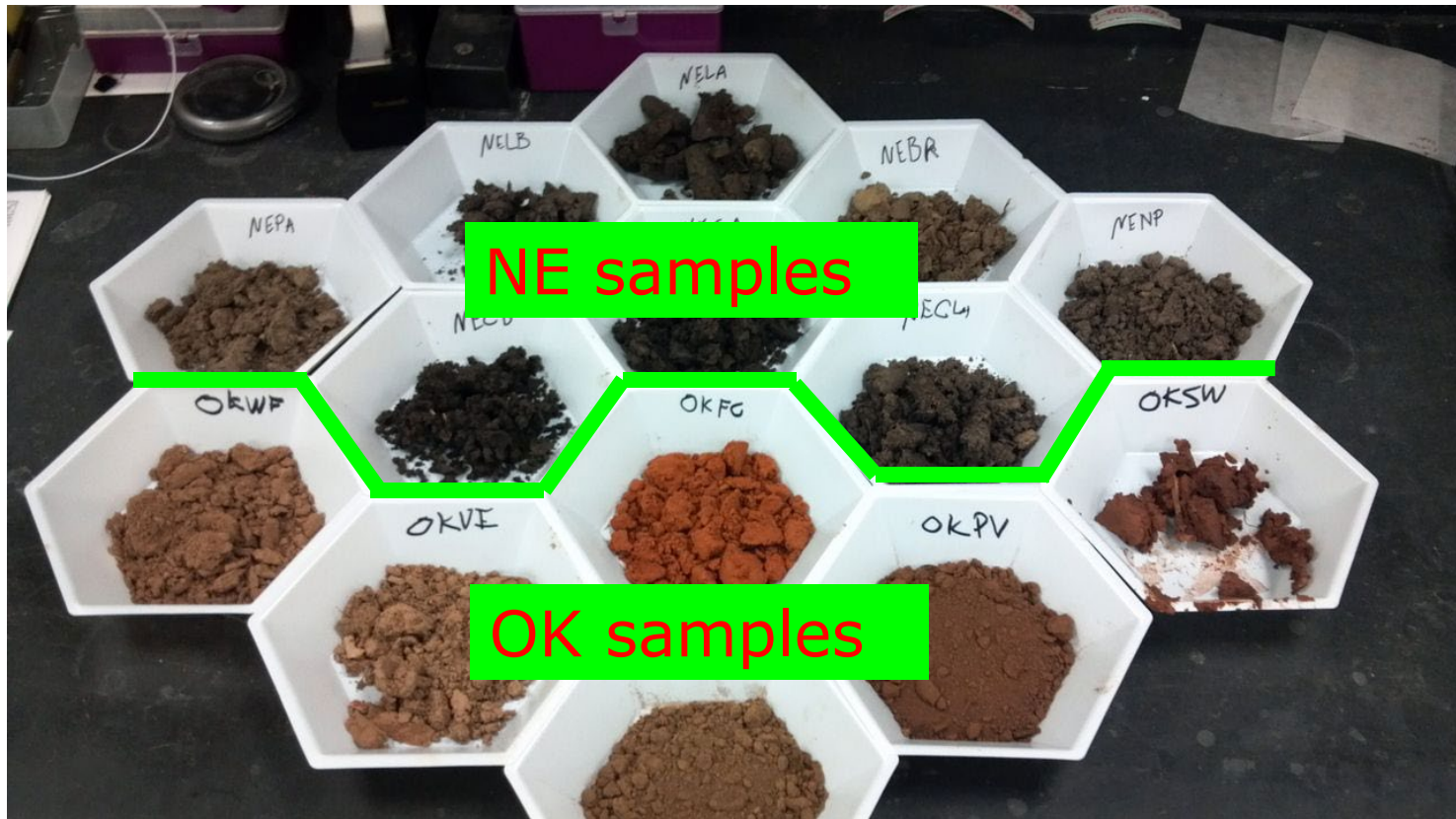


Sampling sites and soybean yield



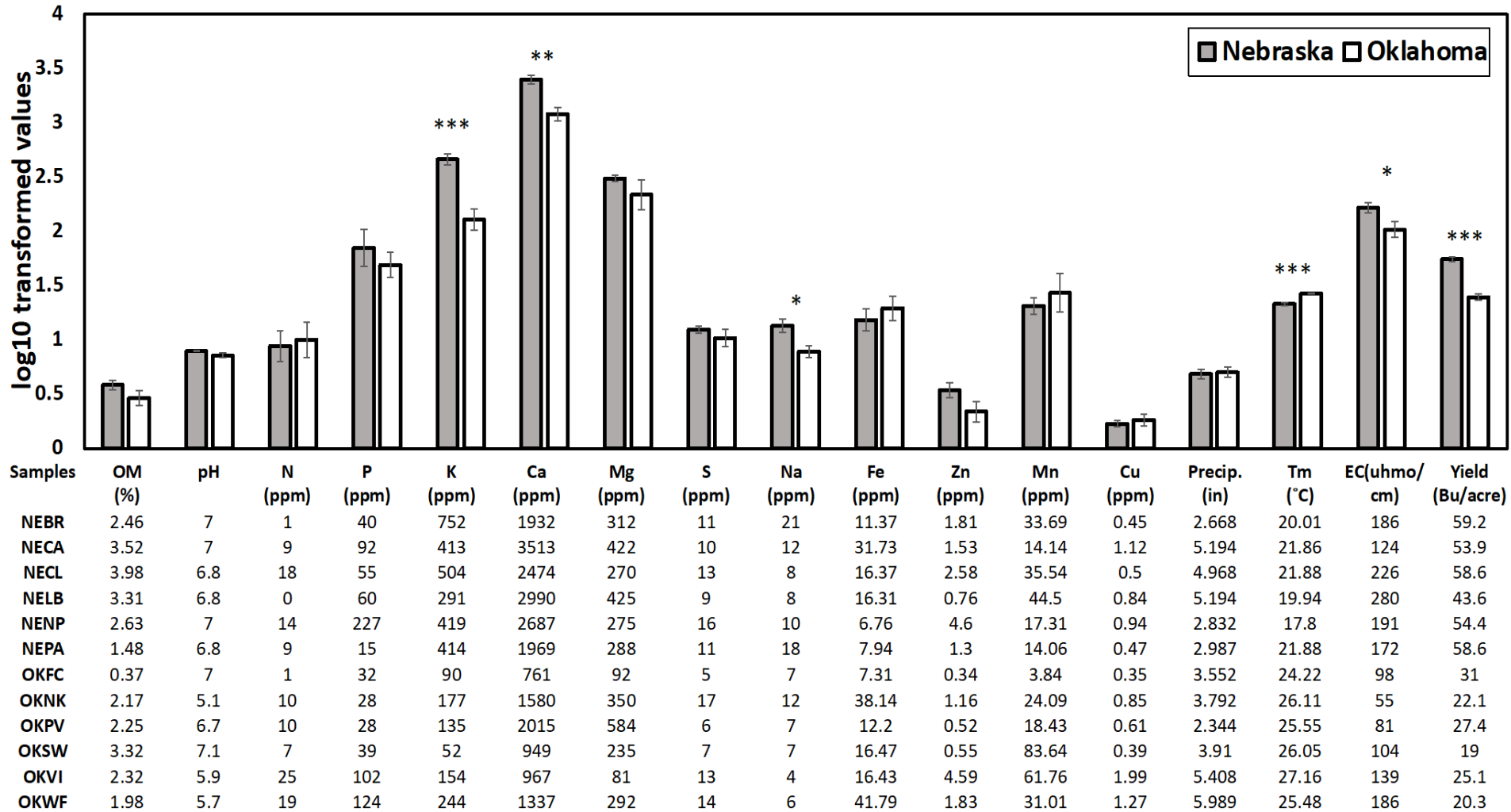


Soil properties

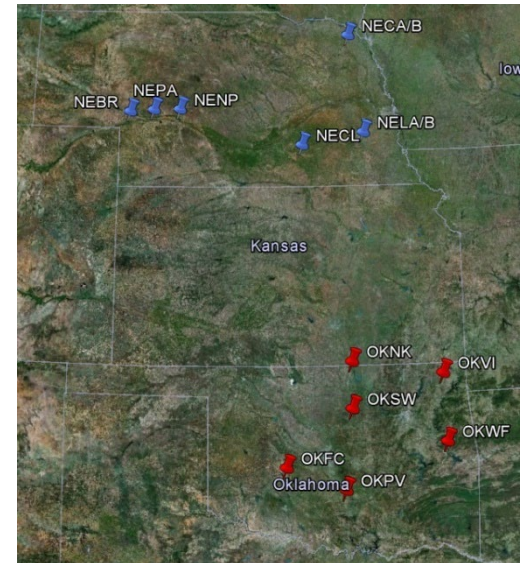




Soil physicochemical properties

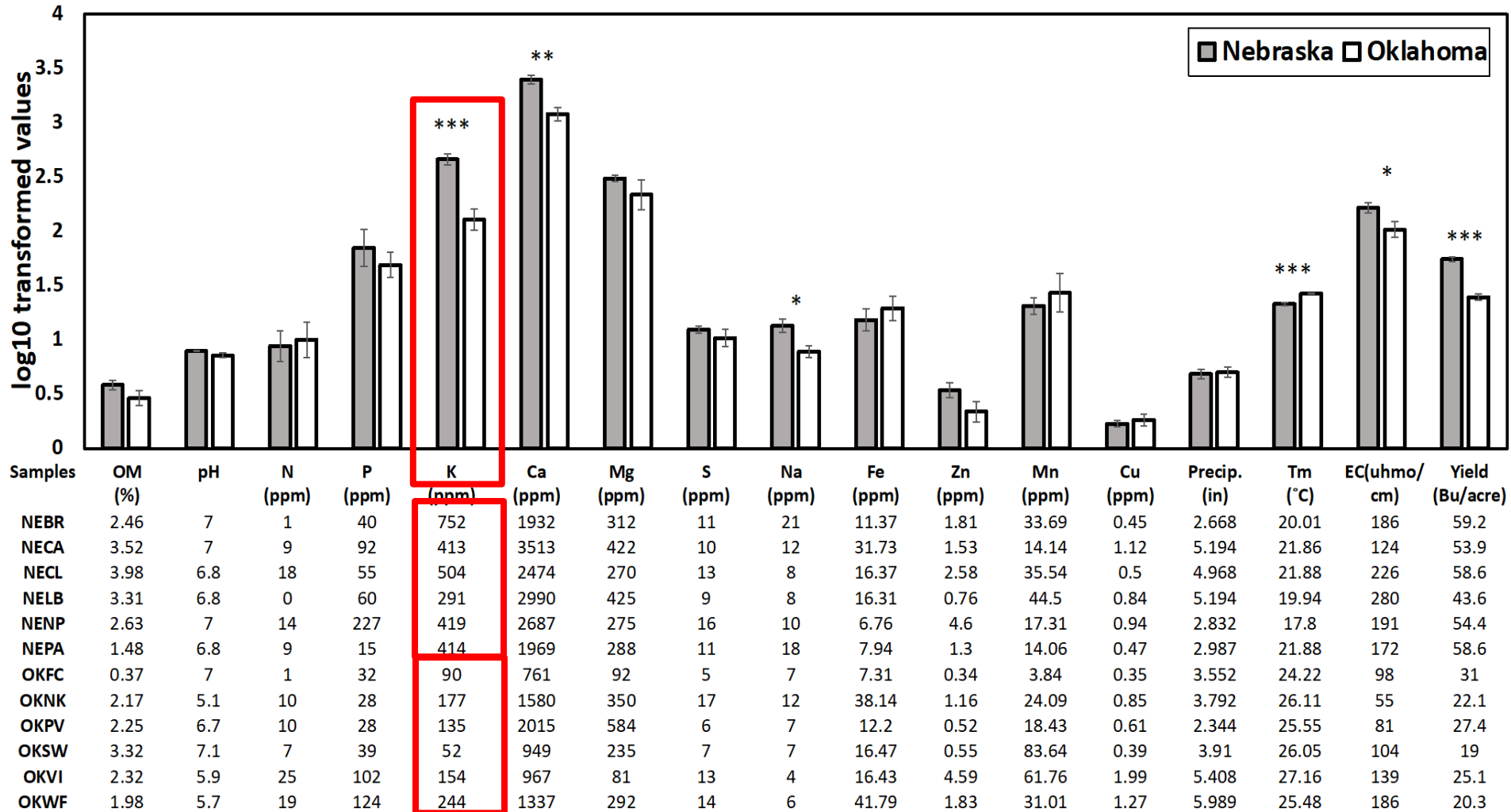


PCA analysis of physico-chemical composition variables (NE & OK)





Soil physicochemical properties

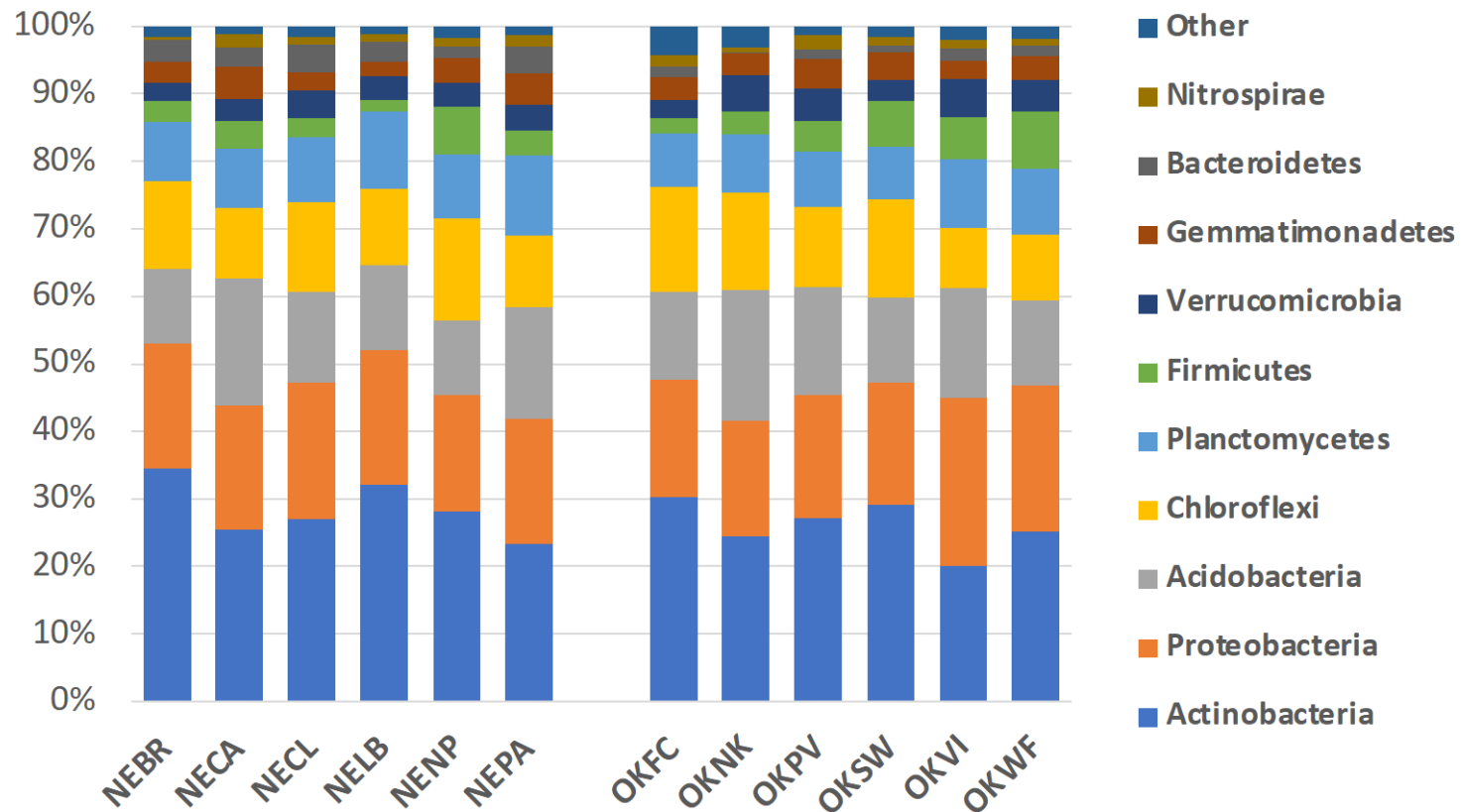




Relative abundance of top 10 phyla

(A)

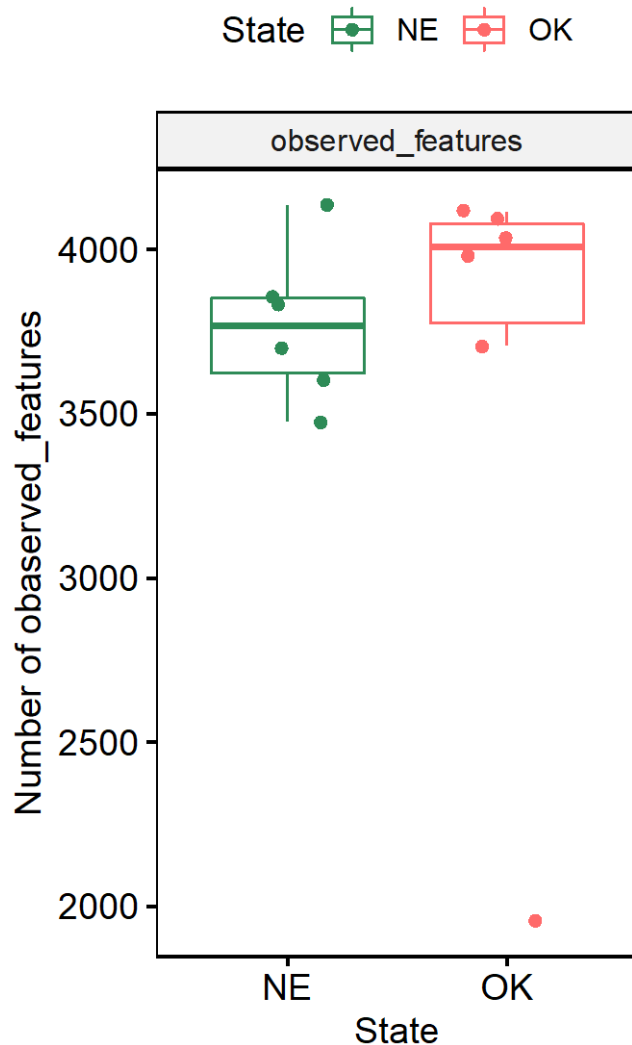
Relative Abundance of Top 10 Phyla





Alpha-diversity

(A)



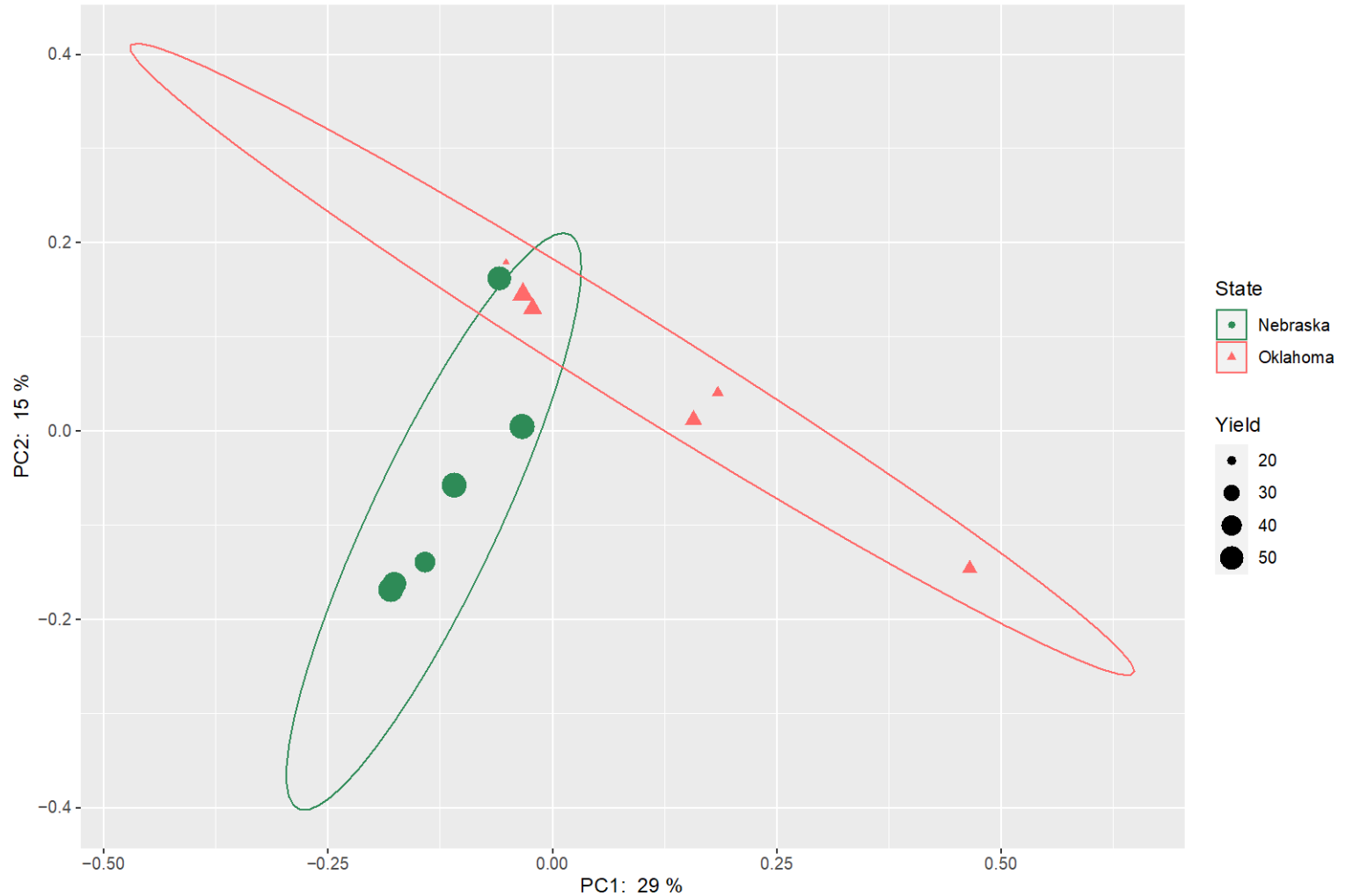
- ❑ Surprising results.
- ❑ We expected higher microbial diversity in NE than OK, but the result was opposite.
- ❑ This derived us to conduct the co-occurrence network analysis in which all factors were considered rather than evaluating only microbial diversity.



Beta-diversity

(B)

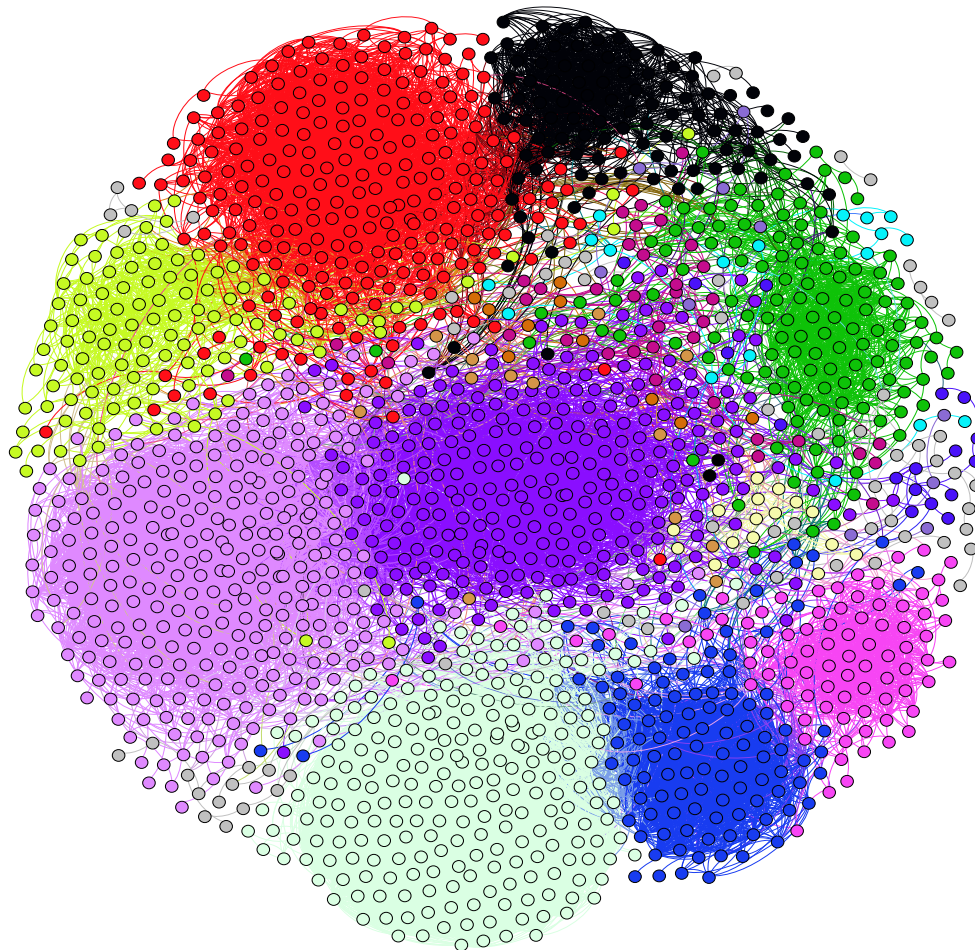
Unweighted UniFrac PCoA





Network analysis

- ❑ Co-occurrence network of rhizosphere microbial communities in NE and OK

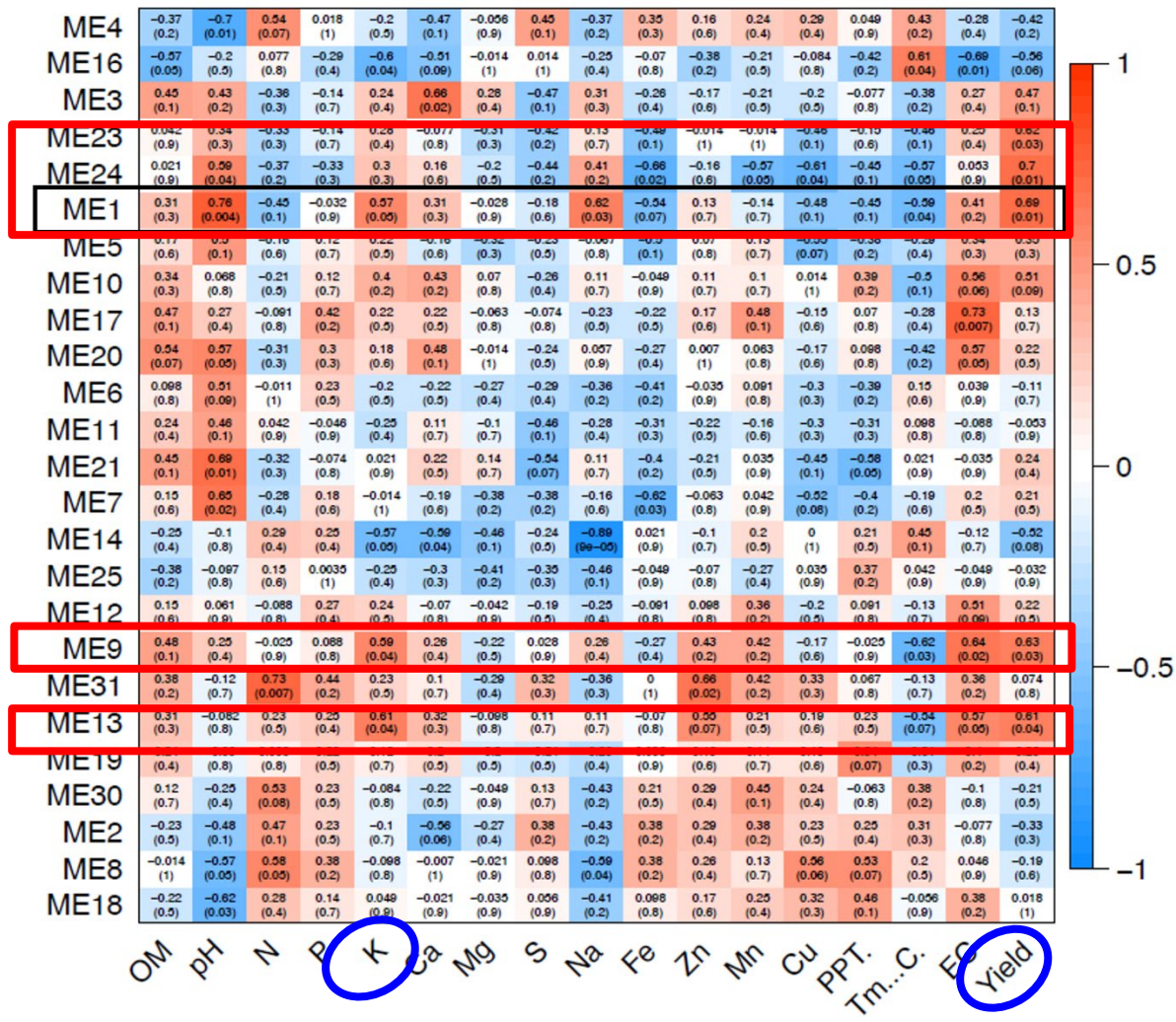




Soybean yield-correlated modules

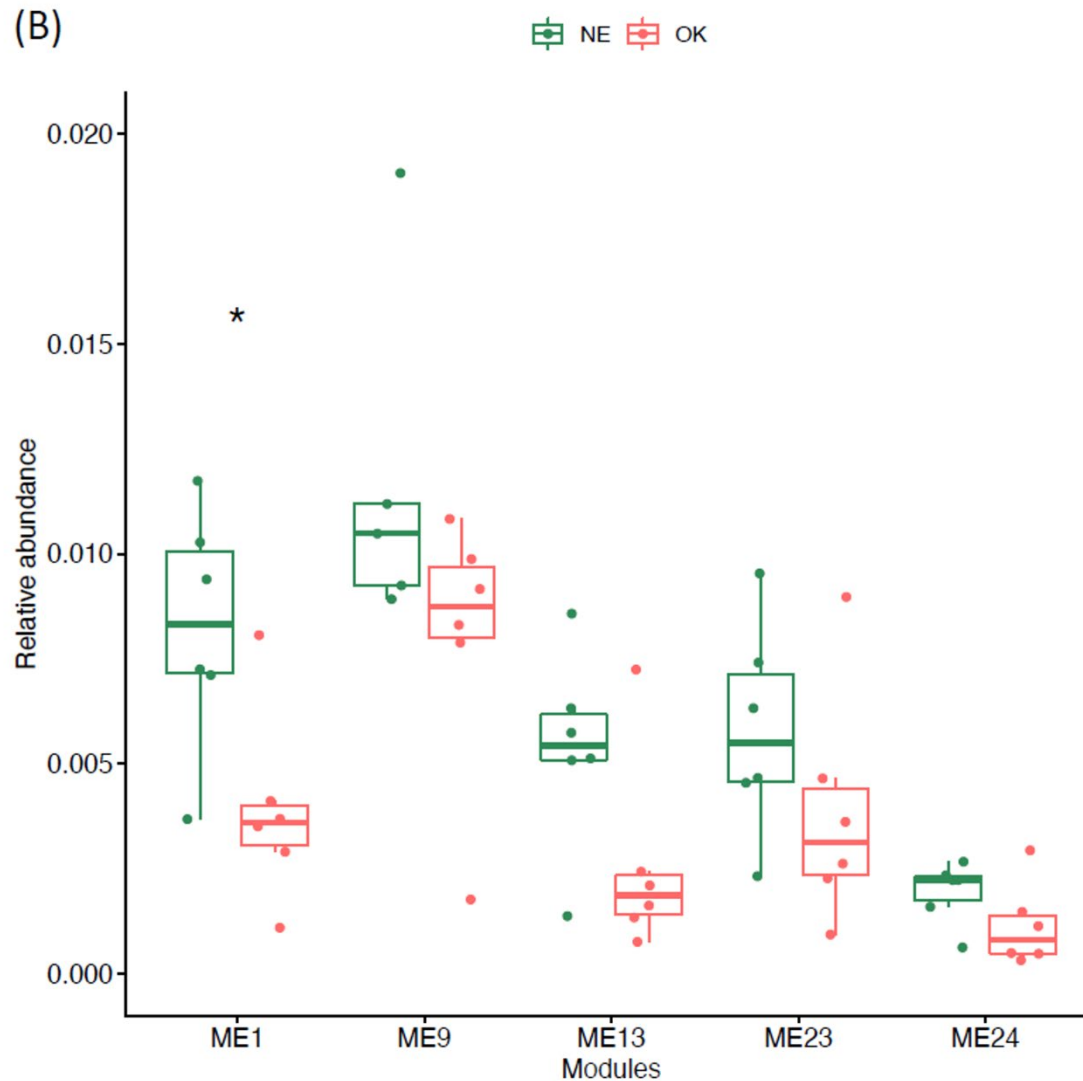
(A)

Module-trait relationships in NE-OK samples

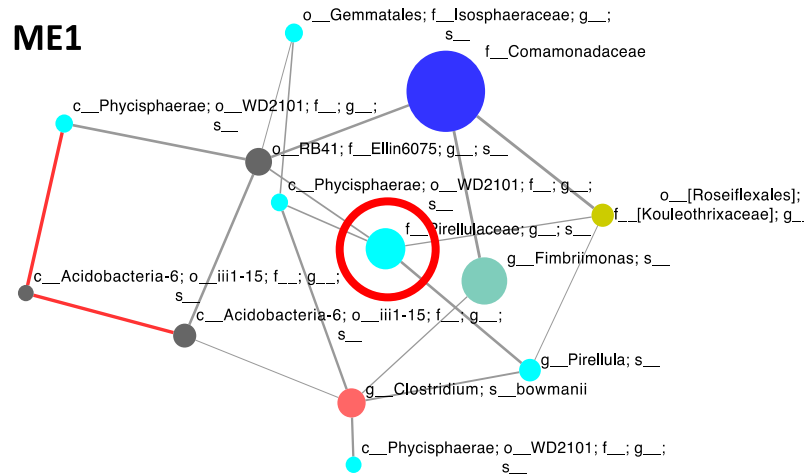




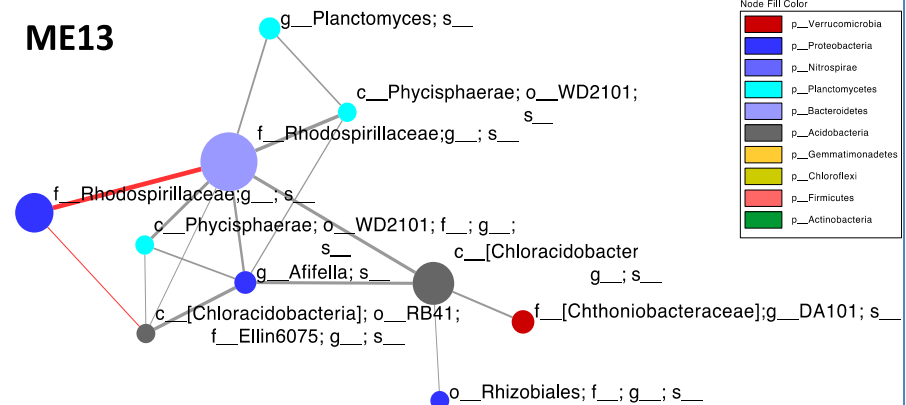
Soybean yield-correlated modules



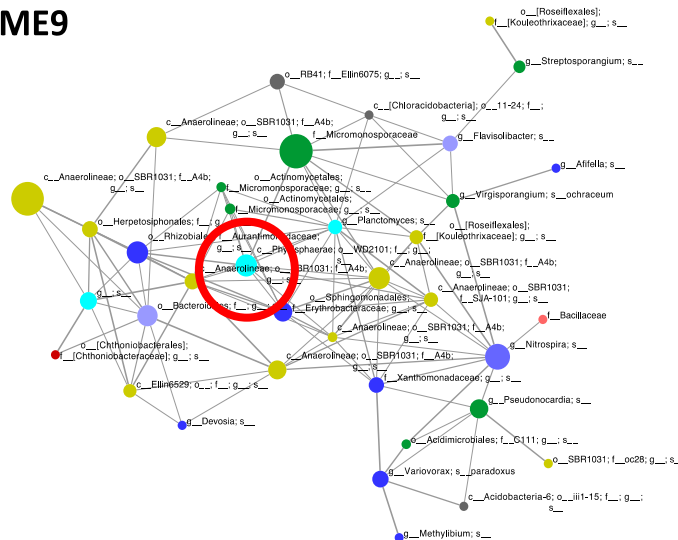
ME1



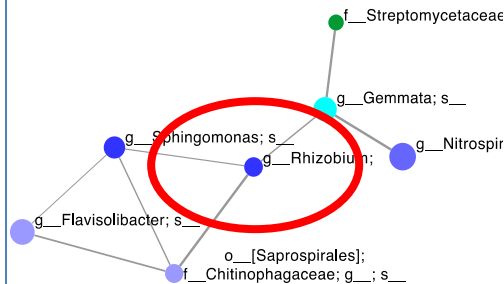
ME13



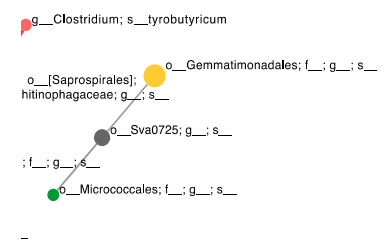
ME9



ME23



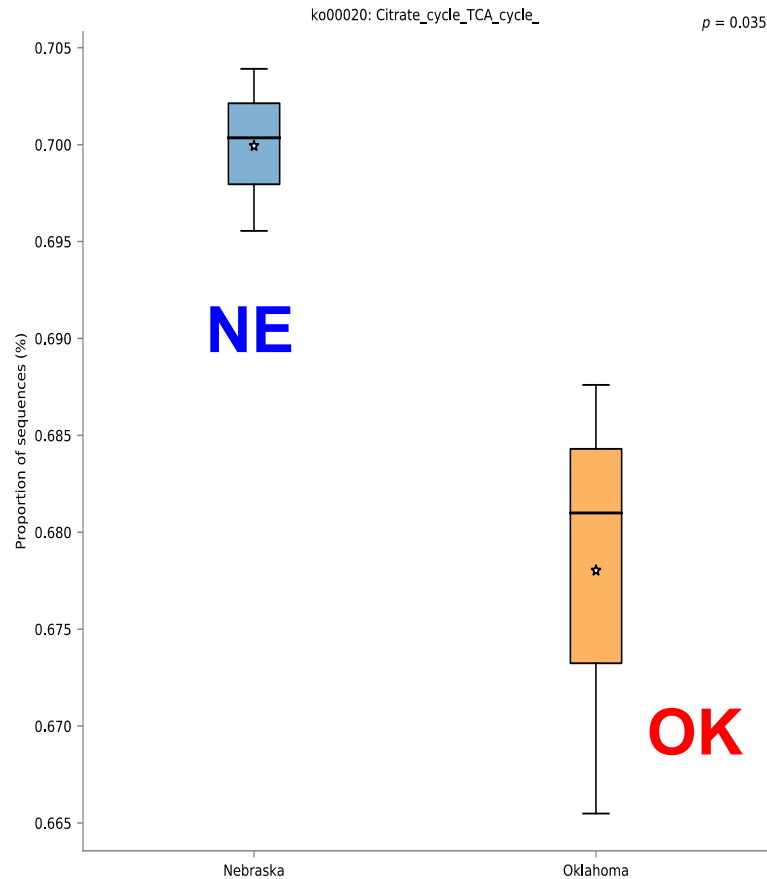
ME24



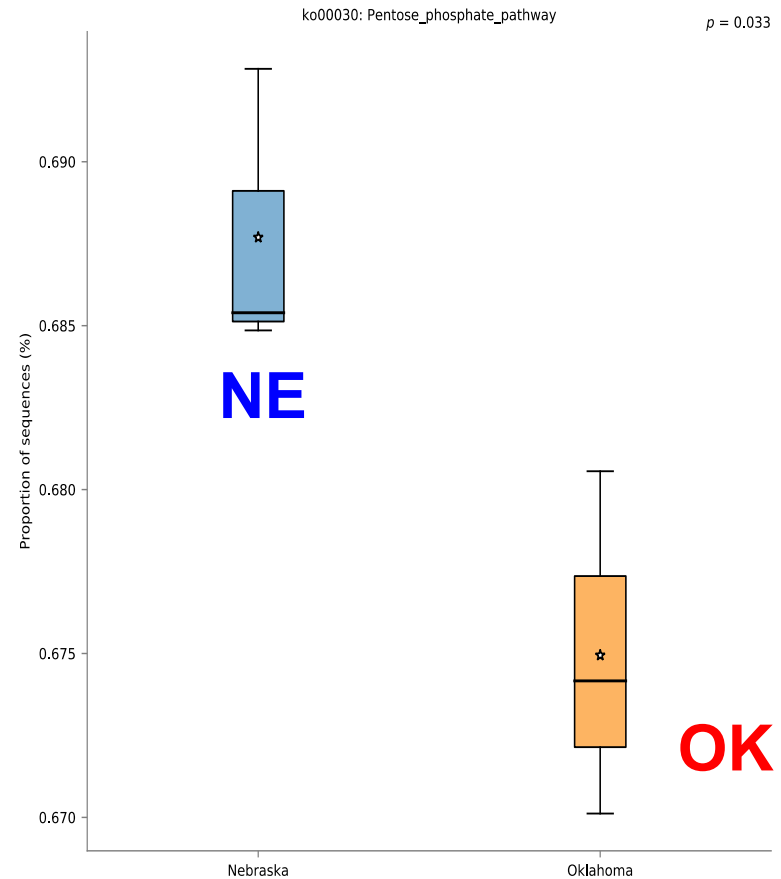


Functional prediction

TCA cycle



Pentose phosphate pathway





Conclusions

- ❑ TXVA is a novel drought-tolerant inoculant to enhance soybean yield under non-irrigated conditions.
- ❑ Potassium is a key macronutrient for soybean cultivation in the high-yield state NE.
- ❑ Higher alpha-diversity was observed in OK.
- ❑ In the co-occurrence microbial network analysis, 5 modules associated with soybean yield revealed key microorganisms at different phylogenetic levels.



Switch to Part-III

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Food and human gut microbiomes (HGM)

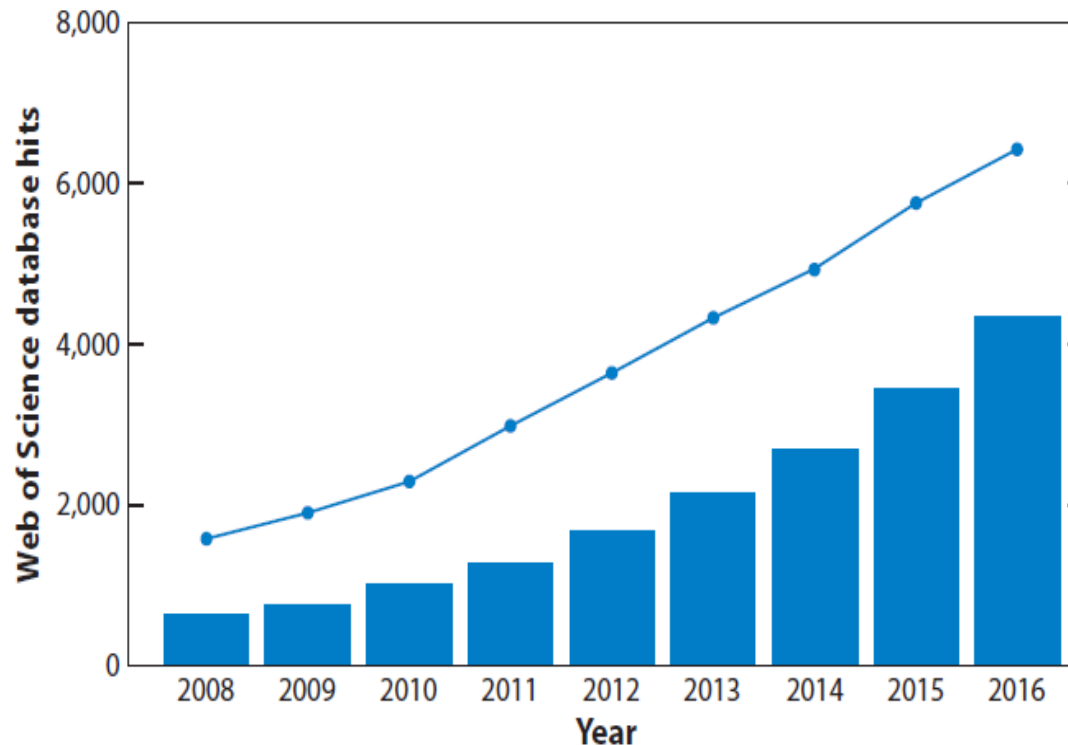


Figure 1

Number of hits found in Web of Science (<https://apps.webofknowledge.com>) database for search terms “food microbiome” or “food microbiota” (*bar graph*), and “high-throughput sequencing” (*line graph*) in articles published from 2008 to 2016 (search on April 2017).



Relevance, significance, and potential impacts of food and HGM

- ❑ Significant microbial diversity across nutritious substances that people eat or drink: basically, each niche has its own ecosystem.
- ❑ Population changes/shifts can be used as indicators of food quality status.
- ❑ Microbiomes can be used for contamination/safety surveillance.



Fermented products characterized using metagenomics approaches

Table 1

Fermented products characterized using high-throughput 16S rRNA gene sequencing.

Type	Product	Product	Domain	Country	Ref.
Food	Dairy	Mongolian dairy products	Bacteria	Mongolia	Oki et al. (2014)
	Condiment	Kochujang (Korean condiment)	Bacteria	Republic of Korea	Nam et al. (2012b)
	Fish	Narezushi (Japanese fish with rice)	Bacteria	Japan	Kiyohara et al. (2012)
		Kaburazushi (Japanese medieval sushi)	Bacteria	Japan	Koyanagi et al. (2013)
		Flatfish jeotgal (Korean fish)	Bacteria	Republic of Korea	Jung et al. (2014)
	Legume	Doenjang (Korean soybean pastes)	Bacteria	Republic of Korea	Nam et al. (2012a)
		Lu-Doh-Huang (Taiwanese mung beans)	Bacteria	Taiwan	Chao et al. (2013)
	Meat	Italian salami	Bacteria	Italy	Polka et al. (2015)
	Seafood	Several different seafood	Bacteria/archaeae	Republic of Korea	Roh et al. (2010)
		Ojingeo jeotgal (Korean squid)	Bacteria	Republic of Korea	Jung et al. (2013a)
	Seed	Cocoa bean	Bacteria/fungi	Belgium	Illeghems et al. (2012)
		Meju (Korean soybean bricks)	Bacteria	Republic of Korea	Kim et al. (2011)
	Vegetable	Kimchi (Korean vegetables)	Bacteria	Republic of Korea	Park et al. (2012)
			Bacteria	Republic of Korea	Jung et al. (2011)
		Nukadoko (Korean vegetable in rice bran)	Bacteria	Japan	Sakamoto et al. (2011)
Liquid	Alcohol	Makgeolli (Korean alcoholic beverage)	Bacteria/fungi	Republic of Korea	Jung et al. (2012)
		Fen liquor (Chinese liquor)	Bacteria/fungi	Chinese	Li et al. (2013)
		Shaoxing wine (Chinese rice wine)	Bacteria	China	Xie et al. (2013)
	Condiment	Chinese soy sauce	Bacteria/yeast	Malaysia	Sulaiman et al. (2014)
	Milk/fruit	Kefir (milk or fruit juice beverage)	Bacteria/yeast	8 regions	Marsh et al. (2013b)
				Ireland	Dobson et al. (2011)
				Ireland	Marsh et al. (2013a)
				Turkey	Nalbantoglu et al. (2014)
				Brazil	Leite et al. (2012)
	Plant	Chicha (maize-based beverage)	Bacteria	Argentina	Elizaquivel et al. (2014)
	Tea	Kombucha (Mongolian tea)	Bacteria	Mongolia	Marsh et al. (2014)
		Pu'er (Chinese tea)	Bacteria	China	Lyu et al. (2013)



Why fermented soybean foods?

- ☐ Unique flavors.
- ☐ Health benefits: lower cholesterol levels and anti-cancer activities.
- ☐ It would be good probiotics as well as prebiotics.



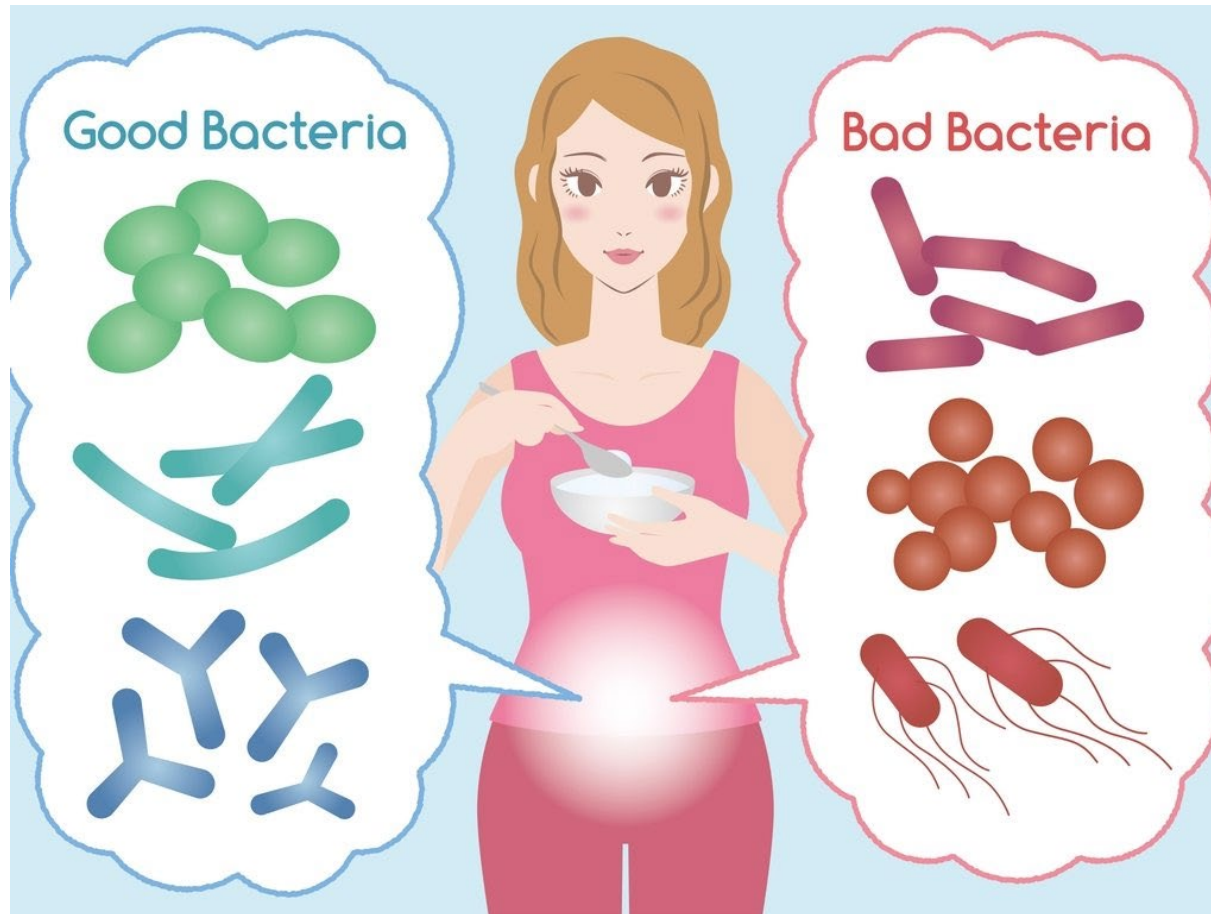
Prebiotics vs. probiotics

- ❑ **Prebiotics:** compounds in food that induce the activity of beneficial microorganisms.

- ❑ **Probiotics:** live microorganisms that provide health benefits when consumed, generally by improving the gut microbiota.



Impact of prebiotics or probiotics on the human gut microbiota



<https://www.thebestofhealth.co.uk>



Which foods are the best sources?

PROBIOTICS vs. PREBIOTICS

Both Are Necessary for A Healthy Gut

Probiotics are the good bacteria living in your gut. They help your body break down food and support gut health, as well as overall wellness.

PLANT-BASED PROBIOTIC FOODS



NATTO



COCONUT KEFIR



SAUERKRAUT



TEMPEH



KIMCHI



MISO



PICKLED VEGGIES
(NON-PASTEURIZED)



NON-DAIRY
YOGURT

Prebiotics are the food for the good bacteria. They come from the non-digestible fiber in certain foods.

PLANT-BASED PREBIOTIC FOODS



ASPARAGUS



GARLIC



BANANAS



JICAMA



CHICORY
ROOT



JERUSALEM
ARTICHOKE



ONION/
LEEKS



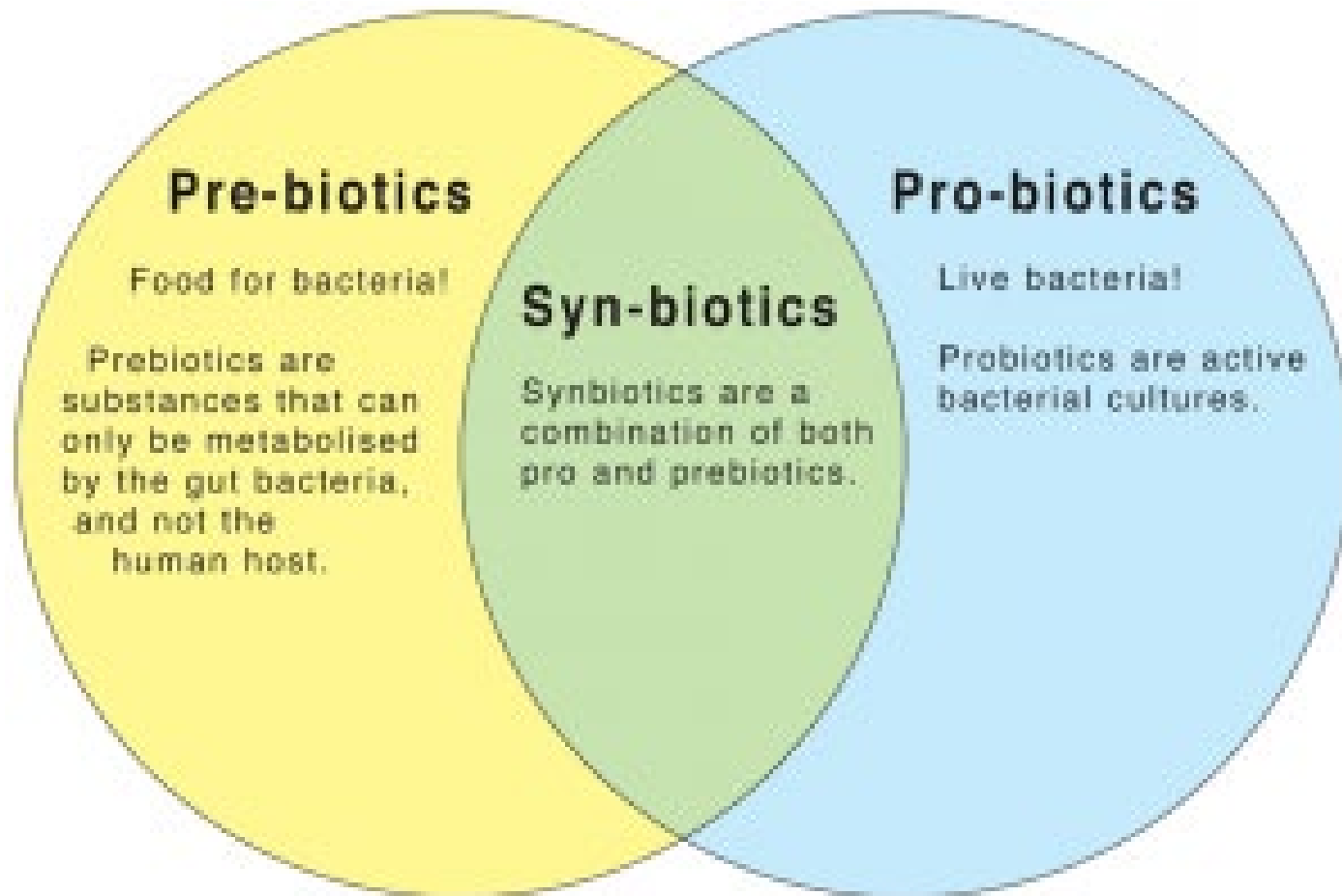
LEAFY GREENS &
DANDELION GREENS

FOOD REVOLUTION
NETWORK

<https://foodrevolution.org/blog/probiotics-and-prebiotics/>

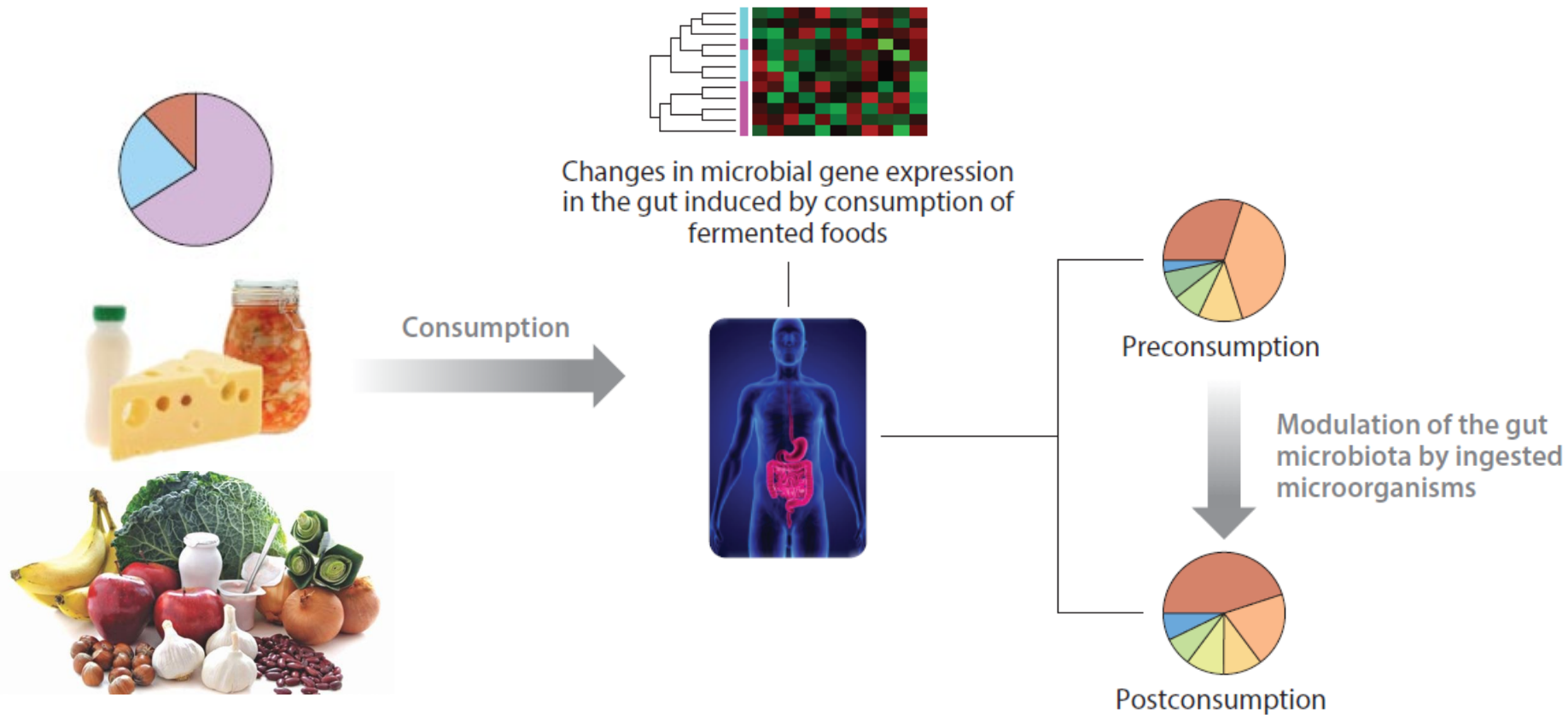


Fermented soybean products (FSPs) as syn-biotics





Impact of prebiotics and probiotics on the human gut microbiota





Isolation of lactic acid bacteria (LAB) from Kimchi



Mixed microbial community in Kimchi



Enrichment procedure for LAB



Screening for better LAB



1. Physiological characterization.
2. Biochemical properties.
3. Microscopy.
4. Animal test.



1. Genomics.
2. Transcriptomics.
3. Proteomics.
4. Metabolomics.
5. Mutagenesis.
6. Mechanism of function.



Screening for LAB in Kimchi

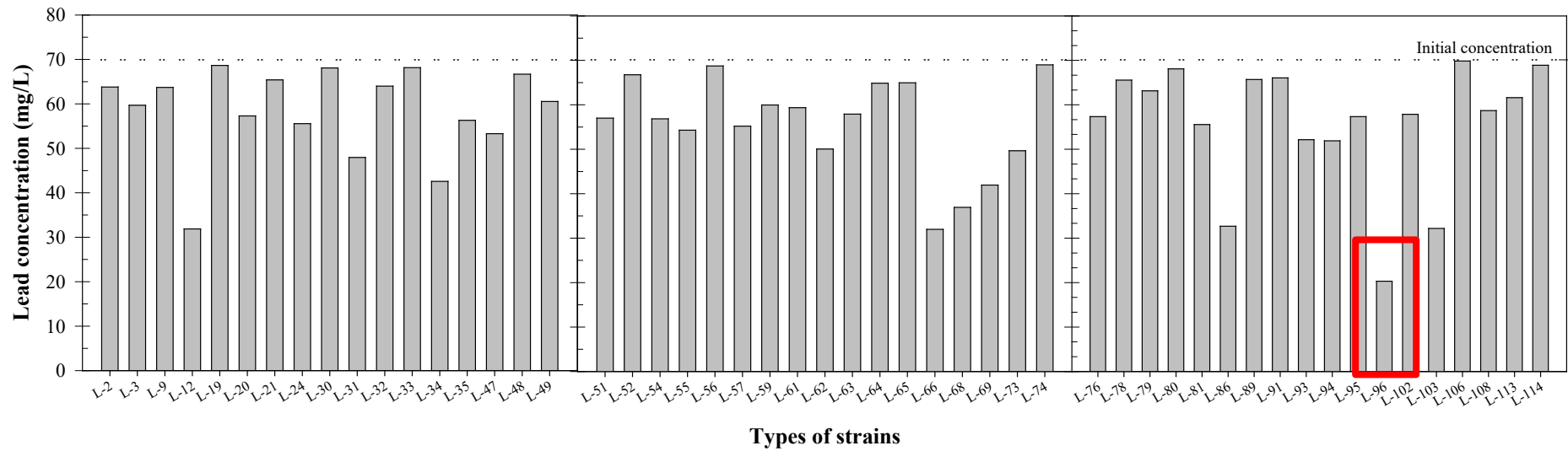


Figure 1. Screening of LAB for Pb removal



Lead (Pb) removals by LAB strains

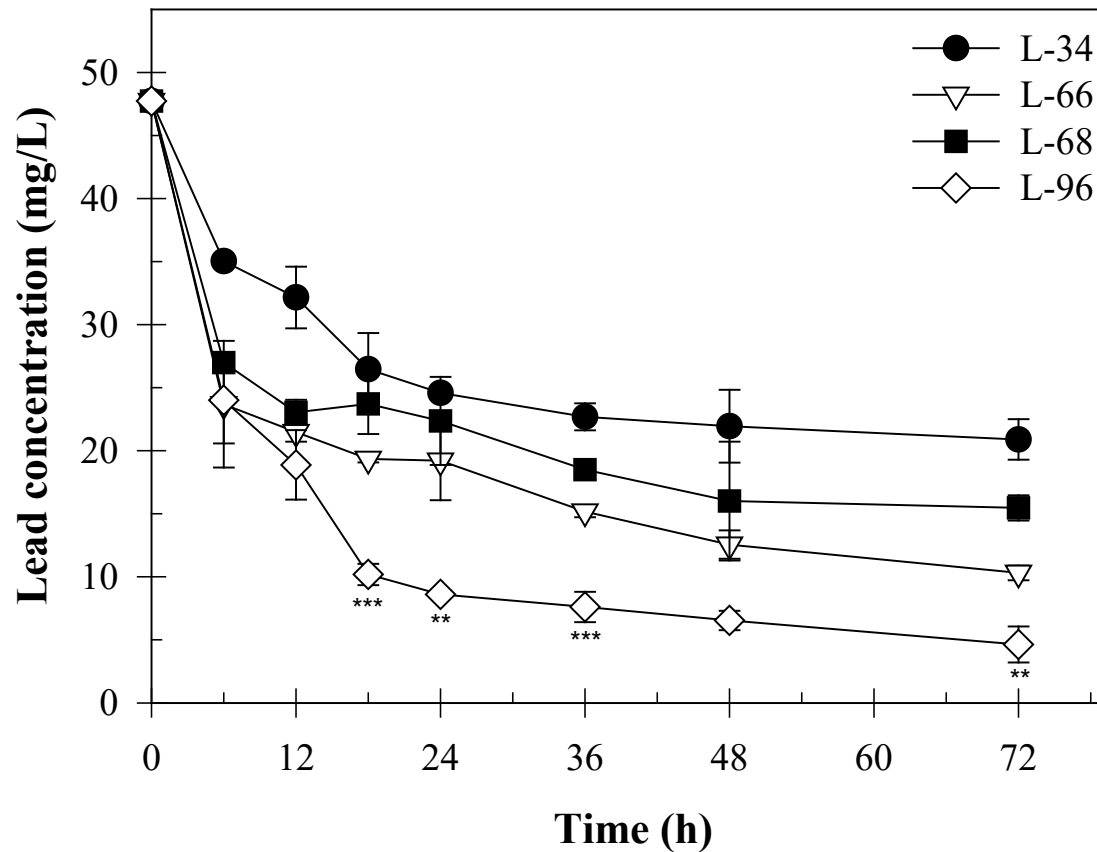


Figure 2. Pb removal (mean $\pm$ SD) by four strains of Pb-tolerant LAB.
** $P < 0.01$ and *** $P < 0.001$: significant differences among the strains.



Lead (Pb) removals by L-96 strain

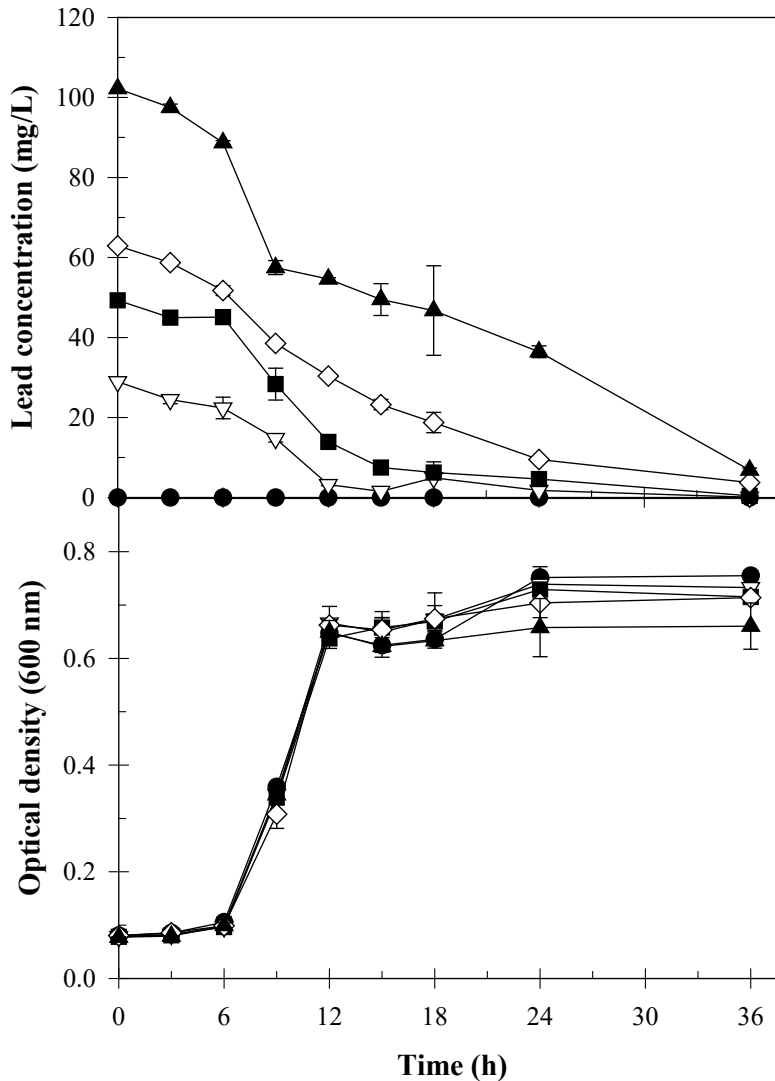


Figure 3. Pb removal and cell density (mean $\pm$ SD) during growth of L-96 LAB in MRS broth. (● = 0 [mg Pb]/L; ▽ = 30 [mg Pb]/L; ■ = 50 [mg Pb]/L; ◇ = 70 [mg Pb]/L; ▲ = 100 [mg Pb]/L).



Biosorption of Pb on L-96 biomass

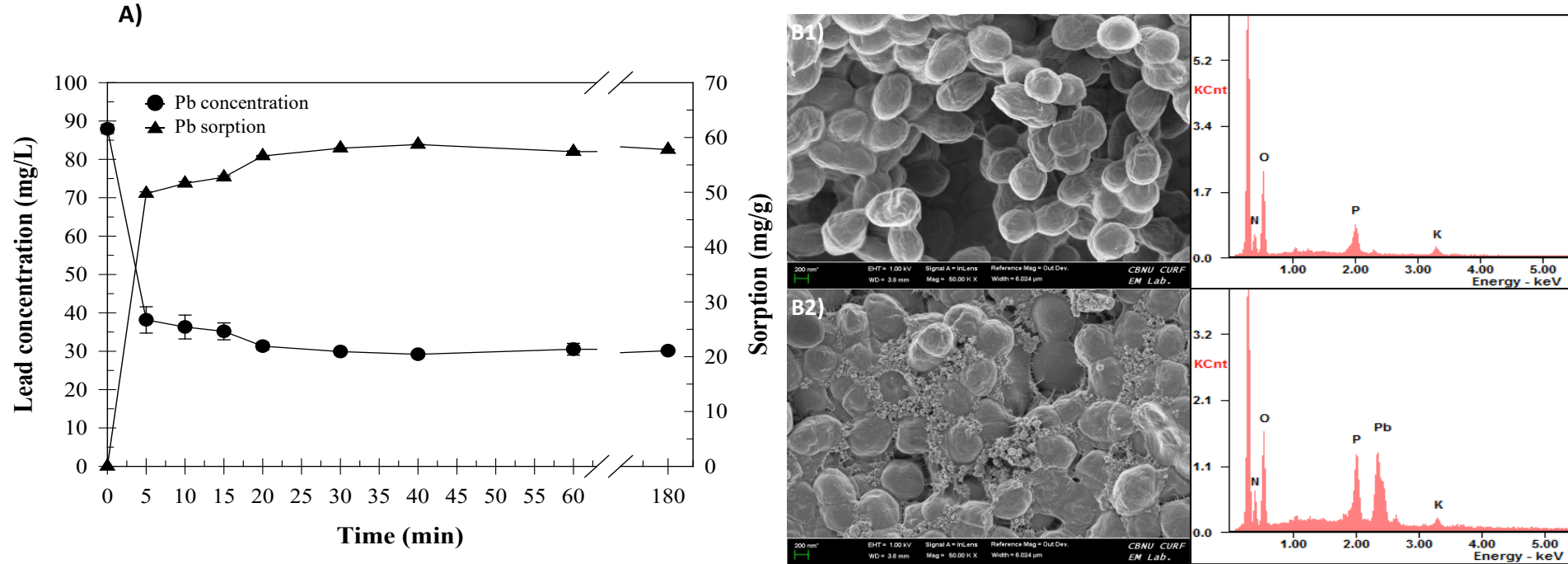


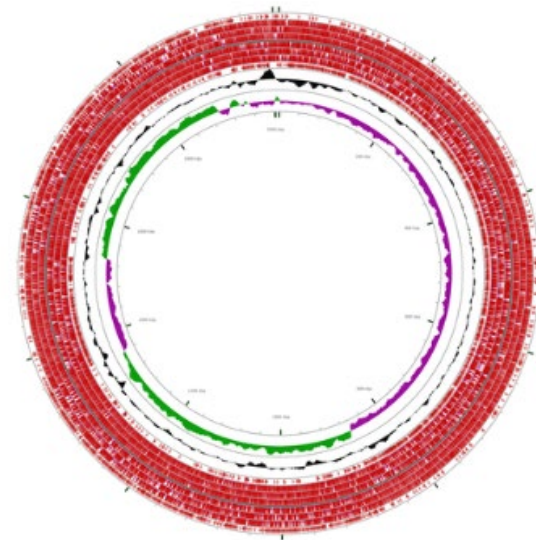
Figure 4. Biosorption assay with L-96 biomass. ● residual Pb concentration [mg/ml], ▲ Pb-sorption amount on biomass [mg/ml]). Biosorption event of L-96 was observed by SEM-EDS analysis (B) before (B1) and after (B2) Pb treatment.



Genome of L-96 strain

- ❑ Sequenced using MiSeq platform
- ❑ Joint Genome Institute (JGI) IMG database used for annotation and genome analysis

	<i>L. mesenteroides</i>
Genome size	2,002,085 bp
GC content	37.62%
Protein coding gene	1,996



Leuconostoc mesenteroides L-96



A new project in collaboration with MIFI and UTA Health Service Center

Title: Effects of fermented soybean products (FSPs) on human gut microbiomes

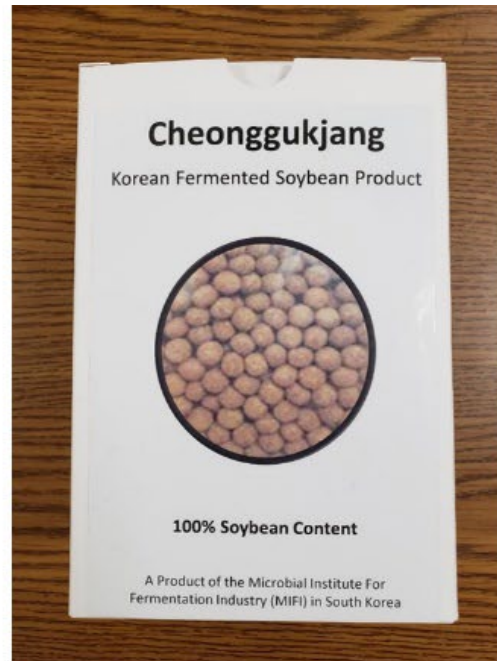
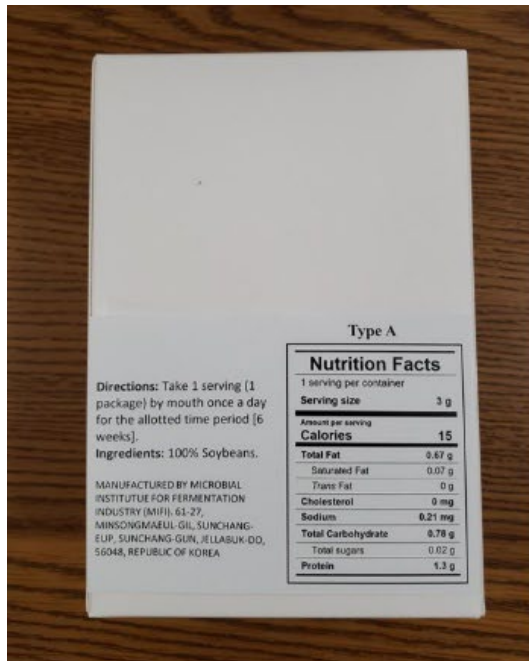
Fermented soybean products (FSPs) pellets called “cheonggukjang pills”





Effects of fermented soybean products (FSPs) on human gut microbiomes

- ❑ Human subjects research: Participants will take FSPs for **6 weeks** and submit their stool samples every 2 weeks (**a total of 5 times**).
- ❑ Participants will receive a **total of \$300 cash** stipend upon sample submission.



Seeking Participants!

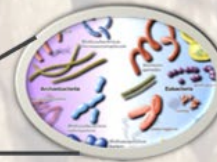
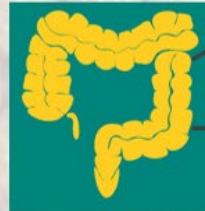
Human Gut Microbiome Project

Lead by Dr. Woo-Suk Chang, Associate Professor of Biology

We are excited to announce a new study that will be taking place here on campus and we invite you to participate. This study hopes to understand how fermented soybean pellets/tablets (FSP) influence the community of microbes in a person's gut. The duration of the entire study will be for 8 weeks. You will take the FSP everyday for the first 6 weeks.

You will be asked to collect and submit stool samples a total of 5 times (e.g., every other week) throughout this study. In addition to this, as you turn in your stool samples to the University Health Services Center, you will be asked to have your blood drawn for a total of 5 times.

In return for your participation, you will receive, in total, a **\$300 cash stipend** upon sample submission.



If you are interested in participating, please go to <https://utaedu.questionpro.com/t/AUedaZqSrZ> to fill out a questionnaire and determine your eligibility. You may also scan the QR code below.





Field work collaborators

- **Dr. Ben Fallen - USDA-ARS, Soybean and Nitrogen Fixation Unit.**
- **Dr. Tommy Carter - USDA-ARS, Soybean and Nitrogen Fixation Unit.**
- **Dr. James Grichar – Texas A&M AgriLife Research.**
- **Andy Scott, Johnson Farm, Fike Farm, Vanderpool Farm in South Texas.**
- **Dr. Avat Shekoofa – University of Tennessee.**
- **Dr. Pengyin Chen – University of Missouri.**
- **Dr. Tessie Wilkerson – Mississippi State University.**
- **Dr. Trey Price – Louisiana State University.**
- **Dr. Leandro Mozzoni – University of Arkansas.**
- **Dr. Shawn Clark – Pine Tree Research Station in Arkansas.**
- **All collaborators in Team Drought.**



Human gut microbiome collaborators

- **Dr. Doyeon Jeong – Director, MIFI.**
- **Dr. Angela Middleton – Director, UTA Health Service Center.**
- **Munira Rangwalla – Chief medical technologist, UTA Health Service Center.**
- **Jasmin Nguyen, Medical technologist II, UTA Health Service Center.**
- **Dr. Heejong Yang – Program manager, MIFI.**
- **Maymi Kyaw – UTA student.**
- **Meaghan Rose – Chang Lab.**
- **Shannon Moncier – Chang Lab.**
- **All staff members in UTA Health Service Center and MIFI.**



Questions?





Questions?

2022

