

INTRODUCTION

- Many microorganisms exist naturally or as possible contaminations near pulse farms, such as irrigation, manure treated soil, farm equipment, and production handling practices.
- Organisms have the potential to remain dormant for years in the form of bacterial spores.
- Limited information exists regarding the microorganisms present on pulse crops.

OBJECTIVES

- To complete a microbial analysis of pulse samples from varying producers in the Palouse and Northern Plains regions of the United States.
- Use matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry to identify individually isolated microbial colonies.

METHOD

- Pulse samples collected as part of the 2023 Pulse quality Survey; individual samples were grouped according to region. Typically, 5 samples per region were defined as a sample.
- Each sample individually placed into sterile sample bags and diluted to 10^{-1} with phosphate buffer saline (PBS).
- All samples were placed into an ultrasonic bath at 37 KHZ at 50% power for 6 minutes to dislodge surface soil and attached microflora.
- Samples were removed and diluted to 10^{-3} . Evaluation of microbial growth was determined using tryptic soy agar (TSA), rose Bengal agar (RBA), MacConkey agar (MAC), and mannitol egg-yolk polymyxin agar (MYPA). Plates were incubated for 24 – 48 hours.
- MALDITOF analysis was completed on isolated colonies in the Animal Disease Research & Diagnostic Laboratory at South Dakota State Univ.

RESULTS

Table 1: Mean microorganism growth of pulse samples per agar type

Chickpea Regional Growth (Mean Log CFU/g)					
Agar Type	Region 1	Region 2	Region 3	Region 4	Region 5
TSA	4.10	3.11	3.59	3.32	3.60
RBA	2.51	0.374	0.84	2.46	2.44
MAC	4.18	1.26	3.20	2.76	2.86
MYPA	3.29	2.82	2.54	3.05	1.86

Pea Regional Growth (Mean Log CFU/g)

Agar Type	Region 1	Region 2	Region 3	Region 4	Region 5
TSA	3.09	3.20	2.93	2.24	3.27
RBA	2.62	0.34	0	1.52	2.6
MAC	3.08	2.23	2.60	1.27	2.11
MYPA	2.71	2.90	2.53	1.50	2.14

Table 2: Average moisture percentage of pulse seeds used in study.

<i>Pulse Type:</i>	<i>Average</i>	<i>Standard Deviation:</i>
Chickpea:	8.93 %	1.53 %
Pea:	9.59%	1.61%



Figure 1: Growth of general bacteria on Tryptic Soy Agar



Figure 2: Growth of Coliforms on MacConkey Agar



Figure 3: Growth of yeast and mold on Rose Bengal Agar

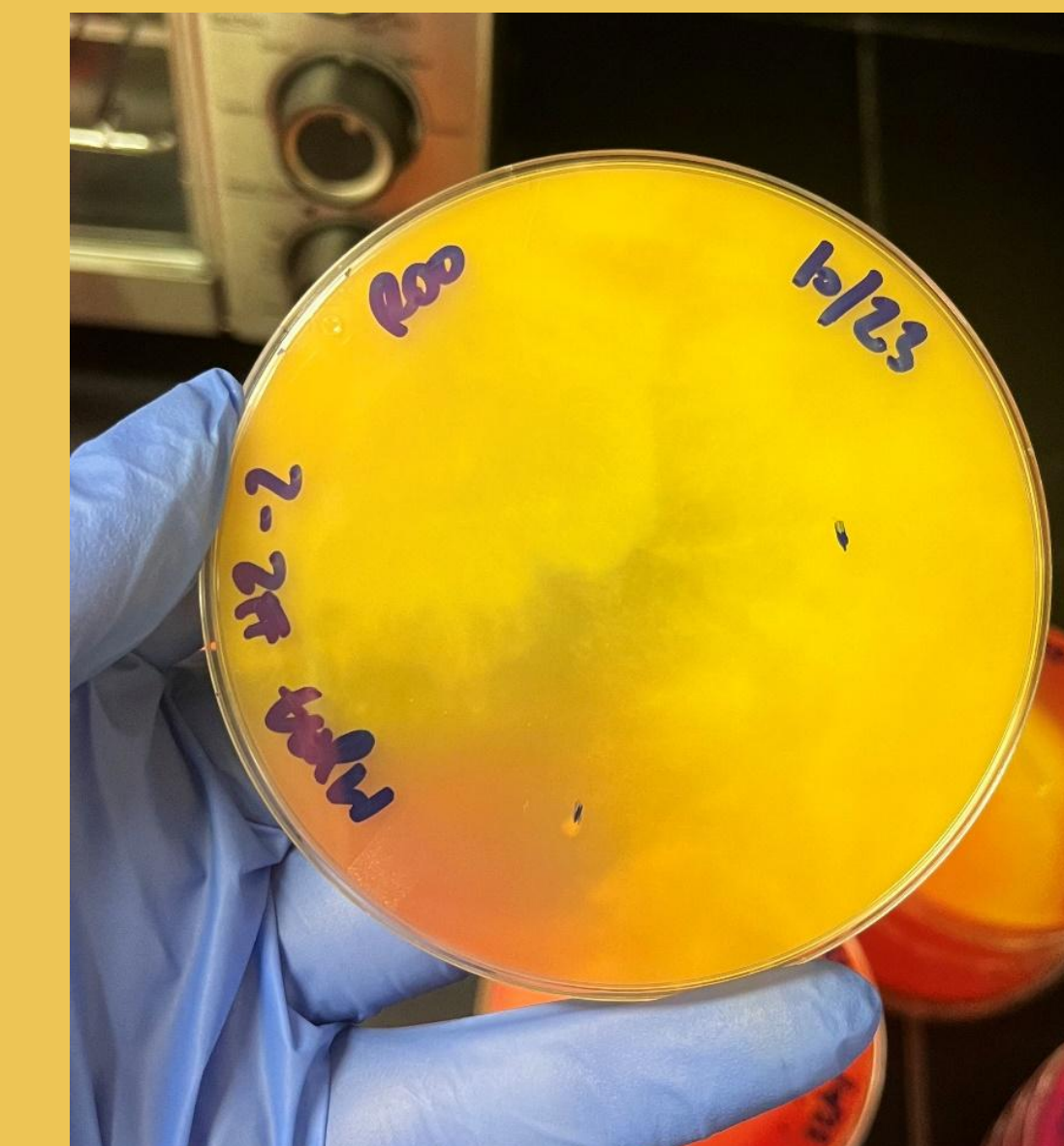


Figure 4: Growth of *Bacillus* Isolates on Mannitol Egg-Yolk Polymyxin

SUMMARY AND CONCLUSIONS

- To ensure product safety, pulse moisture content must remain below 14% for chickpea and 16% for pea. Therefore, seed samples are resistant to organism growth under current conditions in study.
- Agar variation allows for a range of microorganism isolation through different nutrient compositions
- Microbial populations of pulses remained consistent per individual region but shifted within large geographical regions.
- MALDI-TOF analysis characterized opportunistic pathogenic organisms on chickpeas, including *Alternaria Sp.*, *P. agglomerans*, and potential *B. cereus*.
- Due to *B. pumilus* being an environmentally resistant resident soil bacterium, *B. pumilus* is the most identified *bacillus* strain of MALDI-TOF analysis.
- Original source of the microorganisms were likely from soil microbiome, farm equipment, and product handling.
- Prevalence of diverse organisms in region samples and MALDI-TOF characterization provides evidence of need for microbial quality and safety parameters in pulse production and handling facilities.

ACKNOWLEDGEMENTS

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REFERENCES



Proportion of Possible Bacillus Isolates via MALDITOF

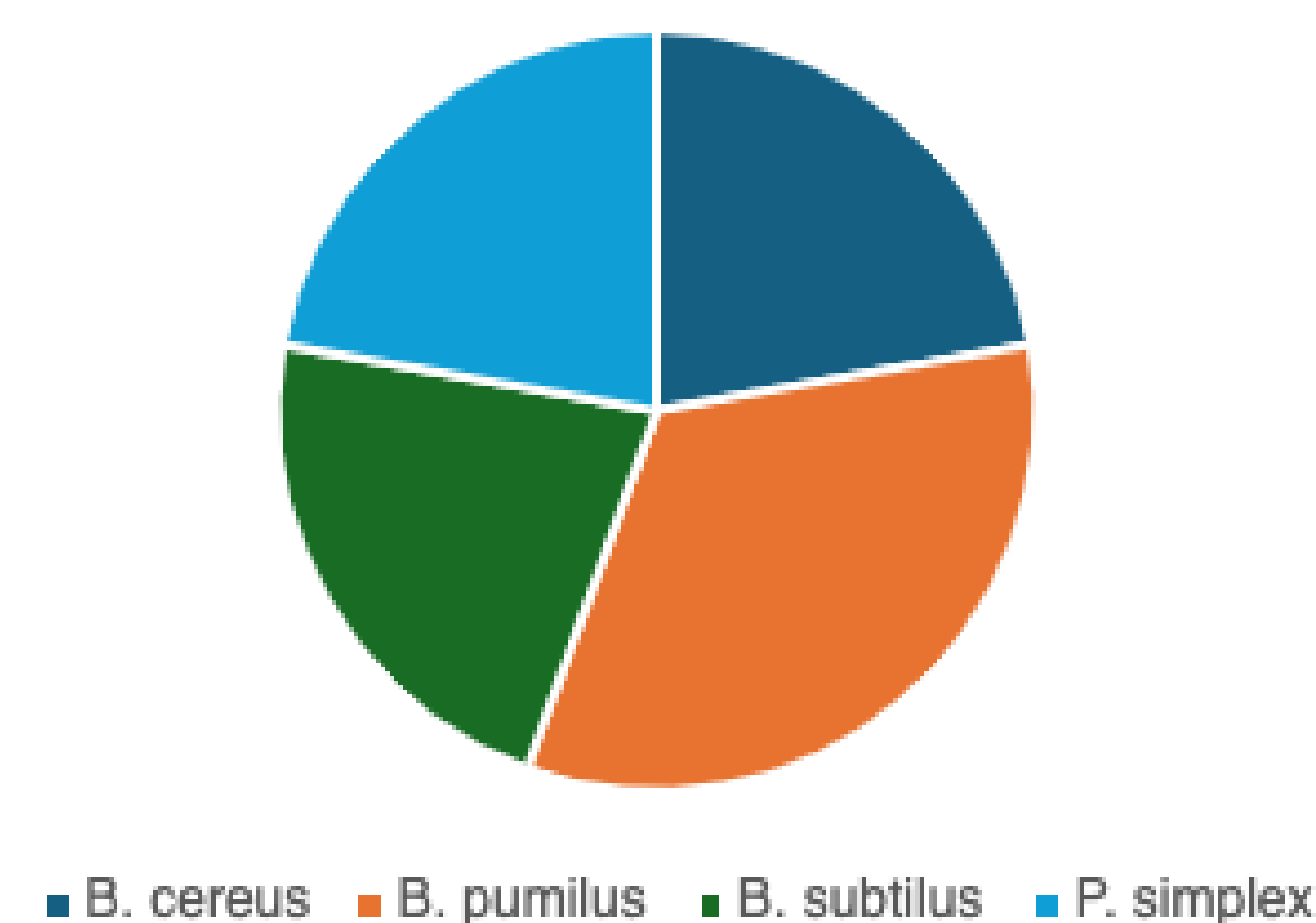


Figure 5: Proportion of organisms isolated through MALDITOF chickpea analysis which remain under the genus *Bacillus*.

Proportion of Total Organism Isolates via MALDITOF

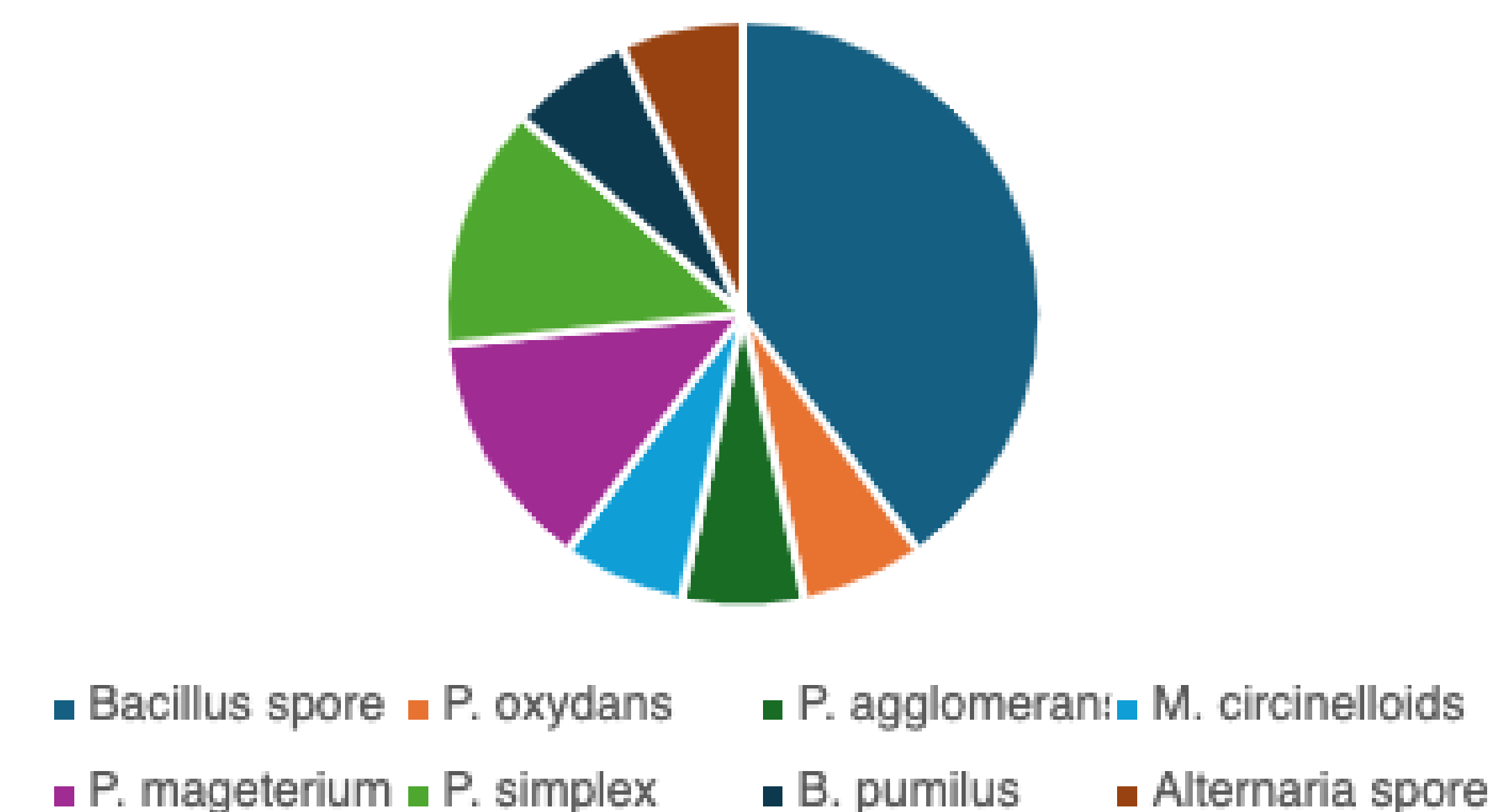


Figure 6: Total proportion of organisms isolated through MALDITOF chickpea analysis, regardless of Taxonomic family.