University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

School of Biological Sciences: Dissertations, Theses, and Student Research

Biological Sciences, School of

7-2024

Investigating the Abilities of Potentially Beneficial Bacteria for Increasing Nitrogen-Use Efficiency in Maize

Lexie R. Foster
University of Nebraska-Lincoln, lfoster7@unl.edu

Follow this and additional works at: https://digitalcommons.unl.edu/bioscidiss

Part of the Agricultural Science Commons, Agronomy and Crop Sciences Commons, Bacteria Commons, and the Plant Biology Commons

Foster, Lexie R., "Investigating the Abilities of Potentially Beneficial Bacteria for Increasing Nitrogen-Use Efficiency in Maize" (2024). *School of Biological Sciences: Dissertations, Theses, and Student Research*. 132.
https://digitalcommons.unl.edu/bioscidiss/132

This Thesis is brought to you for free and open access by the Biological Sciences, School of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in School of Biological Sciences: Dissertations, Theses, and Student Research by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

INVESTIGATING THE ABILITIES OF POTENTIALLY BENEFICIAL BACTERIA FOR INCREASING NITROGEN-USE EFFICIENCY IN MAIZE

by

Lexie R. Foster

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Biological Sciences

Under the Supervision of Professor Daniel Schachtman

Lincoln, Nebraska

July 2024

INVESTIGATING THE ABILITIES OF POTENTIALLY BENEFICIAL BACTERIA FOR INCREASING NITROGEN-USE EFFICIENCY IN MAIZE

Lexie Rae Foster, M.S.

University of Nebraska, 2024

Advisor: Daniel P. Schachtman

Global agriculture relies heavily on the use of synthetic nitrogen fertilizer to meet the current global food demand. Unfortunately, the average nitrogen-use efficiency (NUE) of maize (*Zea mays*) is approximately 50 percent. Improving the NUE of maize is essential for meeting the growing global food demand while also decreasing the negative environmental impacts caused by losses of nitrogen fertilizer due to runoff and volatilization. Harnessing the symbiotic relationship between plants and the soil microbiome may be one method for increasing the NUE in crops such as maize. In the present study, a set of potentially beneficial bacterial species were investigated for their ability to improve NUE-related traits in maize grown under nitrogen deficient conditions. Two bacterial isolates, *Arthrobacter* sp. and *Pseudomonas kribbensis* exhibited plant-growth promoting capabilities in the Mo17 maize genotype grown under nitrogen-deficient conditions. The time points at which the two bacterial isolates offered a significant effect differed, as the *Arthrobacter* sp. offered a significant growth effect at 14 days of growth, while *Pseudomonas kribbensis* offered a significant growth effect starting at 21 days of growth. While *Arthrobacter* sp. and *Pseudomonas kribbensis* offered plant-growth promotion in the Mo17 maize genotype under low nitrogen, other genotypes were not positively influenced, suggesting a specific plant genotype and bacterial species relationship. Exploring the plant-growth promoting effects of bacterial isolates sampled from nitrogen-deficient maize genotypes is one avenue in increasing the NUE of crops.

Contents

List of Figures ix

1 Acknowledgements x

2 Project Funding xii

3 Introduction 1

3.1 References . . . 3

4 Literature Review 4

4.1 Nitrogen in Plants . . . 4

4.2 Plant Responses to Nitrogen Deficient Growth Conditions . . . 6

4.3 The Soil Microbiome and its Members' Effects on the Soil Nitrogen Cycles, Nitrogen Availability for the Plants, and Symbiotic Relationships with Plants 9

4.4 The Role of Indole-3-Acetic Acid in Increasing NUE in Plants . . . 12

4.5 Incorporating the Rhizosphere into Plant Breeding . . . 14

4.6 The Use of Microbial Inoculants for Increasing Plant Fitness Under Nitrogen Deficiency . . . 15

4.7 References . . . 16

5 Materials and Methods 28

5.1 Initial Bacterial Isolate Screening . . . 28

5.1.1 Identification of the 64 Bacterial Isolates . . . 28

5.1.2 Bacterial Isolates . . . 29

5.1.3 Bacterial Growth . . . 29

5.1.4 Mo17 Maize Seed Sterilization and Germination . . . 30

5.1.5 Potting Mix Sterilization . . . 30

5.1.6 Seedling Priming Bacterial Inoculation 31

5.1.7 Maize Planting 31

5.1.8 Maize Growth Conditions 32

5.1.9 Maize Root and Shoot Sampling 32

5.1.10 Breaking the 64 Isolates up into Separate Growth Experiments 33

5.1.11 Statistical Analysis 33

5.2 Sanger Sequencing 33

5.2.1 Confirming the Identities of the Selected Bacterial Isolates 33

5.3 Two-Week Validation Experiment for Selected Bacterial Isolates 34

5.3.1 Breaking the Selected Bacterial Isolates into Two Experimental Groups 34

5.3.2 Growth Chamber Experimental Layouts for Groups 1 and 2 35

5.3.3 Seed Germination for Group 1 and Group 2 Bacterial Isolates 36

5.3.4 Bacterial Growth for Group 1 and Group 2 Bacterial Isolates 37

5.3.5 Seedling Priming for Group 1 and Group 2 Bacterial Isolates 37

5.3.6 Potting Mix Sterilization 37

5.3.7 Maize Planting 38

5.3.8 Maize Growth Conditions 38

5.3.9 Maize Root and Shoot Sampling 38

5.3.10 Statisical Analysis 38

5.4 4-Week Growth Validation Experiment 38

5.4.1 Soil Water Holding Capacity (SWHC) Trial 39

5.4.2 Bacterial Selection 40

5.4.3 Maize Genotype Selection 40

5.4.4 Experimental Design 41

5.4.5 Bacterial Growth and Inoculation 42

5.4.6 Maize Planting and Growth Conditions 42

5.4.7 Hoagland Solution Soil Drench Treatment 43

5.4.8 Plant Phenotyping at 2-, 3-, and 4-Week Timepoints 43
5.4.9 Statisical Analysis 44
5.5 Analysis of Bacterial Genomes 111 and 730 44
5.5.1 DNA Extraction 45
5.5.2 Library Preparation 45
5.5.3 Genome Sequencing 46
5.5.4 Hybrid Genome Assembly 46
5.5.5 Genome Annotation 46

6 Results 47

6.1 Identification of 64 Potentially Beneficial Bacterial Isolates 47
6.2 Initial Assay of the 64 Potentially Beneficial Bacterial Isolates 55
6.3 Sanger Sequencing 60
6.4 2-Week Validation Experiments for Selected Bacterial Isolates 60
6.5 4-week Validation Experiment for Selected Bacterial Isolates 64
6.5.1 Results from the Soil Water Holding Capacity Experiment 64
6.5.2 Effect of Bacterial Isolates on the Dry Shoot Weight of Inoculated Maize Genotypes 65
6.5.3 Phenotyping of above ground biomass of maize plants during 4 week experiment 72
6.6 Bacterial Genome Analysis of Bacterial Isolates 111 and 730 88

7 Discussion 90

8 Conclusion 101

9 References 102

10 Supplementary Tables 107

List of Figures

1 Growth chamber layout for the 2-week validation experiment for the group 1 bacterial isolates. 36

2 Growth chamber layout for the 2-week validation experiment for the group 2 bacterial isolates. 36

3 Growth chamber layout for the 4-week validation experiment for the selected bacterial isolates (including the uninoculated low N and high N controls) and maize genotypes. (Notation: Maize Genotype-Bacterial Isolate) 42

4 Initial Assay Round 1 Dry Shoot Weight with six Bacterial Isolates. Dry shoot weight (grams) results from round 1 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. Boxed bacterial isolates were selected for the validation studies. . . . 56

5 Initial Assay Round 2 Dry Shoot Weight with eight Bacterial Isolates. Dry shoot weight (grams) results from round 2 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. Boxed bacterial isolates were selected for the validation studies. . . . 57

6 Initial Assay Round 3 Dry Shoot Weight with two Bacterial Isolates. Dry shoot weight (grams) results from round 3 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. 57

7 Initial Assay Round 4 Dry Shoot Weight with eighteen Bacterial Isolates. Dry shoot weight (grams) results from round 4 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. Boxed bacterial isolates were selected for the validation studies. . . . 58

8 Initial Assay Round 5 Dry Shoot Weight with four Bacterial Isolates. Dry shoot weight (grams) results from round 5 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. Boxed bacterial isolates were selected for the validation studies. . . . 58

9 Initial Assay Round 6 Dry Shoot Weight with twenty-six Bacterial Isolates. Dry shoot weight (grams) results from round 6 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. Boxed bacterial isolates were selected for the validation studies. . . 59

10 Initial Assay Round 7 Dry Shoot Weight with eleven Bacterial Isolates. Dry shoot weight (grams) results from round 7 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. 59

11 Dry shoot weight (grams) of the Mo17 maize genotype inoculated with Group 1 bacterial isolates in the 2-week validation experiment 61

12 Dry shoot weight (grams) of the Mo17 maize genotype inoculated with Group 2 bacterial isolates in the 2-week validation experiment 62

13 Soil Water Holding Capacity (SWHC) Experiment. Three SWHC amounts were tested to decide which SWHC amount (70, 80, or 90 percent) to water the plants in the four-week validaion experiment at (Different letters indicate a significant difference $p < 0.05$). 64

14 4-Week Validation Experiment - Dry shoot weight (grams) of the Mo17 maize genotype inoculated with bacterial isolates and the low N control. (Different letters indicate a significant difference $p < 0.05$). 68

15 4-Week Validation Experiment - Dry shoot weight (grams) of the Ames 27065 maize genotype inoculated with bacterial isolates and the low N control. (Different letters indicate a significant difference $p < 0.05$). 69

16 4-Week Validation Experiment - Dry shoot weight (grams) of the NSL 30867 maize genotype inoculated with bacterial isolates and the low N control. (Different letters indicate a significant difference $p < 0.05$). 69

17 4-Week Validation Experiment - Dry shoot weight (grams) of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and uninoculated conditions (low N control). (Different letters indicate a significant difference $p < 0.05$). 70

18 4-Week Validation Experiment - Dry shoot weight (grams) of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 111. (Different letters indicate a significant difference $p < 0.05$). 70

19 4-Week Validation Experiment - Dry shoot weight (grams) of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 730. (Different letters indicate a significant difference $p < 0.05$). 71

20 4-Week Validation Experiment - Dry shoot weight (grams) of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 4589. (Different letters indicate a significant difference $p < 0.05$). 71

21 Correlation analysis between the sampled dry shoot weights of the 28-day old plants and the shoot biomass images at 27-days of growth. $r = 0.93693$ 73

22 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the three inoculated maize genotypes with the bacterial isolates 111, 730, and 4589, and the low N control. 75

23 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Mo17 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control. 76

24 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Ames 27065 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control. 77

25 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the NSL 30867 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control 78

26 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and uninoculated conditions (low N control). (Different letters indicate a significant difference $p < 0.05$). 78

27 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 111. (Different letters indicate a significant difference $p < 0.05$). 79

28 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 730. (Different letters indicate a significant difference $p < 0.05$). 79

29 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 4589. (Different letters indicate a significant difference $p < 0.05$). 80

30 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the three inoculated maize genotypes with the bacterial isolates 111, 730, and 4589, and the low N control. 82

31 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Mo17 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control (Different letters indicate a significant difference $p < 0.05$). 83

32 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Ames 27065 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control. 84

33 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the NSL 30867 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control (Different letters indicate a significant difference $p < 0.05$). 85

34 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and uninoculated conditions (low N control). (Different letters indicate a significant difference $p < 0.05$). 85

35 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 111. (Different letters indicate a significant difference $p < 0.05$). 86

36 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 730. (Different letters indicate a significant difference $p < 0.05$). 86

37 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 4589. (Different letters indicate a significant difference $p < 0.05$). 87

38 Genome Sequencing Metrics for Bacterial Isolate 111 89

39 Genome Sequencing Metrics for Bacterial Isolate 730 90

1 Acknowledgements

Reflecting on the past two years of graduate school, I can see not just how much I have learned academically and about my subject, but also how much I have gained as a person and how I want to live my life. I have many thanks to give to the people in my life that have supported me during this time as well as moving forward.

I first want to thank my advisor Dr. Daniel Schachtman for the opportunity to work and grow in your lab. From your advice and direction on my project, to sharing stories about biking and traveling, I am very appreciative of having the chance to learn from your direction and getting to know you and Ellen.

Thank you to my committee members, Dr. Jinliang Yang and Dr. Karrie Weber, for your advice and insight into my project. Dr. Yang, I always really enjoyed the few days out of the summer collaborating with your team on the rhizosphere harvests!

A big thanks to the people in the Schachtman Lab who kept things fun and lighthearted during the long days of lab work. I am very grateful to have been given the opportunity to work with Jingjie Hao, Emma Bergmeyer, Kaitlin Bachle, Dania Vieira Branco Ozorio, Hussnain Mukhtar, and Rylee McDonnell, throughout different points in my graduate school career.

And last but certainly not least, a huge thank you to the people closest to me in my life. Thank you to my Mom and Dad, the two people who sparked and nurtured my love for learning, the outdoors, nature, and exploring the farm. Thank you to my three older sisters, Jami, Kelsey, and Audrey, for being the role models I always have looked up to in my life and the three best friends a girl could ask for growing up and still today. Thank you to my

boyfriend Maxx for all the books read, songs listened to, and miles ran and biked together over the past two years. And finally, a thank you to Muffin, Bear, and an unexpected Lilac, for bringing such light into my life. I love all of you.

2 Project Funding

This research project was made possible by research assistant funding from the Agricultural Research Division.

3 Introduction

Plants utilize nitrogen in the composition of their proteins, nucleic acids, chlorophyll, and other secondary products within the plant (Glass 2009). The U.S. rates of nitrogen fertilizer use in agriculture have increased steadily since the 1940s (Cao et al., 2018). Unfortunately, the nitrogen-use efficiency (NUE) of plants, such as maize (*Zea mays*), is only anywhere from 25-50 percent (Javed et al., 2022), meaning that a high proportion of the applied nitrogen is lost to the environment in the form of volatilization or leaching (Lan et al., 2022). Improving the NUE in maize is essential not only for decreasing negative environmental impacts of nitrogen use, but also for meeting the global food demand.

Harnessing the symbiotic relationship between plants and the soil microbiome is one method for decreasing excessive usage of nitrogen and for increasing the NUE of maize. Based on a previous study (Meier et al. 2022), a group of amplicon sequence variants (ASVs) of soil bacterial species was identified from the rhizospheres of inbred maize plants grown under nitrogen-deficient conditions. Plants can recruit bacterial communities to their rhizospheres through root exudation (Coskun et al. 2017). When plants, such as maize, encounter a nutrient deficient environment, such as insufficient nitrogen quantities for growth, plants can recruit specific bacterial species that may offer a benefit to their overall plant health (Coskun et al. 2017). The bacterial species identified in Meier et al. 2022 were hypothesized to be under selection by the maize plant genomes, as the bacterial species were recruited to the rhizospheres of maize grown in nitrogen-deficient conditions. The ASVs identified in Meier et al. 2022 were matched to 16s rRNA sequences in the Schachtman Lab Culture Collection and identified as 64 potentially beneficial bacterial isolates that were selected for further testing.

The goal of this research project was to investigate the potential of the 64 bacterial iso-

lates as plant-growth promoting bacteria for maize genotypes grown in nitrogen-deficient conditions. To test the plant-growth promoting abilities of the 64 bacterial isolates, plant inoculation studies were carried out using seed and plant inoculation methods outlined in Chai et al. 2022. The effects of each bacterial isolate on the shoot biomass weight of nitrogen-deficient maize were used to help start identifying bacterial isolates that may be plant-growth promoting rhizobacteria for maize genotypes grown in low nitrogen environments.

Identifying bacterial species that offer a plant-growth promoting effect on nitrogen-deficient maize plants is one avenue towards increasing NUE in global crops. Increasing NUE in crops, such as maize, will not only ensure that the global food demand is met, but also decrease negative environmental impacts brought on by nitrogen fertilizer overuse. The identification of plant-growth promoting bacterial species will offer new avenues for plant breeding, such as breeding for plant root exudates that select for beneficial bacterial species under nutrient-deficient conditions. Developing plant inoculations containing plant-growth promoting bacteria is another avenue that can be explored with beneficial bacterial species. This project is the investigation of a set of bacterial species and their potential as plant-growth promoting bacteria in nitrogen-deficient maize.

3.1 References

References

[1] Cao, P., Lu, C., Yu, Z. Historical nitrogen fertilizer use in agricultural ecosystems of the contiguous United States during 1850-2015: application rate, timing, and fertilizer types. *Earth Syst. Sci. Data.* (2018). 10:969-984. Doi:10.5194/essd-10-969-2018

[2] Chai, Y.N., Futrell, S., Schachtman, D.P. Assessment of Bacterial Inoculant Delivery Methods for Cereal Crops. *Front. Microbiol.* (2022). 13:791110. Doi:10.3389/fmicb.2022.791110

[3] Coskun, D., Britto, D.T., Shi, W., Kronzucker, H.J. How Plant Root Exudates Shape the Nitrogen Cycle. *Trends in Plant Science.* (2017). 22(8):661-673 DOI:10.3390/genes9030125

[4] Glass, A.D. Nitrate uptake by plant roots. *Botany.* 87(7): 659-667. Doi:10.1139/B09-014

[5] Javed, T., I, I., Singhal, R.K., Shabbir, R., Shah, A.N., Kumar, P., Jinger, D., Dharmappa, P.M., Shad, M.A., Saha, D., Anuragi, H., Adamski, R., Siuta, D. Recent Advances in Agronomic and Physio-Molecular Approaches for Improving Nitrogen Use Efficiency in Crop Plants. *Front. Plant Sci.* (2022). 13:877544. Doi:10.3389/fpls.2022.877544

[6] Lan, X., Zhu, L., Yuan, Q. Long-Term Variation of Greenhouse Gas N2O Observed by MLS during 2005-2020. *Remote Sens.* (2022). 14, 955. Doi:10.3390/rrs14040955

[7] Meier, M.A., Xu, G., Lopez-Guerrero, M.G., Li, G., Smith, C., Sigmon, B., Herr, J.R., Alfano, J.R., Ge, Y., Schnable, J., Yang, J. Association analyses of host genetics, root-colonizing microbes, and plant phenotypes under different nitrogen conditions in maize. *eLife.* (2022). 11:e75790. Doi:10.7554/eLife.75790

4 Literature Review

4.1 Nitrogen in Plants

Nitrogen (N) is an essential element in the growth, functioning, and health of plants (Luo et al., 2020). Nitrogen in the soil can either be found in its organic or inorganic forms. Inorganic nitrogen species include ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) (Soumare et al., 2020). Plants can utilize nitrogen in the form of either NH_4^+ or more commonly, NO_3^- (Novoa et al., 1981). NO_3^- acts as a signaling molecule allowing plants to sense the presence or absence of NO_3^- in the surrounding soil, thus, activating NO_3^- related genes and enabling NO_3^- transporters to uptake the NO_3^- from the soil (Aluko et al., 2023). The nitrogen content within the soil at a given moment is variable, therefore there are at least three distinguishable NO_3^- transport systems within a plant's roots that monitor the appropriate amount of nitrogen to uptake from the soil. The constitutive high-affinity transport systems (CHATS) are responsible for a constant uptake of NO_3^- when NO_3^- concentrations are low in the soil. The inducible high-affinity transport systems (IHATS) transporters are activated when NO_3^- concentrations are low in the soil. Finally, the low-affinity transport systems (LATS), are active when NO_3^- concentrations are high in the soil (Crawford et al., 1998). Ammonium transporters (AMTs) are responsible for the ammonium uptake in plants (Yang et al., 2023).

The nitrogen within a plant can have different fates, depending on the age and growth needs of the plant. The long-distance transport of nitrogen throughout the plant is carried out by low-affinity nitrate transporters (*NRT1s*), such as *NRT1.5* and *NRT1.8* in Arabidopsis plants, as these transporters load nitrate to and from the root and shoot of the Arabidopsis plant (Li et al., 2010; Lin et al., 2008). The role of nitrogen in a plant includes being incorporated into the composition of proteins, nucleic acids, chlorophyll and other

secondary products within the plant (Glass 2009), as well as carrying out processes such as photosynthesis (Ya-wei et al., 2019). For the plant to be able to use the NO_3^- it takes up from the soil, the plant's NO_3^- and NO_2^- reductases convert NO_3^- into NH_4^+, which can then go through the glutamine-synthetase and glutamate synthase (GS-GOGAT) pathway to make essential amino acids for the plant (Raddatz et al., 2020).

In the U.S., the rates of nitrogen fertilizer have steadily increased since the 1940s. The rate of nitrogen fertilizer use went from 0.28 g N m^{-2} y^{-1} in 1940 to 9.54 g N m^{-2} y^{-1} in 2015 (Cao et al., 2018). The production of synthetic nitrogen fertilizer is based on the Haber-Bosch process, developed in the early 1900s by German chemist Fritz Haber and German chemist and engineer Carl Bosch. Today on a global scale, NH_3 is synthesized, with the help of an iron catalyst, by combining hydrogen and nitrogen at an extremely high temperature and pressure (Wood & Cowie, 2004). Nitrogen is commonly applied to the soils in the form of NH_3. The fate of applied NH_3 that is not assimilated by the plant is often times volatilized into the atmosphere. Nitrous oxide (N_2O), a greenhouse gas primarily emitted from soils, has a global warming potential 298 times greater than CO_2, making it a cause for concern in global climate change (Lan et al., 2022). Soils contribute to approximately 53 percent of the global anthropogenic emission of N_2O (Denman et al., 2007). In addition to N_2O being a greenhouse gas, N_2O along with other reactive nitrogen species, such as NO_3^-, NH_3, and $NH_4{}^+$ can interact with other compounds to create pollutants such as ozone (O_3) and particulate matter (PM), further decreasing air quality (Peel et al., 2013). The pH of soils is also affected by nitrogen fertilizer application, as long-term N use can significantly reduce the soil pH in the topsoil layer (0-15 cm), which may further lead to decreases in yield due to poor soil health (Schroder et al., 2011). Even with the increasing application of nitrogen fertilizer to agricultural fields, the nitrogen-use efficiency (NUE) of plants is only anywhere from 25-50 percent of applied N (Javed et al., 2022). Increasing the NUE of crops is important not only for decreasing the negative environmental impact of nitrogen fertilizer

application, but also for maintaining the health of the plant, as nitrogen is a key macronutrient.

4.2 Plant Responses to Nitrogen Deficient Growth Conditions

Plants have different mechanisms for adapting to nitrogen-deficient growth conditions. For example, one mechanism in which a plant may respond to nitrogen-deficient soil is by increasing their root growth. This mechanism, the stress-initiated nitrate allocation to roots (SINAR), is controlled by the nitrate transporters *NRT1.5* and *NRT1.8* (Zhang et al., 2014). When a plant is subjected to nitrogen-deficient conditions, its *NRT1.8* transporter may work to move nitrate from the xylem back into the roots (Li et al., 2010). For example, under low-nitrogen conditions, the roots of rapeseed plants elongated, cells in the elongation zone of the root tips became larger in size, and there were denser cells in the meristematic zone (Qin et al. 2019). In another study, the fresh root weight, lateral root density, and root surface area of strawberry plants grown under low nitrogen conditions, all increased (Zhang et al., 2023). Similarly, researchers found an increase in root fresh weight and lateral root number in nitrogen-deficient wheat plants (Lv et al., 2021). There are different mechanisms that may be responsible for this increase in root biomass under low nitrogen conditions. For example, in the previously mentioned studies for strawberry and wheat plants (Zhang et al., 2023, Ly et al., 2021), the researchers also found there to be a heightened accumulation of indole-3-acetic acid (IAA) and jasmonic acid (JA) in the nitrogen-deficient plants. To produce IAA in plants, IAA is first converted from tryptophan (Trp) into indole-3-pyruvate (IPA) by the TAA amino transferases family. IPA is then converted into IAA by the YUC flavin monooxygenases family (Zhao et al., 2012). Indole-3-acetic acid (IAA) has been characterized as having influence in the regulation of certain plant processes, such as cell division, cell elongation, and vascular differentiation (Zhang et al., 2021). One mechanism

in which plants may optimize their growth in nitrogen-deficient conditions is by increasing their IAA production, directly influencing their root growth and indirectly increasing their root biomass to scavenge for nitrogen sources. JA is a plant phytohormone that regulates responses to changing nutrient conditions in plants (Shikha et al., 2023). One way in which JA may influence a nitrogen-deficient plant is by recruiting beneficial mycorrhizal associations through JA exudation, such as in tomato plants, which may help uptake nitrogen from the soil for the plant (Sánchez-Bel et al., 2018). In addition, under nitrogen-deficient conditions, the increased production of JA can regulate the expression levels of *NRT1.5* and *NRT1.8*, nitrate transporters responsible for the transportation of NO_3^- in and out of the xylem and roots in the plant (Kamali et al. 2022). The increased concentrations of IAA and JA observed in the nitrogen-deficient wheat and strawberry plants is one example of a way in which plants can adapt and optimize their processes in low nitrogen environments to influence root growth.

While the root biomass may be increased as the plant adapts to search the soil for more nitrogen, the shoot biomass of the plant may decrease, in response to nitrogen-deficient conditions. Decreasing the shoot biomass to maintain or in some cases, increase root biomass in low nitrogen conditions, may be a plant's method of maximizing its scarce nitrogen sources to obtain more nitrogen resources from the soil (Chun et al. 2004). For example, a particular study found that when maize plants are grown under low nitrogen conditions, the shoot growth was reduced by as much as 63 percent on the 12th day of a low nitrogen treatment, while the root dry weight increased under the low nitrogen treatment (Gao et al. 2015). Similar results were presented in another study, as researchers found that the root to shoot ratio was increased by 67 percent in the low nitrogen conditions, compared to the nitrogen sufficient conditions (Mu et al. 2017). In the same study, the researchers also found that the photosynthetic rate of the nitrogen deficient maize was reduced by 83 percent (Mu et al. 2017). A plant's decrease in photosynthetic rate in nitrogen deficient conditions may

be caused by the subsequent decrease in the important photosynthetic enzymatic activities of Rubisco and PEPcase (Wei et al. 2016). In a study investigating the photosynthetic and chlorophyll fluorescence abilities of maize cultivars varying in low nitrogen resilience, researchers found that nitrogen-deficient conditions decreased the chlorophyll content at the seedling stage, with this stress increasing over the lifespan of the plants (Ya-wei et al., 2019). In addition, the low-N tolerant maize cultivar was found to have a higher net photosynthetic rate, compared to the low-N sensitive maize cultivar.

An important plant quality that is affected by N-deficient conditions, as it directly impacts the global food supply, is the plants' grain yield. In one study, researchers found that maize grown under high nitrogen conditions had a grain yield/ha increase of 14.53 percent and a 13.8 percent increase in grain yield/plant, compared to the nitrogen-controlled maize, grown in medium nitrogen levels (Abdel-Lattif, H.M., et al., 2019). In the same study, the plants grown in low nitrogen conditions resulted in a 4.3 percent decrease in grain yield/ha and a 4.26 percent decrease in grain yield/plant, compared to the control plants. Interestingly, in the same study, the maize grown in low nitrogen conditions had a 27.53 percent increase in NUE. Similarly, in another study, researchers found that, on average, there was a 137 percent decrease in maize yield grown in nitrogen-deficient conditions, compared to high nitrogen (Raza et al., 2022). This significant increase in grain yield is one of the main reasons for the increase of nitrogen fertilizer use over the past century (Cao et al. 2018).

4.3 The Soil Microbiome and its Members' Effects on the Soil Nitrogen Cycles, Nitrogen Availability for the Plants, and Symbiotic Relationships with Plants

The soil habitat harbors a large and diverse variety of microorganisms, collectively referred to as the soil microbiome. This soil microbiome community consists of prokaryotes, fungi, protists, and viruses (Bardgett et al. 2014). The rhizosphere, the area of soil in direct proximity with a plant's roots, that is therefore, influenced by the plant's nutrients and oxygen availability, forms the intersection between a host plant and its microbes (Trivedi et al. 2020). To potentially select for certain microorganisms to inhabit their rhizospheres, plants may release chemical compounds to determine the beneficial microbial members that will inhabit their respective rhizospheres (Coskun et al. 2017). These chemical compounds are referred to as root exudates, and the composition of root exudates may include sugars, simple polysaccharides, amino acids, organic acids, and phenolic compounds. These root exudates can influence the soil microorganisms surrounding the exuding roots (Bertin et al. 2003). In a study investigating the root exudate chemistry and microbial members present in a wild oat plant (*Avena barbata*), researchers found that the chemical compounds released from the plant differed with each growth stage and that there were positive and negative microbial respondents to these exudates (Zhalnina et al. 2018). Plants, such as switchgrass (*Panicum virgatum* L.), can release exudates that competitive bacterial species can utilize, therefore, allowing the plant to potentially select for its microbial members (Mao et al. 2014). The genotype and growth stage of a plant can also affect the bacterial community composition of the plant's rhizosphere, as the root exudate profiles and abundances differ based on genotype and growth stage, therefore, selecting for distinct bacterial communities in the different genotypes and at certain growth stages (Lopes et al., 2022; Sutherland et al. 2021). When plants encounter a less-than-favorable environment due to a nutrient deficiency, such as low nitrogen, they perhaps can select for microbes that will offer a ben-

eficial effect to overcome their deficit (Coskun et al. 2017). For example, in a particular study investigating how a nitrogen deficiency affects the microbial community of six different sorghum cultivars' rhizospheres, researchers found that the relative abundances and diversity of sorghum rhizospheres decreased, perhaps due to the sorghum plants selecting for specific bacterial groups that would offer a beneficial effect in low nitrogen conditions (Wu et al. 2020).

The microorganisms within the soil can sometimes play either a beneficial or pathogenic role toward the plant species whose roots they are inhabiting (Lau et al. 2012; Peeters et al. 2013). For example, a well-studied relationship between a plant host and its nitrogen-fixing bacterial companion, is the symbiotic relationship between leguminous plants and rhizobia bacteria (Clúa et al. 2018). The formation of root nodules, the root structures where the conversion of atmospheric nitrogen by the rhizobia bacteria takes place, into a nitrogen form the plant can utilize, is initiated by nodulation (Nod) factors released by the rhizobia bacteria (Jones et al. 2007). The yield performance in a leguminous crop, such as soybeans, is associated with the plant's ability to form root nodules (Jin et al. 2022). Plants are able to recruit beneficial bacterial species to their rhizospheres through the production and release of specific root exudates, for example the recruitment of beneficial *Bacillus subtillis* through malic acid exudation from the roots of Arabidopsis (*Arabidopsis thaliana*) plants. (Rudrappa et al., 2008). Plant-growth promoting capabilities of *Bacillus subtillis* include but are not limited to, nitrogen-fixation, phosphorus solubilization, and cytokinin production (Blake et al., 2021). In various maize genotypes, *Azospirillum brasilense* has been shown to improve crop growth and increase NUE in nitrogen-deficient growth conditions (Zeffa et al. 2019), perhaps due to the synthesis of phytohormones and upregulating stress-tolerance related genes in plants (Fukami et al. 2017).

Just as plants can select for the microbial members represented in their rhizosphere, abiotic

factors can also play a role in the microbial members that inhabit a host plant's rhizosphere (Berg et al. 2009). The addition of nitrogen fertilizer is perhaps one the most important abiotic factors that must be considered. In a six-year nitrogen fertilization regime study, researchers found that long-term addition of nitrogen fertilizer can modify both the above ground plant diversity of that soil area, as well as, certain below ground factors, such as soil acidity (Zeng et al. 2016). Similarly, in another study investigating the effect of six different nitrogen addition treatments on soil properties, including bacterial alpha diversity, researchers found that long-term addition of nitrogen significantly changed the soil pH, and the highest nitrogen addition rate significantly reduced the bacterial alpha diversity of the soil (Song et al. 2023). The input of nitrogen fertilizer decreases the soil's pH through microbial oxidation of these ammonium-based fertilizers (Barak et al. 1997). A decreased pH of the soil can significantly affect and shift the bacterial communities inhabiting the soil area (Zhang et al. 2017). In addition, altered above-ground plant species may affect the microbial members inhabiting the soil environment's resident rhizospheres, depending on the level of interaction between resident plants and bacterial species (Kowalchuk et al. 2002). It is speculated that nitrogen addition alters these soil bacterial communities, potentially preventing the inhabiting plants from selecting the bacterial species that best support their health. Conversely, if the nitrogen addition alters the above ground composition of plant species, different bacterial species may be favored.

Among the functions of soil microbes, certain microbial species can play a role in the nitrogen cycle below ground, further benefiting the plants inhabiting the same soil area (Franche et al. 2009). Nitrifying bacteria in the soil can convert NH_3 into NO_3^-, a nitrogen form plants can directly utilize, in the process known as nitrification (Fiencke et al. 2005). During nitrification, aerobic NH_3-oxidizing bacteria convert unstable NH_3 into NO_2^-, while NO_2^--oxidizing bacteria convert the NO_2^- into NO_3^-. In addition, within the soil nitrogen cycle, bacteria also carry out denitrification, a process in which NO_3^- is reduced to N_2O or nitrogen

gas (Martienssen & Schöps, 1999). Certain soil bacteria can also carry out a process known as dissimilatory nitrate reduction to ammonium (DNRA), in which NO_3^- is reduced to NH_4^+ using the pentaheme cytochrome *c* nitrite reductase (NrfA) enzyme (Wang et al., 2024). Additionally, certain bacterial species, such as *Clostridium perfringens*, are able to use NO_3^- as an electron acceptor, during anaerobic respiration processes such as fermentation (Hasan et al., 1975). A direct way in which plants can benefit from a function of certain bacteria is by utilizing the nitrogen that is available from biological nitrogen fixation (BNF). BNF is the process in which specific bacterial species can convert di-nitrogen gas (N_2) into NH_3, where it can be converted into a nitrogen form a plant can readily use, such as NH_4^+ (Soumare et al. 2020). These nitrogen-fixing bacteria contain the nitrogenase enzyme, which consists of the iron (Fe) and molybdenum iron (MoFe) proteins, that catalyzes the reduction of N_2 to NH_3 (Howard et al. 1996). The nitrogenase enzyme is encoded by the following genes: *nifD*, *nifk*, and *nifH* (Zehr et al. 2003). The presence of these genes in bacterial species' genomes could perhaps mean that the bacterial species has the nitrogen-fixing ability. As mentioned previously, a well-known bacterial group that has nitrogen-fixing capabilities for its host plant, legumes, is rhizobia bacteria (Clúa et al. 2018). In addition to rhizobia bacteria that associate with plant roots directly for nitrogen fixation, there are certain bacterial species that are free-living in the soil that fix nitrogen that plants can then utilize. Examples of these free-living nitrogen-fixers include *Azospirillum*, *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Azoarcus* spp., and *Azotobacter* (Steenhoudt et al., 2000). Bacteria in the *Azospirillum* genus have been shown to fix nitrogen for grasses such as maize.

4.4 The Role of Indole-3-Acetic Acid in Increasing NUE in Plants

Another response that certain plant growth promoting bacterial species have in response to nitrogen deficient conditions is the increased production of indole-3-acetic acid (IAA). IAA, often referred to as auxin, regulates plant processes such as cell division, cell elongation, vascular differentiation, and phototropism (Zhang et al., 2021). IAA production can

take place in plants and bacteria through a variety of different pathways. For example, the production of IAA in *Azospirillum brasilense* SM takes place via the indole-3-pyruvic acid (IPyA) pathway. In their investigation, the researchers found that the production levels of IAA in the *A. brasilense* bacteria were significantly increased under nitrogen deficient conditions (Malhotra et. al 2009). Based on this finding, IAA-producing bacterial species may be best utilized or selected for by plants growing in soils lacking adequate nitrogen resources for sufficient plant growth. Similarly, other researchers found that low nitrogen conditions significantly enhanced the IAA production in bacterial species *Serratia* sp. ZM (Ouyang et al. 2017). Indole-3-pyruvate decarboxylate, which is encoded by the ipdC gene, is the key enzyme in the IPyA pathway. When this gene was knocked out in *Azospirillum brasilense* Sp245, the production of IAA was strongly reduced, therefore, making this gene a possible target for enhancing the effect that IAA-producing bacterial species have on their plant growth promoting abilities (Spaepen et al. 2008).

The production of IAA by bacteria can stimulate a range of plant growth promoting products in the bacteria's host plant. A heavily investigated aspect of a plant that is influenced by the IAA production of its corresponding rhizospheric bacteria, is the increased growth of the root system. In one study, researchers found that IAA production by *Azospirillum brasilense* SM promoted the development of lateral roots and apical meristem divisions, which in turn, led to the lengthening of the plant roots (Malhotra et. al 2009). Root elongation, influenced by the production of rhizospheric bacterial IAA, may be a product of a plant grown in nitrogen deficient conditions. The lengthening of the root system may be the effect of the plant searching for further sources of nitrogen. To support this theory, one study found that maize genotypes inoculated with *Azospirillum brasilense* Ab-V5 under low nitrogen conditions, experienced a higher IAA concentration and NUE, compared to uninoculated plants (Zeffa et al. 2019).

4.5 Incorporating the Rhizosphere into Plant Breeding

Past crop breeding has neglected the role of the plants' microbiome, in selecting for traits to breed for. U.S. maize yields have increased from 1930 to 1960 with an average gain of 63 kg^{-1} ha^{-1}, as well as an increase of an average gain of 110 kg^{-1} ha^{-1} from 1960 to 2000 (Woli et al., 2018). Genetic changes could, in part, be attributed to this yield increase, as the number of new maize hybrids increased to greater than 85 percent in the years 1959 onward, with a complete transition in hybrids from 1989 to 1999 (Assefa et al. 2012). In addition to genetic factors contributing to yield changes, nitrogen fertilizer application also correlates significantly with yield increases. From 1940 to 2015, N fertilizer levels have increased from 0.28 g N m^{-2} y^{-1} to 9.54 g N m^{-2} y^{-1} (Cao et al. 2018).

To begin incorporating the role of the plant microbiome when it comes to breeding for healthier, more nutrient-sufficient plants, one avenue crop breeders may take is targeting the root exudates of the designated plant, to allow the plant to target specific beneficial rhizobacterial members through their root exudation profiles and recruit these beneficial bacteria to their rhizospheres. There have been complex shifts in the rhizosphere bacterial communities from teosinte to our modern maize hybrids through the directed breeding of this crop. In one study, researchers found that through domestication of teosinte into modern maize plants, the plant-microbe interactions have been significantly affected, while the microbe-microbe interactions within the plant's rhizosphere have been further impacted by agricultural intensification (Schmidt et al. 2020). This shift in microbial communities across the domesticated stages of each crop may be due to a shift in root exudates caused by crop breeding. Similarly, the abundance of nitrogen-fixing bacteria known as diazotrophs, identified by the presence of the *nifH* gene, has significantly decreased in maize inbred genotypes ranging from 1949 to 1986 (Favela et al. 2021). This decline in the presence of nitrogen-fixing bacteria in the rhizospheres of maize genotypes from the 1940s to 1980s may

be explained by the steady increase of synthetic nitrogen application starting in the 1940s and reaching its modern levels in the 2000s (Cao et al. 2018). In a study investigating the root exudates released from 10 wheat genotypes corresponding to the key steps in domestication of tetraploid wheat, it was discovered that the changes in rhizosphere metabolites were associated with differences among the genotypes (Iannucci et al., 2017). These changes in root exudation in modern wheat varieties could be due to the altering of root architecture, a product of modern crop breeding. Modern wheat varieties have been discovered to have smaller root systems than historic varieties (Fradgley et al. 2020). This reduction in root system size could contribute to a reduction in root exudate release. Incorporating a plant's rhizospheric bacterial community into plant breeding strategies is one avenue researchers might consider for breeding healthier, more nutrient-efficient crops.

4.6 The Use of Microbial Inoculants for Increasing Plant Fitness Under Nitrogen Deficiency

Utilizing the plant-growth promoting capabilities in certain bacterial species is a method in which researchers and producers are turning to with the goal of increasing the nitrogen-use efficiency of high-value crops, such as maize. Recent studies have been investigating the role and potential of using microbial inoculants, in place of or in addition to synthetic nitrogen fertilizers, to improve nitrogen-related traits of crops. These studies have taken place both in field and greenhouse conditions. In a particular study investigating the effect of two *Pseudomonas* strains on vegetative growth and yield of various maize genotypes, researchers found that both *Pseudomonas kilonensis* and *Pseudomonas protegens* significantly increased the plant height, leaf length, and the yield of maize, compared to the uninoculated plants (Alori et al. 2019). *Pseudomonas* strains have been reported to have proteolytic and chitinolytic activity, along with siderophore production, thus, providing beneficial effects to certain host plants (Georgieva et al. 2018). Similarly, in another study, scientists in-

vestigated the effects of two microbial inoculants, *Azospirillum lipoferum* and *Azotobacter chroococcum*, on certain NUE-related traits of the maize hybrid 647. Their results showed that the microbial inoculants increased the dry shoot weight of the maize anywhere from 63 to 115 percent (Biari et al. 2008). Both *Azospirillum* and *Azotobacter* species have been reported to provide beneficial mechanisms to host plants, including nitrogen fixation and phytohormone production. (Fukami et al. 2018; Hindersah et al. 2020). The effect of microbial inoculants may also be affected by the nutrient status of the soil they are used in, as demonstrated in a study investigating the use of microbial inoculants in two soils differing in nutrient availability (Egamberdiyeva et al. 2005). The researchers found that when plants were inoculated with a combination of the strains *Pseudomonas alcaligenes* PsA15, *Bacillus polymyxa* BcP26, and *Mycobacterium pheli* MbP18, the shoot dry weight increased 17-30 percent in nutrient-poor soil, whereas the shoot dry weight did not increase in nutrient-dense soil (Egamberdiyeva et al. 2005). This could be caused by plants favoring nutrient-stabilizing capabilities of bacteria in nutrient-deficient soils, while other abilities, such as hormone production are favored in nutrient-dense soils (Beschoren da Costa et al. 2014). When grown with certain bacterial species, plant hosts appear to benefit from various beneficial traits provided by the bacterial species. The results taken from microbial inoculant studies further support the premise that bacterial species incorporated into a host plant's rhizosphere can offer a beneficial effect in nutrient deficient conditions, such as low nitrogen environments.

4.7 References

References

[1] Abdel-Lattif, H.M., Taha, M.H., Atta, M.M.M. Nitrogen Use Efficiency and Grain Protein of Corn Affected by Low Nitrogen Application. *Egypt. J. Agron.* (2019). 41(1):69-78.

Doi:10.21608/agro.2019.9752.1151

[2] Alori, E.T., Babalola, O.O., Prigent-Cambaret, C. Impacts of Microbial Inoculants on the Growth and Yield of Maize Plant. *The Open Agriculture Journal.* (2019) 13(1):1-8. DOI: 10.2174/1874331501913010001

[3] Aluko, O.O., Kant, S., Adedire, O.M., Li, C., Yuan, G., Liu, H., Wang, Q. Unlocking the potentials of nitrate transporters at improving plant nitrogen use efficiency. (2023). *Front. Plant Sci.* 14:1074839. Doi: 10.3389/fpls.2023.1074839

[4] Assefa, Y., Roozeboom, K.L., Staggenborg, S.A., Du, J. Dryland and Irrigated Corn Yield with Climate, Management, and Hybrid Changes from 1939 through 2009. *Crop Ecology & Physiology.* (2012). 104(2):473-482. Doi:10.2134/agronj2011.0242

[5] Barak, P., Jobe, B.O., Krueger, A.R., Peterson, L.A., Laird, D.A. Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. *Plant and Soil.* (1997). 197:61-69. doi:10.1023/A:1004297607070

[6] Bardgett, R., van der Putten, W. Belowground biodiversity and ecosystem functioning. *Nature.* 515, 505-511 (2014). doi:10.1038/nature13855

[7] Berg, G., Smalla, K. Plant Species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbial Ecol.* (2009). 68(1):1-13. doi: 10.1111/j.1574-6941.2009.00654.x

[8] Bertin, C., Yang, X., Weston, L.A. The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil.* 256, 67-83 (2003). doi.org:10.1023/A:1026290508166

[9] Beschoren da Costa, P., Granada, C.E., Ambrosini, A., Moreira, F., de Souza, R., dos Passos, J.F.M., Arruda, L., Passaglia, L.M.P. A Model to Explain Plant Growth Promotion Traits: A Multivariate Analysis of 2,211 Bacterial Isolates. *PloS ONE.* (2014). 9(12) DOI:10.1371/journal.pone.0116020

[10] Biari, A., Gholami, A., Rahmani, H.A. Growth Promotion and Enhanced Nutrient Uptake of Maize (*Zea mays* L.) by Application of Plant Growth Promoting Rhizobacteria in Arid Region of Iran. *Journal of Biological Sciences.* (2008). 8(6):1015-1020. DOI: 10.3923/jbs.2008.1015.1020

[11] Blake, C., Christensen, M.N., Kovács, A.T. Molecular Aspects of Plant Growth Promotion and Protection by *Bacillus subtillis. MPMI.* (2021). 34:15-25. Doi:10.1094/MPMI-08-20-0225-CR

[12] Cao, P., Lu, C., Yu, Z. Historical nitrogen fertilizer use in agricultural ecosystems of the contiguous United States during 1850-2015: application rate, timing, and fertilizer types. *Earth Syst. Sci. Data.* (2018). 10:969-984. Doi:10.5194/essd-10-969-2018

[13] Chun, L. Mi, G., Li, Chen, F., Zhang, F. Genetic analysis of maize root characteristics in response to low nitrogen stress. *Plant and Soil.* (2004). 276:369-382. Doi:10.1007/s11104-005-5876-2

[14] Clúa, J., Roda, C., Zanetti, M.E., Blanco, F.A. Compatibility between Legumes and Rhizobia for the Establishment of a Successful Nitrogen-Fixing Symbiosis. *Genes.* (2018). 9. Doi:10.3390/genes9030125

[15] Coskun, D., Britto, D.T., Shi, W., Kronzucker, H.J. How Plant Root Exudates Shape the Nitrogen Cycle. *Trends in Plant Science.* (2017). 22(8):661-673 Doi:10.3390/genes9030125

[16] Crawford, N.M., Glass, A.D.M. Molecular and physiological aspects of nitrate uptake in plants. *Trends in Plant Science.* (1998). 3(10):389-395. Doi: 10.1016/S1360-1385(98)01311-9

[17] Denman, K.L., Brasseur, A., Chidthaisong, P., Ciais, P.M., Cox, R.E., Dickinson, D., Hauglustaine, C., Heinze, E., Holland, D., Jacob, U., Lohmann, S., Ramachandran, P.L.,

da Silva Dias, S.C., Wofsy and X. Zhang. Couplings Between Changes in the Climate System and Biogeochemistry. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. (2007).

[18] Egamberdiyeva, D. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Applied Soil Ecology.* (2005). 36(2-3):184-189. Doi:10.1016/j.apsoil.2007.02.005

[19] Favela, A., Bohn, M.O., Kent, A.D. Maize germplasm chronosequence shows crop breeding history impacts recruitment of the rhizosphere microbiome. *ISME J.* (2021). 15:2454-2464. Doi:10.1038/s41396-021-00923-z

[20] Fiencke, C., Spieck, E., Bock, E. Nitrifying Bacteria. *Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment.* (2002). 255-276.

[21] Fradgley, N., Evans, G., Biernaskie, J.M., Cockram, J., Marr, E.C., Oliver, A.G., Ober, E., Jones, H. Effects of breeding history and crop management on the root architecture of wheat. *Plant and Soil.* (2020). 452(1):587-600. Doi:10.1007/S11104-020-04585-2

[22] Franche, C., Lindström, K., Elmerich, C. Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and Soil.* (2009). 321:35-59. doi:10.1007/s11104-008-9833-8

[23] Fukami, J., Cerezini, P., Hungria, M. *Azospirillum*: benefits that go far beyond nitrogen fixation. *AMB Expr.* (2018). 8:73. Doi:10.1186/s13568-018-0608-1

[24] Gao K, Chen F, Yuan L, Zhang F, Mi G. A comprehensive analysis of root morphological changes and nitrogen allocation in maize in response to low nitrogen stress. *Plant Cell Environ.* (2015) 38(4):740-50. Doi:10.1111/pce.12439

[25] Georgieva, T., Evstatieva, Y., Savov, V., Bratkova, S., Nikolova, D. Assessment of plant growth promoting activities of five rhizospheric *Pseudomonas* strains. *Biocatalysis and Agricultural Biotechnology.* (2018). 16:285-292. Doi:10.1016/j.bcab.2018.08.015

[26] Glass, A.D. Nitrate uptake by plant roots. *Botany.* 87(7): 659-667. Doi:10.1139/B09-014

[27] Hasan, S.M., Hall, J.B. The Physiological Function of Nitrate Reduction in *Clostridium perfringens. Journal of General Microbiology.* (1975). 87(1):120-128. Doi:10.1099/00221287-87-1-120

[28] Hindersah, R., Kamaluddin, N.N., Samanta, S., Banerjee, S., Sarkar, S. Role and perspective of *Azotobacter* in crops production. *Sains Tanah Journal of Soil Science and Agroclimatology.* (2020). 17(2):170-179. Doi:10.20961/stjssa.v17i2.45130

[29] Howard, J.B., Rees, D.C. Structural Basis of Biological Nitrogen Fixation. *Chem. Rev.* (1996). 96(7):2965-2982. Doi:10.1021/cr9500545

[30] Iannucci, A., Fragasso, M., Beleggia, R., Nigro, F., Papa, R. Evolution of the Crop Rhizosphere: Impact of Domestication on Root Exudates in Tetraploid Wheat (*Triticum turgidum* L.) *Front. Plant Sci.* (2017). 8:2124. Doi:10.3389/fpls.2017.02124

[31] Javed, T., I, I., Singhal, R.K., Shabbir, R., Shah, A.N., Kumar, P., Jinger, D., Dharmappa, P.M., Shad, M.A., Saha, D., Anuragi, H., Adamski, R., Siuta, D. Recent Advances in Agronomic and Physio-Molecular Approaches for Improving Nitrogen Use Efficiency in Crop Plants. *Front. Plant Sci.* (2022). 13:877544. Doi:10.3389/fpls.2022.877544

[32] Jin, Y., He, J., Zhu, Y., Siddique, K.H.M., Nodule Formation and Nitrogen Use Efficiency Are Important for Soybean to Adapt to Water and P Deficit Conditions. *Agriculture.* (2022). 12(9):1326. Doi:10.3390/agriculture12091326

[33] Jones, K., Kobayashi, H., Davies, B. How rhizobial symbionts invade plants: the *Sinorhizobium-Medicago* model. *Nat Rev Microbial* 5, 619-633 (2007). Doi:10.1038/nrmicro1705

[34] Kamali, S., Singh, A. Jasmonates as Emerging Regulators of Plants Response to Variable Nutrient Environment. *Critical Reviews in Plant Sciences.* (2022). 41(4):271-285. Doi:10.1080/07352689.2022.2109866

[35] Kowalchuk, G.A., Buma, D.S., de Boer, W., Klinkhamer, P.G.L., van Veen, J.A. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Antonie Van Leeuwenhoek.* (2002). 81:509-520. Doi:10.1023/A:1020565523615

[36] Lan, X., Zhu, L., Yuan, Q. Long-Term Variation of Greenhouse Gas N2O Observed by MLS during 2005-2020. *Remote Sens.* (2022). 14, 955. Doi:10.3390/rrs14040955

[37] Lau, J.A., Lennon, J.T. Rapid responses of soil microorganisms improve plant fitness in novel environments. *Biological Sciences.* (2012). 109(35):14058-14062. Doi:10.1073/pnas.120231910

[38] Li, J.Y., Fu, Y.L., Pike, S.M., Bao, J., Tian, W., Zhang, Y., Chen, C.Z., Zhang, Y., Li, H.M., Huang, J., Li, L.G., Schroeder, J.I., Gassman, W., Gong, J.M. The *Arabidopsis* Nitrate Transporter NRT1.8 Functions in Nitrate Removal from the Xylem Sap and Mediates Cadmium Tolerance. *The Plant Cell.* (2010). 22(5):1633-1646. Doi:10.1105/tpc.110.075242

[39] Lin, S.H., Kuo, H.F., Canivenc, G., Lin, C.S., Lepetit, M., Hsu, P.K., Tillard, P., Lin, H.L., Wang, Y.Y., Tsai, C.B., Gojon, A., Tsay, Y.F. Mutation of the Arabidopsis *NRT1.5* Nitrate Transporter Causes Defective Root-to-Shoot Nitrate Transport. *The Plant Cell.* (2008). 20(9):2514-2528. Doi:10.1105/tpc.108.060244

[40] Lopes, L.D., Wang, P., Futrell, S.L., Schachtman, D.P. Sugars and Jasmonic Acid Concentration in Root Exudates Affect Maize Rhizosphere Bacterial Communities. *Applied and Environmental Microbiology.* (2022). 88(18): Doi:10.1128/aem.00871-22

[41] Luo, L., Zhang, Y., Xu, G. How does nitrogen shape plant architecture? *Journal of Experimental Botany.* (2020). 71(15):4415-4427. Doi:10.1093/jxb/eraa187

[42] Lv, X., Zhang, Y., Hu, L. Low-Nitrogen Stress Stimulates Lateral Root Initiation and Nitrogen Assimilation in Wheat: Roles of Phytohormone Signaling. *J Plant Growth Regul.* (2021). 40:436-450. Doi:10.1007/s00344-020-10112-5.

[43] Malhotra, M., Srivastava, S. Stress-responsive indole-3-acetic acid biosynthesis by *Azospirillum brasilense* SM and its ability to modulate plant growth. *European Journal of Soil Biology.* (2009). 45(1):73-80. Doi: 10.1016/j.ejsobi.2008.05.006

[44] Mao, Y., Li, X., Smyth, E.M., Yannarell, A.C., Mackie, R.I. Enrichment of specific bacterial and eukaryotic microbes in the rhizosphere of switchgrass (*Panicum virgatum* L.) through root exudates. *Environmental Microbiology Reports.* (2014). 6(3):293-306. Doi:10.1111/1758-2229.12152

[45] Martienssen, M., Schöps, R. Population Dynamics of Denitrifying Bacteria in a Model Biocommunity. *Wat. Res.* (1999). 33(3):639-646.

[46] Mu, X., Chen, Q., Chen, F., Yuan, L., Mi, G. A RNA-Seq Analysis of the Response of Photosynthetic System to Low Nitrogen Supply in Maize Leaf. *Int J Mol Sci.* (2017) 5;18(12):2624. Doi:10.3390/ijms18122624

[47] Novoa, R., Loomis, R.S. Nitrogen and Plant Production. *Plant and Soil.* (1981). 58:177-204. Doi.org/10.1007/BF02180053

[48] Ouyang, L., Pei, H., Xu, Z. Low nitrogen stress stimulating the indole-3-acetic acid biosynthesis by *Serratia* sp. ZM is vital for the survival of the bacterium

and its plant growth-promoting characteristic. *Arch Microbiol.* (2017). 199:425-432. Doi:10.1007/s00203-016-1312-7

[49] Peel, J.L., Haeuber, R., Garcia, V., Russell, A.G., Neas, L. Impact of nitrogen and climate change interactions on ambient air pollution and human health. *Biochemistry.* (2013). 114:121-134. Doi:10.1007/s10533-012-9782-4

[50] Peeters, N., Guidot, A., Vailleau, F., Valls, M. *Ralstonia solanacearum*, a widespread bacterial plant pathogen in the post-genomic era. *Molecular Plant Pathology.* (2013). 14(7):651-662. Doi:10.1111/mpp.12038

[51] Qin, L., Walk, T.C., Han, P., Chen, L., Zhang, S., Li, Y., Hu, X., Xie, L., Yang, Y., Liu, J., Lu, X., Yu, C., Tian, J., Shaff, J.E., Kochian, L.V., Liao, X., Liao, H. Adaption of Roots to Nitrogen Deficiency Revealed by 3D Quantification and Proteomic Analysis. *Plant Physiology.* (2019). 179(1):329-347. Doi:10.1104/pp.18.00716.

[52] Raddatz, N., Morales de los Rios, L., Lindahl, M., Quintero, F.J., Pardo, J.M. Coordinated Transport of Nitrate, Potassium, and Sodium. *Front. Plant Sci.* (2020). 11:247. Doi:10.3389/fpls.2020.00247

[53] Raza, S., Farmaha, B.S. Contrasting corn yield responses to nitrogen fertilization in southeast coastal plain soils. *Front. Environ. Sci.* (2022). 10:955142. Doi:10.3389/fenvs.2022.955142

[54] Rudrappa, T., Czymmek, K.J., Paré, P.W., Bais, H.P. Root-Secreted Malic Acid Recruits Beneficial Soil Bacteria. *Plant Physiology.* (2008). 148:1547-1556. Doi:10.1104/pp.108.127613

[55] Sánchez-Bel, P., Sanmartin, N., Pastor, V., Mateu, D., Cerezo, M., Vidal-Albalat, A., Pastor-Fernández, J., Pozo, M.J., Flors, V. Mycorrhizal tomato plants fine tunes the

growth-defence balance upon N depleted root environments. *Plant Cell Environ.* (2018). 41:406-420. Doi:10.1111/pce.13105

[56] Schmidt, J.E., Rodrigues, J.L.M., Brisson, V.L., Kent, A., Gaudin, A.C.M. Impacts of Directed Evolution and Soil Management Legacy on the Maize Rhizobiome. *Soil Biology and Biochemistry.* (2020). 145:107794. Doi:10.1016/j.soilbio.2020.107794

[57] Schroder, J.L., Zhang, H., Girma, K., Raun, W.R., Penn, C.J., Payton, M.E. Soil Acidification from Long-Term Use of Nitrogen Fertilizers on Winter Wheat. *Soil Fertility & Plant Nutrition.* (2011). 75(3):957-964. Doi:10.2136/sssaj2010.0187

[58] Shikha, D., Jakhar, P., Satbhai, S.B. Role of jasmonate signaling in the regulation of plant responses to nutrient deficiency. *Journal of Experimental Botany.* (2023). 74(4):1221-1243. Doi:10.1093/jxb/erac387

[59] Soumare, A., Diedhious, A.G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., Kouisni, L., Exploiting Biological Nitrogen Fixation: A Route Towards a Sustainable Agriculture. *Plants.* (2020) 9(8):1011. Doi:10.3390/plants9081011

[60] Spaepen, S., Dobbelaere, S., Croonenborghs, A. Effects of *Azospirillum brasilense* indole-3-acetic acid production on inoculated wheat pants. *Plant Soil.* (2008). 312:15-23. Doi:10.1007/s11104-008-9560-1

[61] Steenhoudt, O., Vanderleyden, J. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. *FEMS Microbiology Reviews.* (2000). 24(4):487-506. Doi:10.1111/j.1574-6976.2000.tb00552.x

[62] Sutherland, J., Bell, T., Trexler, R.V., Carlson, J.E., Lasky, J.R. Host genomic influence on bacterial composition in the switchgrass rhizosphere. *Molecular Ecology.* (2021). 31(14):3934-3950. Doi:10.1111/mec.16549

[63] Trivedi, P., Leach, J., Tringe, S., Sa, T., Singh, B., Plant-microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology.* 18:11(607-621) (2020). Doi:10.1038/s41579-020-0412-1

[64] Wang, C., He, T., Zhang, M., Zheng, C., Yang, L., Yang, L. Review of the mechanisms involved in dissimilatory nitrate reduction to ammonium and the efficacies of these mechanisms in the environment. *Environmental Pollution.* (2024). 345. Doi:10.1016/j.envpol.2024.123480.

[65] Wei, S., Wang, X., Shi, D., Li, Y., Zhang, J., Liu, P., Zhao, B., Dong, S. The mechanisms of low nitrogen induced weakened photosynthesis in summer maize (*Zea mays* L.) under field conditions. *Plant Physiology and Biochemistry.* (2016). 105:118-128. Doi:10.1016/j.plaphy.2016.04.007

[66] Woli, K.P., Sawyer, J.E., Boyer, M.J., Abendroth, L.J., Elmore, R.W. Corn Era Hybrid Macronutrient and Dry Matter Accumulation in Plant Components. *Agronomic Application of Genetic Resources.* (2018). 110:1648-1658. Doi:10.2134/agronj2018.01.0025

[67] Wood, S., Cowie, A. A Review of Greenhouse Gas Emission Factors for Fertiliser Production. *Research and Development Division, State Forests of New South Wales, Cooperative Research Centre for Greenhouse Accounting, International Energy Agency Bioenergy Task* 38. (2004). 38(1):20.

[68] Wu, A.L., Jiao, X.Y., Wang, J.S., Dong, E.W., Guo, J., Wang, L.G., Sun, A.Q., Hu, H.W. Sorghum rhizosphere effects reduced soil bacterial diversity by recruiting specific bacterial species under low nitrogen stress. *Science of the Total Environment.* (2020). 770:144742. Doi:10.1016/j.scitotenv.2020.144742

[69] Ya-wei, W., Qiang, L., Rong, J., Wei, C., Xiao-lin, L., Fan-lei, K., Yong-pei, K., Hai-chun, S., Ji-chao, Y. Effect of low-nitrogen stress on photosynthesis and chlorophyll fluo-

rescence characteristics of maize cultivars with different low-nitrogen tolerances. *Journal of Integrative Agriculture.* (2019). 18(6):1246-1256. Doi:10.1016/S2095-3119(18)62030-1

[70] Zeffa DM, Perini LJ, Silva MB, de Sousa NV, Scapim CA, Oliveira ALM, Amaral Júnior ATD, Azeredo Gonçalves LS. *Azospirillum brasilense* promotes increases in growth and nitrogen use efficiency of maize genotypes. *PLoS One.* (2019) 18;14(4):e0215332. Doi:10.1371/journal.pone.0215332

[71] Zehr JP, Jenkins BD, Short SM, Steward GF. Nitrogenase gene diversity and microbial community structure: a cross-system comparison. *Environ Microbiol.* (2003). 5(7):539-54. Doi:10.1046/j.1462-2920.2003.00451.x

[72] Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., Chu, H. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biology and Biochemistry.* 2016. 92:21-49. Doi:10.1016/j.soilbio.2015.09.018

[73] Zhalnina K, Louie KB, Hao Z, Mansoori N, da Rocha UN, Shi S, Cho H, Karaoz U, Loqué D, Bowen BP, Firestone MK, Northen TR, Brodie EL. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nat Microbiol.* 2018. 3(4):470-480. Doi:10.1038/s41564-018-0129-3.

[74] Zhang, G-B., Hong-Ying, Y., Gong, J-M. The Arabidopsis Ethylene/Jasmonic Acid-NRT Signaling Module Coordinates Nitrate Reallocation and the Trade-Off between Growth and Environmental Adaptation. *The Plant Cell.* (2014). 26:3984-3998. Doi:10.1105/tpc.114.129296

[75] Zhang, B.H., Li, P.S., Wang, Y.Y., Wang, J.J., Liu, X.L., Wang, X.Y., Hu, X.M. Characterization and synthesis of indole-3-acetic acid in plant growth promoting *Enterobacter sp. RSC Adv.* (2021). 11(31601). Doi:10.1039/d1ra05659j

[76] Zhang, W., Zhang, J., Lei, W., Zhao, L., Wang, S., Shi, M., Wei, M. Low Nitrogen Stress Promotes Root Nitrogen Uptake and Assimilation in Strawberry: Contribution of Hormone Networks. *Horticulturae.* (2023). 9(2):249. Doi:10.3390/horticulturae9020249

[77] Zhang, Y., Shen, H., He, X., Thomas, B.W., Lupwayi, N.Z., Hao, X., Thomas, M.C., Shi, X. Fertilization Shapes Bacterial Community Structure by Alteration of Soil pH. *Frontiers in Microbiology.* (2017). 8. Doi:10.3389/fmicb.2017.01325

[78] Zhao, Y. Auxin Biosynthesis: A Simple Two-Step Pathway Converts Tryptophan to Indole-3-Acetic Acid in Plants. *Molecular Plant.* (2012). 5(20):334-338. Doi:10.1093/mp/ssr104

5 Materials and Methods

5.1 Initial Bacterial Isolate Screening

5.1.1 Identification of the 64 Bacterial Isolates

The goal of the initial assay was to select individual bacterial isolates, from the list of 64 potentially beneficial bacterial isolates, that appear to provide an increase in maize plant shoot growth under nitrogen deficient conditions. In the paper, "*Association analyses of host genetics, root-colonizing microbes, and plant phenotypes under different nitrogen conditions in maize*" (Meier et al. 2022), amplicon sequence variants (ASVs) of soil microbes were identified from rhizosphere samples based on associations with maize genes. These microbes were interpreted to possibly contribute to increasing plant fitness,. The host plants' genomes from these rhizosphere samples likely underwent negative or positive selection to favor specific microbial groups (referred to as rhizobiome traits) by removing deleterious alleles or increasing desirable alleles. The methods used in this paper to identify these recruited ASVs did not explicitly test which traits or phenotypes would be affected, and therefore, the goal of this research project was to investigate whether these ASVs were recruited under low nitrogen conditions due to a beneficial phenotypic effect offered by the microbe to the plant. The ASVs from this study were matched to 16S sequences in the Schachtman Lab Culture Collection and from this matching, 64 potentially beneficial bacterial isolates were identified. It was uncertain whether any of the 64 bacterial isolates that were identified, offered any kind of benefit towards the plant in nitrogen deficient conditions. The goal of the initial screening was to identify certain bacterial isolates, out of the 64, to be further analyzed.

5.1.2 Bacterial Isolates

The 64 bacterial isolates that were identified from the Schachtman Lab culture collection were tested in the initial screening. The Schachtman Lab Culture Collection consists of over 4,000 isolates including endophytes and soil microbes collected from plant roots, soil, and the rhizospheres of various plants, sampled from a diverse range of soil environments. The 4,000+ isolates within the culture collection are stored in individual glycerol stocks in a -80°C freezer, for long-term storage.

5.1.3 Bacterial Growth

Within the Schachtman Lab culture collection, information about 2,500 of the stored isolates is recorded, such as their genus, species, collection year, sample type, collection location, and growth medium. Depending on the medium type listed in the Schachtman Lab culture collection database, the 64 bacterial isolates were inoculated and grown in one of the following media types: yeast extract-peptone-dextrose (YPD), yeast mannitol agar (YMA), trypticase soy agar (TSA), reasoner's 2A agar (R2A), or Ashby's Nitrogen-free medium (Stella & Suhaimi 2010). To begin the microbial growth of each bacterial isolate, the glycerol stock was removed from the -80°C freezer. A loop of the frozen bacterial isolate culture was taken from the glycerol stock and inoculated into 2 mL of its desired liquid medium type. The tube containing the bacterial inoculant was placed on the rotary shaker at 220 rpm at room temperature (24°C). After 24 hours of growth or until the liquid media was turbid with bacterial growth, serial dilutions at 10x, 100x, 1000x, 10,000x, and 100,000x were carried out and then plated onto the desired media plates. The plates sat at room temperature (24°C) until single colonies grew on the agar surface. When single colonies appeared on the serial dilution plates, colony forming units (CFU) counts were recorded and a single colony was taken from the plate and inoculated into 3 mL of its desired medium, prior to

being placed on the rotary shaker at 220 rpm at room temperature (24°C). After 24 hours of growth or until the liquid was turbid with bacterial growth, the 3 mL of bacterial culture was inoculated into 50 mL of fresh desired medium and placed on the rotary shaker at 220 rpm at room temperature (24°C) for 24-48 hours of growth.

5.1.4 Mo17 Maize Seed Sterilization and Germination

The Mo17 inbred maize seed variety was selected for testing in the initial screening of the 64 bacterial isolates. The Mo17 maize seed was surface sterilized for 48 hours using chlorine gas, produced by mixing 4 mL of concentrated hydrochloric acid (HCl) with 100 mL of bleach in a desiccator placed inside a fume hood. The chlorine gas was replaced at the 24-hour mark. After the 48-hour surface-sterilization was complete, the Mo17 seed was placed in aerated water for imbibing overnight at room temperature (24°C). Upon the completion of imbibing, the seeds were placed in petri dishes lined with paper at the bottom and moistened with sterile water. Prior to germination, 0.5 mL Captan mixture (0.2 percent conc.) was also applied to the maize seeds in the petri dishes for fungal control. The petri dishes were sealed with micropore tape and placed at 30°C for 24-48 hours until the seeds germinated, indicated by the presence of the radicle and the plumule beginning to emerge.

5.1.5 Potting Mix Sterilization

For each pot, 500 g of calcined clay were added as a simulated soil matrix. To ensure sterility before the planting and of the system, the pots (11cm in height, 13cm in diameter) containing the 500 g of calcined clay were autoclaved 3 times (cycle: P03 vacuum, 25 minutes sterilization at 121°C , 10 minutes drying).

5.1.6 Seedling Priming Bacterial Inoculation

The goal of the initial screening was to narrow down to individual bacterial isolates, from the list of 64 potentially growth-beneficial bacterial isolates, that appeared to have a positive growth effect on the maize grown in nitrogen-deficient conditions. Because of this goal, germinated maize seeds were inoculated with only one of the 64 bacterial isolates for each test. This ensured that only one bacterial isolate was tested in each trial for growth promoting effects on maize under low nitrogen conditions.

The inoculation of the Mo17 maize seed with the individual bacterial isolates was carried out following the seedling priming inoculation technique previously described (Chai et al. 2022). The maize seed germination was timed to occur at the same time that the 50 mL bacterial cultures were turbid with growth. When the seeds germinated, and the 50 mL bacterial culture of the bacterial isolate was turbid, germinated seeds were placed in a new, clean petri dish and approximately 10 mL of the liquid bacterial culture was applied to the seeds in the petri dish. The petri dish was sealed with micropore tape and placed on the rotary shaker at 80 rpm for 12 hours at room temperature (24°C), to ensure the inoculation of the germinated maize with the desired bacterial isolate. For the uninoculated plants used as controls in the high N and low N conditions (high N control and low N control, respectively), germinated seedlings were inoculated with sterile R2A medium containing no bacterial isolate and placed on the shaker at 80 rpm at room temperature (24°C) for 12 hours, to replicate the same conditions the inoculated seedlings underwent.

5.1.7 Maize Planting

After 12 hours the inoculated seeds were planted. A sterilized pot containing the sterilized 500 g of calcine clay was placed into a sterile growth bag (*Nasco-Whirl-PAK*) with an

AeraSeal film placed on its front. To apply the *AeraSeal* film to the *Nascco-Whirl-PAK* bag, sterilized scissors were used to cut a hole in the bag large enough for the film, and the film was then placed over the hole. This was carried out in a laminar fume hood. The sterile germination bag allows for a fully sterile growth system. The *AeraSeal* film on the *Nasco-Whirl-PAK* bag ensured that the diffusion of carbon dioxide and oxygen into and out of the bags took place, for plant respiration, photosynthesis and growth. A volume of 450 mL half-strength Hoagland's nutrient solution (1.95 mM N) (Hoagland & Arnon, 1950) was added to the calcine clay, bringing the system to 90 percent soil water holding capacity (SWHC). The high N control plants were given 450 mL full-strength Hoagland solution (15.50 mM N). An inoculated maize seed was sown into the soil at a 1-inch depth. The germination bag was rolled to close the top and then placed in the growth chamber. The plants were planted in a laminar flow hood to maintain sterility.

5.1.8 Maize Growth Conditions

The maize plants inside the germination bags were placed inside the growth chamber for 14-15 days before measuring the biomass of the plants. The conditions of the growth chamber were kept at 26°C during the day and 18°C during the night, with a 16-hour light period. No additional water or nutrients were added to the system throughout the growth period.

5.1.9 Maize Root and Shoot Sampling

At 14 days of growth, the maize plants were taken out of the germination bags and the fresh roots and fresh shoots were separated and their weights recorded. The roots and fresh shoots were placed in a drying oven at 68°C and then at least 48 hours later, the dry roots and dry shoots weights were recorded.

5.1.10 Breaking the 64 Isolates up into Separate Growth Experiments

The testing of all 64 isolates was a large undertaking, therefore the initial screening of the 64 bacterial isolates was broken up into smaller, more manageable experiments with each experiment having bacterial isolate-inoculated plants, high N and low N control plants with 3-8 replicates for each treatment, depending on the initial assay round. The high N and low N uninoculated controls in each smaller experiment served as a benchmark to ensure that the treatments in each individual experiments clearly showed a response to low N conditions.

5.1.11 Statistical Analysis

The statistical analysis of the screening experiments was carried out in R (R Developmental Core et al. 2018) and SAS (SAS Institute Inc. et al. 2023). To confirm that the low nitrogen conditions showed a decrease in growth compared to high N conditions, an unpaired t-test was performed.

To select the 15 bacterial isolates for further testing, one-way ANOVA tests were also performed on each individual experiment to determine whether the dry shoot weight (grams) was influenced by the bacterial isolate inoculant. The Tukey's HSD post hoc pairwise comparison was performed following the one-way ANOVA to compare the mean differences of the dry shoot weights of the bacterial isolates. Plants grown with bacterial isolates that appeared to offer a significant growth effect on the dry shoot weight compared to the uninoculated low N contol were selected for further experiments.

5.2 Sanger Sequencing

5.2.1 Confirming the Identities of the Selected Bacterial Isolates

Upon selection of the 15 bacterial isolates that appeared to be offering some beneficial growth effect for nitrogen-deficient maize, Sanger sequencing was carried out on the 16S

ribosomal RNA (rRNA) gene of each of the selected bacterial isolates to confirm their genus and species. The selected bacterial isolates ((Table 1, Table 2)) were grown following the procedure in (Section 5.1.3). For each bacterial isolate that was selected, a 50 µl PCR reaction was carried out with two primers of the 16s rRNA bacterial gene: 27 forward and 1492 reverse primers. The 2x Hi Fidelity Taq (NEB) Mix was used as the enzyme in the PCR reaction. The following cycles were performed for the PCR reaction: 1) 95 degrees Celsius 5min, 2) 30x cycles: 95 degrees Celsius 1min, 3) 65.9 degrees Celsius 1 min, 72 degrees Celsius 2min, 72 degrees Celsius 10 min. Upon completion of the PCR cycles, a gel electrophoresis was used to confirm the success of the PCR reactions. The PCR products were then cleaned up with the Qiagen PCR clean up kit (Qiagen). The final volume of the cleaned PCR product was 50µl and the concentration of this product was measured on a Qubit. The three primers, 27F, 515F, and 1492R were used in the full-length Sanger Sequencing of the 16S rRNA gene for each bacterial isolate. Upon receiving the sequencing results, the sequences produced from the three primers were aligned and combined to produce a whole 16s rRNA gene sequence for each bacterial isolate. The sequences were run through the NCBI database (N.R. Coordinators et al., 2018) and the bacterial species identity with the highest similarity was selected as the bacterial isolate identity.

5.3 Two-Week Validation Experiment for Selected Bacterial Isolates

5.3.1 Breaking the Selected Bacterial Isolates into Two Experimental Groups

The selected bacterial isolates were split into two groups, for 2 separate growth experiments (Table 1, Table 2).

Bacterial Isolate ID	Isolate Identity
111	*Arthrobacter sp.*
702	*Pseudomonas koreensis*
708	*Leifsonia aquatica*
726	*Pseudomonas sp. Csya02*
730	*Pseudomonas kribbensis*
1204	*Chitinophaga pinensis*
2829	*Janthinobact- erium lividum*
4509	*Dyadobacter fermentans*
4589	*Sphingomonas sp.*
4606	*Acidovorax sp.*

Table 1: Group 1 Bacterial Isolates for 2-Week Validation Experiment

Bacterial Isolate ID	Isolate Identity
571	*Burkholderia cepacian*
574	*Burkholderia cepacian*
606	*Mesorhizobi- um erdamanii*
1138	*Ralstonia pickettii*
4487	*Comamonas sediminis*

Table 2: Group 2 Bacterial Isolates for 2-Week Validation Experiment

5.3.2 Growth Chamber Experimental Layouts for Groups 1 and 2

The selected bacterial isolates were placed in a randomized complete block design (RCBD), with each of the four benches separated into 2 blocks. There were 8 blocks total in the growth chamber, with each bacterial isolate having one replication in each block (Figure 1; Figure 2). There were 8 replications for each bacterial isolate and 16 replications for both the high N control and the low N control. In the initial screening of the 64 bacterial isolates, bacterial isolate 478 appeared to offer no positive growth effect while bacterial isolate 1611 appeared to offer a decrease in shoot growth. Therefore, 478 was included as a neutral control, as it was expected to not have an effect on dry shoot weight in inoculated plants, and 1611 was selected as a negative control, as it was expected to show a decrease in dry shoot weight in inoculated plants (Figure 1). Bacterial Isolates 1147 (*Herbaspirillum huttiense*)

and 2725 (*Pseudomonas* sp.) were also selected for testing in the Group 2 experiment. Bacterial isolate 1147 is a bacterial isolate previously found by our lab to have *nif* genes, therefore it was selected as a positive control, as it was predicted to have a positive effect on inoculated plants' dry shoot weight. Bacterial isolate 2725 was tested previously in our lab and found to have a positive effect on plant growth, therefore, it was selected as a second positive control (Figure 2).

Bench 1							
Block 1				Block 2			
Low N Control	High N Control	4589	Low N Control	702	730	Low N Control	1611
4606	High N Control	708	702	4509	Low N Control	1204	708
2829	730	4509	1611	High N Control	726	High N Control	111
1204	111	478	726	4606	2829	4589	478

Bench 2							
Block 3				Block 4			
478	High N Control	4509	730	Low N Control	730	726	478
1611	1204	726	High N Control	1204	4589	High N Control	4509
Low N Control	4606	702	2829	4606	708	Low N Control	High N Control
111	4589	Low N Control	708	2829	702	1611	111

Bench 3							
Block 5				Block 6			
111	1611	2829	1204	High N Control	High N Control	4606	Low N Control
High N Control	4509	726	4589	4509	1204	708	Low N Control
4606	Low N Control	708	702	730	702	1611	111
Low N Control	478	High N Control	730	2829	478	726	4589

Bench 4							
Block 7				Block 8			
4589	2829	4606	708	High N Control	2829	Low N Control	111
730	4509	Low N Control	High N Control	4589	High N Control	4606	708
1611	726	478	111	1204	730	726	478
Low N Control	702	1204	High N Control	702	1611	Low N Control	4509

Figure 1: Growth chamber layout for the 2-week validation experiment for the group 1 bacterial isolates.

Bench 1					
Block 1			Block 2		
Low N Control	High N Control	571	2725	Low N Control	High N Control
Low N Control	1147	2725	571	574	606
4487	High N Control	1138	4487	1138	High N Control
606	574		1147	Low N Control	

Bench 3					
Block 5			Block 6		
574	4487	High N Control	Low N Control	4487	2725
1138	Low N Control	Low N Control	574	571	606
1147	2725	571	High N Control	1147	1138
High N Control	606		Low N Control	High N Control	

Bench 2					
Block 3			Block 4		
High N Control	574	4487	1147	Low N Control	574
1147	606	Low N Control	High N Control	1138	Low N Control
1138	High N Control	2725	4487	606	High N Control
Low N Control	571		571	2725	

Bench 4					
Block 7			Block 8		
4487	High N Control	Low N Control	1147	Low N Control	Low N Control
1147	High N Control	571	High N Control	571	High N Control
606	1138	2725	4487	606	2725
574	Low N Control		574	1138	

Figure 2: Growth chamber layout for the 2-week validation experiment for the group 2 bacterial isolates.

5.3.3 Seed Germination for Group 1 and Group 2 Bacterial Isolates

The Mo17 maize seed was sterilized and germinated following the protocol in (Section 5.1.4).

5.3.4 Bacterial Growth for Group 1 and Group 2 Bacterial Isolates

The Group 1 bacterial isolates were grown following the protocol in (Section 5.1.3) in R2A medium. The Group 2 bacterial isolates were grown in single-colony patches on nitrogen-limited combined carbon (NLCC) medium plates (0.8g/L K_2HPO_4, 0.2g/L KH_2PO_4, 0.1g/L NaCl, 28.0mg/L $Na_2FeEDTA$, 25.0mg/L $Na_2MoO_4H_2O$, 100mg/L Yeast Extract, 5.0g/L Mannitol, 5.0g/L Sucrose, 0.5mL/L (60 percent, v/v sodium lactate, 0.2g/L $MgSO_47H_2O$, 0.06g/L $CaCl_2$).

5.3.5 Seedling Priming for Group 1 and Group 2 Bacterial Isolates

The Group 1 bacterial isolates were inoculated onto the germinated Mo17 maize seeds following the protocol in (Section 5.1.6). For the Group 2 bacterial isolates, on the day of the seedling priming bacterial inoculation, the single colony patches grown on the NLCC medium plates for each of the Group 2 bacterial isolates were inoculated into phosphate-buffered solution (PBS) (8g/L NaCl, 0.2g/L KCl, 1.44g/L Na_2HPO_4, 0.24g/L KH_2PO_4), until the OD for each bacterial culture reached an OD_{600} value of 1. Upon reaching the desired OD_{600} value, the germinated seedlings were inoculated with each of the bacterial isolate PBS cultures in petri dishes that were then sealed with micropore tape and placed on the shaker at 80 rpm at room temperature (24°C) overnight to ensure thorough inoculation of the seedlings with the desired bacterial isolates.

5.3.6 Potting Mix Sterilization

The potting mix was sterilized following the protocol in (Section 5.1.5).

5.3.7 Maize Planting

The inoculated maize seedlings were planted following the procedure in (Section 5.1.7).

5.3.8 Maize Growth Conditions

The plants were grown for two weeks in the same growth conditions outlined in (Section 5.1.8).

5.3.9 Maize Root and Shoot Sampling

At the end of the 2-week growth period, the roots and shoots of the maize plants were sampled following the protocol in (Section 5.1.9).

5.3.10 Statisical Analysis

The statistical analysis of the 2-week growth experiments was carried out in R (R Developmental Core et al. 2018) and SAS (SAS Institute Inc. et al. 2023). A linear mixed model analysis was performed to determine if the dry shoot weight of the maize grown with the bacterial isolate was significantly higher than the dry shoot weight of the maize grown in low nitrogen conditions (the low N control). A post-hoc analysis was performed to determine which specific treatment groups were significantly different from one another.

5.4 4-Week Growth Validation Experiment

To further evaluate the potentially beneficial effects of the selected bacterial isolates, a four-week growth experiment was carried out to assess whether a longer growth period encourages

the emergence of a significant phenotypic effect that is caused by the bacterial isolates.

5.4.1 Soil Water Holding Capacity (SWHC) Trial

First a four-week long growth-trial was done to determine the best soil water holding capacity (SWHC) to grow the maize plants at for the four-week growth experiment. Plants were grown in pots (19 cm in height, 15 cm in diameter) for a total of four weeks inside a growth chamber kept at 26°C during the day and 18°C during the night, with a 16-hour light period. The simulated soil matrix for the plants was a 2/3 peat and 1/3 fine vermiculite mixture. This soil mixture was tested and used in the 4-week validation experiment due to its ability to enhance microbiota-driven soil fertility and crop productivity (Wu et al., 2021). To best replicate the growth conditions that would be used in the four-week bacterial inoculation growth experiment, the soil was autoclaved three times to ensure sterility.

Three SWHC levels were tested in this trial: 70 percent, 80 percent, and 90 percent, with four plants per each SWHC level, for a total of 12 plants grown in the experiment (4 plants x 3 SWHC levels = 12 total plants). Mo17 maize seeds were imbibed for 24 hours and placed in petri dishes in an oven set at 30°C, until they were germinated. Three germinated seeds were planted in each pot. To ensure evenness across the treatments, when the plants were about two inches tall, the pots were thinned down to one plant each. To determine the SWHC for each level, 100 percent SWHC was measured by slowing adding water to 1000 grams of the peat and vermiculite soil mixture until the water could be squeezed out of a handful of soil. For 1000 grams of soil, 1000 grams of water was needed to reach 100 percent SWHC. The plants were watered at their specified SWHC for the entire duration of the growth experiment (four weeks). Full-strength Hoagland solution was added approximately one time each week to the desired SWHC of each plant. After four weeks of growth, the plants were removed from the growth chamber and the dry shoot weights were analyzed

using R (R Developmental Core et al. 2018).

5.4.2 Bacterial Selection

From the prior 2-week growth experiments with the bacterial isolates selected from the initial assay of 64, three bacterial isolates were chosen for the 4-week growth experiment, after the plants they were inoculated in showed either a significant increase in growth under low N conditions (i.e., bacterial isolate 111), or because they appeared to increase the shoot growth under low N, although it was not statistically significant (730 and 4589). The three selected bacterial isolates from the 2-week growth experiments were the following: (Table 3).

Bacterial Isolate ID	**Bacterial Isolate Identity**
111	*Arthrobacter* sp.
730	*Pseudomonas kribbensis*
4589	*Sphingomonas* sp.

Table 3: Bacterial isolates and identities for 4-Week Validation Experiment

5.4.3 Maize Genotype Selection

To assess whether different maize genotypes responded in different ways to the selected bacterial isolates, three different maize genotypes were selected to be inoculated with bacterial isolates 111, 730, and 4589, separately. The three selected maize genotypes were the following: (Table 4). The maize genotypes Ames-27065 and NSL-30867 were selected from the paper, *Sugars and Jasmonic Acid Concentration in Root Exudates Affect Maize Rhizosphere Bacterial Communities*, in which these two maize genotypes were assessed among other genotypes, for their sugar and jasmonic acid exudate concentrations (Lopes et al. 2022). The two maize genotypes selected in addition to Mo17 were significantly different in their

exudate concentrations of both sugar and jasmonic acid.

Maize Genotype Number	Maize Genotype Name
1	Mo17
2	Ames 27065
3	NSL 30867

Table 4: Maize genotype numbers and identities for 4-Week Validation Experiment

5.4.4 Experimental Design

To test the effects of the 3 selected, potentially beneficial bacterial isolates on the 3 selected maize genotypes, a 3x5 factorial experimental design with two treatment factors was implemented. Factor 1 was the maize genotype and factor 2 was the bacterial isolate (111, 730, 4589, high N control, low N control). The following replicated block design was the layout for the plants in the growth chamber for the duration of the 4-week growth experiment (Figure 3). The growth chamber holds 4 benches and each bench was divided into 2 blocks (8 blocks total). Each treatment combination (bacterial isolate x maize genotype) had one replicate in each block, giving 8 relications to each treatment combination.

Block1			Block2		
Mo17-730	Mo17-4589	Ames-111	Mo17-High N	NSL-730	Ames-111
Ames-730	Ames-High N	NSL-Low N	Ames-High N	Mo17-Low N	Mo17-4589
Mo17-Low N	NSL-High N	Mo17-High N	Ames-730	Mo17-730	Ames-Low N
Ames-4589	NSL-111	Ames-Low N	NSL-High N	Mo17-111	Ames-4589
Mo17-111	NSL-730	NSL-4589	NSL-Low N	NSL-4589	NSL-111

Block 3			Block4		
NSL-730	Mo17-Low N	Ames-111	Mo17-High N	Ames-4589	Ames-High N
Mo17-High N	Mo17-111	NSL-Low N	Ames-Low N	Mo17-111	Mo17-4589
Mo17-4589	NSL-High N	Ames-High N	Ames-111	NSL-730	NSL-4589
Ames-730	Mo17-730	NSL-4589	Mo17-730	Mo17-Low N	NSL-High N
Ames-Low N	Ames-4589	NSL-111	NSL-Low N	NSL-111	Ames-730

Block5			Block6		
NSL-High N	NSL-4589	Ames-Low N	Mo17-111	NSL-730	Ames-High N
Mo17-High N	Mo17-111	Ames-4589	Mo17-High N	Ames-4589	Mo17-Low N
Ames-730	Mo17-730	Mo17-4589	Ames-730	NSL-4589	Ames-Low N
NSL-730	NSL-Low N	Mo17-Low N	NSL-High N	Mo17-4589	Ames-111
Ames-111	Ames-High N	NSL-111	NSL-111	Mo17-730	NSL-Low N

Block7			Block8		
NSL-High N	Ames-4589	Mo17-Low N	Mo17-730	Mo17-Low N	Mo17-High N
Ames-Low N	NSL-Low N	Mo17-High N	NSL-111	Mo17-111	Ames-Low N
Ames-111	Ames-High N	Mo17-4589	NSL-Low N	Ames-730	Ames-111
Mo17-730	NSL-111	NSL-4589	Mo17-4589	NSL-4589	Ames-4589
Mo17-111	NSL-730	Ames-730	NSL-High N	Ames-High N	NSL-730

Figure 3: Growth chamber layout for the 4-week validation experiment for the selected bacterial isolates (including the uninoculated low N and high N controls) and maize genotypes. (Notation: Maize Genotype-Bacterial Isolate)

5.4.5 Bacterial Growth and Inoculation

The selected bacterial isolates were grown following the protocol in (Section 5.1.3). The three maize genotypes were all sterilized and germinated following the protocol in (Section 5.1.4). The germinated maize seeds were then inoculated following the protocol in (Section 5.1.6).

5.4.6 Maize Planting and Growth Conditions

After 18 hours on the shaker, the inoculated seeds were removed from the shaker and two seeds were sown into each pot, containing 2 parts peat and 1 part vermiculite at 60 percent SWHC, that had been previously autoclaved three times (cycle: P01 liquid, 20 minutes sterilization), to ensure sterility of the growth system. The pots containing the seeds inoculated with bacterial isolates, as well as the low N control maize plant seeds, were given a half-strength nitrogen Hoagland solution at the time of planting, up to 85 percent SWHC. The uninoculated plants that were grown under high nitrogen conditions were treated with

a full-strength nitrogen Hoagland solution at the time of planting. Upon planting of the two seeds, the pots were covered with 6" plastic saucers and placed in the growth chamber. The growth conditions in the growth chamber were kept at 26°C during the day and 18°C during the night, with a 16-hour light period. The plants were watered and kept around 85-90 percent soil water holding capacity (SWHC) throughout the 28-day growth experiment.

5.4.7 Hoagland Solution Soil Drench Treatment

During the course of the 4-week growth period, the plants were given a Hoagland solution soil drench with the bacterial culture resuspended in Hoagland solution and watered onto the soil of each plant. Twice a week, this treatment was administered. A bacterial culture for each of the three bacterial isolates was grown on the shaker at 200 rpm for 18 hours at room temperature and adjusted to an OD_{600} value of 1. To achieve an OD_{600} value of 0.002 for the Hoagland treatment solution, 1 mL of bacterial culture (OD_{600} of 1) was added to 1 liter of Hoagland solution. 25 mL of the treatment was added, twice each week, over the course of the 4 weeks.

5.4.8 Plant Phenotyping at 2-, 3-, and 4-Week Timepoints

To track the growth progress of the plants at the 2-, 3-, and 4-week growth timepoints, a phenotyping system was utilized to optically compute the shoot biomass of the plants. A low-cost phenotyping system was utilized for this task. The phenotyping system captures 8 images from 360° and through R (R Developmental Core et al. 2018), the biomass of each plant is accurately measured through the processing of the eight images. At 14-, 21-, and 27-days after planting, the plants were photographed to measure their shoot biomass. Upon completion of the collection of images, the images were processed in R (R Developmental Core et al. 2018) and a correlation between the 27-day photographed biomass measurements and the collected dry shoot weights of the 28-day old plants was calculated in *MatLab* (MATLAB 2024). The shoot biomass measurements were analyzed in R to investigate how

each treatment combination affected the shoot biomass of the plant throughout the 4-week growth period. A linear mixed model analysis was performed to determine if the shoot biomass measurements of the maize grown with the bacterial isolate was significantly higher than the shoot biomass measurements of the maize grown in low nitrogen conditions (the low N control) for the 14- and 21-day old plants.

5.4.9 Statisical Analysis

The statistical analysis of the 4-week growth experiment results was carried out in R (R Developmental Core et al. 2018) and SAS (SAS Institute Inc. et al. 2023). A linear mixed model analysis was performed to determine if the dry shoot weight of the maize grown with the bacterial isolate was significantly higher than the dry shoot weight of the maize grown in low nitrogen conditions (the low N control). A post-hoc analysis was performed to determine which specific treatment groups were significantly different from one another.

5.5 Analysis of Bacterial Genomes 111 and 730

In the 2-week validation experiment of the Group 1 bacterial isolates, isolate 111 was found to a have a significant growth effect on the biomass of the shoots compared to the uninoculated plant grown in low nitrogen. In the 4-week validation experiment, bacterial isolate 730 was found to have a significant growth effect on the biomass of the shoots compared to the uninoculated plant grown in low nitrogen. To further investigate the potentially beneficial effects of these two bacterial species, their genomes were sequenced, assembled, and annotated to investigate for the presence of beneficial genes, such as those in the *nif* cluster and for enzymes in various indole-3-acetic acid production pathways.

5.5.1 DNA Extraction

Bacterial isolates 111 and 730 were removed from the -70°C freezer and a quadrant streak was performed on an R2A medium agar plate for each bacterial isolate and placed at room temperature (24°C) for bacterial growth. After 48 hours of growth, a single colony was taken from the R2A plate, inoculated into 3 mL of fresh R2A liquid medium, and placed on the shaker at 200 rpm to grow at room temperature (24°C). Upon turbidity indicating bacterial growth, the 3 mL bacterial culture was inoculated into 50 mL fresh R2A liquid medium and placed on the shaker at 200 rpm to grow at room temperature (24°C). After 18 hours of growth, the bacterial culture was adjusted to an OD_{600} of 1, or the equivalent of 4 - 6 x 10^9 cells. Approximately 10 mL of the OD_{600} bacterial culture was pelleted by centrifugation. After centrifugation, the supernatant was removed from the pelleted bacterial cells. The cells were then resuspended and washed in 1 mL of phosphate-buffered solution (PBS), then pelleted again by centrifugation. The supernatant was removed, and the final pellet was resuspended in 0.5 mL of Zymo 1X DNA/RNA Shield and placed in a 2 mL screw cap tube for shipment to Plasmidsaurus for DNA extraction, library preparation, and genome sequencing. Plasmidsaurus carried out the DNA extraction for both bacterial isolates using the ZymoBIOMICS 96 MagBead DNA Kit.

5.5.2 Library Preparation

Libraries were prepared through constructing an amplification-free long-read sequencing library using v14 library prep chemistry, including minimal fragmentation of the genomic DNA in a sequence independent manner. The Rapid Barcoding Kit 96 V14, part SQK-RBK114.96 was utilized for library preparation.

5.5.3 Genome Sequencing

A hybrid sequencing option was used for sequencing, which consisted of long-read sequencing reads produced by Oxford Nanopore Technologies (ONT), that were then polished by Illumina bacterial genome sequencing reads. The ONT reads were sequenced on the Promethion P24 with R10.4.1 flow cell machine. The Illumina sequencing reads were sequenced on the NextSeq2000, paired-end 2x150bp run configuration machine.

5.5.4 Hybrid Genome Assembly

The bottom 5 percent worst fastq sequencing reads were removed using *Filtlong v0.2.1.* A rough draft of the assembly was created using *Miniasm v0.3* by downsampling the reads to 250 Mb via *Filtlong v0.2.1.* The reads were then re-downsampled to 100x coverage using the *Miniasm* rough draft assembly. *Flye v2.9.1* was used to run an assembly with parameters selected for high quality ONT reads. The assembly generated from *Flye v2.9.1* was polished using the reads generated from the re-downsampling of the *Miniasm v0.3* assembly via *Medaka v1.8.0.* The ONT .fna assembly was polished with Illumina .fastq reads using *Polypolish v0.6.0*, producing a new, polished hybrid genome assembly.

5.5.5 Genome Annotation

Annotation of the bacterial genome assembly was carried out by *Bakta v1.6.1.* Contig analysis was executed using *Bandage v0.8.1.* Completeness and contamination assessments of the genome were performed by *CheckM v1.2.2.* The species identification of the genome was carried out using *Mash v.2.3* against RefSeq genomes+plasmids and *Sourmash v4.6.1* against GenBank.

6 Results

6.1 Identification of 64 Potentially Beneficial Bacterial Isolates

In the paper, *Association analyses of host genetics, root-colonizing microbes, and plant phenotypes under different nitrogen conditions in maize* (Meier et al. 2022), positively and negatively associated amplicon sequence variants (ASVs) of soil microbes, that may contribute to increasing plant fitness, were identified from rhizosphere samples. The host plants' genomes from these rhizosphere samples likely underwent negative or positive selection to favor specific microbial groups (referred to as rhizobiome traits) by removing harmful alleles or increasing desirable alleles. The methods used in this paper to identify these recruited ASVs did not explicitly test which traits or phenotypes would be affected. Therefore, the goal of this research project was to investigate whether these ASVs recruited under low nitrogen conditions impart a beneficial phenotypic effect to maize. The ASVs from this study were searched against the 16S sequences in the Schachtman Lab Culture Collection by the Nebraska Center for Biotechnology Bioinformatics Core and from this, 64 potentially beneficial bacterial isolates were identified (Table 5). The 64 bacterial isolates were not necessarily selected for a benefit to maize under low nitrogen conditions. Therefore this initial screening of the 64 isolates was be carried out to determine if related bacterial isolates found in the culture collection were beneficial to maize under low N conditions.

Table 5: The 64 Identified Bacterial Isolates for Initial Screening

Bacterial Isolate ID	**Genus**	**Species**	**Sampled From**	**Collection Site**
111	*Arthrobacter*	*sp.*	side oats soil	Mead, NE
94	*Mucilaginibac- ter*	*sp.*	big bluestem soil	Mead, NE
707	*Pseudomonas*	*sp. Csya02*	maize roots	Brule, NE
708	*Leifsonia*	*aquatica*	maize roots	Brule, NE
709	*Burkholderia*	*sp. RB142*	maize roots	Brule, NE
726	*Pseudomonas*	*sp. Csya02*	maize roots	Brule, NE
727	*Burkholderia*	*seminalis*	maize roots	Brule, NE
730	*Pseudomonas*	*kribbensis*	maize roots	Brule, NE
731	*Pseudomonas*	*sp. Csya02*	maize roots	Brule, NE
1087	*Mesorhizobi- um*	*erdmanii strain NZP2014*	energy sorghum rhizosphere	Florence, SC
1610	*Kosakonia*	*sp.*	transgenic sorghum rhizosphere	Mead, NE
1611	*Pseudomonas*	*fluorescens*	transgenic sorghum rhizosphere	Mead, NE

Bacterial Isolate ID	**Genus**	**Species**	**Sampled From**	**Collection Site**
1903	*Atlantibacter*	*hermannii*	transgenic sorghum rhizosphere	Mead, NE
478	*Pseudomonas*	*sp.*	switchgrass roots	Mead, NE
616	*Burkholderia*	*seminalis*	maize roots	Brule, NE
621	*Dyella*	*yeoguensis*	maize roots	Brule, NE
1112	*Pantoea*	*dispersa*	energy sorghum roots	Florence, SC
3432	*Pseudomonas*	*brassicacearum*	smooth brome grass roots	Sandhills NE
3552	*Arthrobacter*	*bambusae*	foxtail barley roots	Sandhills NE
4479	*Flavobacteri- um*	*johnsoniae*	transgenic sorghum rhizosphere	Mead, NE
4493	*Dyadobacter*	*sp.*	false purple brome extraradical hyphae	Ithaca, NY

Bacterial Isolate ID	**Genus**	**Species**	**Sampled From**	**Collection Site**
4494	*Dyadobacter*	*sp.*	false purple brome extraradical hyphae	Ithaca, NY
4509	*Dyadobacter*	*fermentans*	false purple brome extraradical hyphae	Ithaca, NY
4540	*Sphingomonas*	*sp.*	false purple brome extraradical hyphae	Ithaca, NY
4579	*Dyadobacter*	*sp.*	false purple brome extraradical hyphae	Ithaca, NY
4605	*Sphingomonas*	*echinoides*	false purple brome extraradical hyphae	Ithaca, NY
4606	*Acidovorax*	*sp.*	false purple brome extraradical hyphae	Ithaca, NY

Bacterial Isolate ID	**Genus**	**Species**	**Sampled From**	**Collection Site**
460	*Pseudomonas*	*sp.*	switchgrass roots	Mead, NE
620	*Burkholderia*	*sp. CR22*	maize roots	Brule, NE
700	*Burkholderia*	*ambifaria*	maize roots	Brule, NE
701	*Burkholderia*	*ambifaria*	maize roots	Brule, NE
702	*Pseudomonas*	*koreensis*	maize roots	Brule, NE
704	*Sphingomonas*	*kyeoggiensis*	maize roots	Brule, NE
725	*Pseudomonas*	*sp. Agri-10*	maize roots	Brule, NE
739	*Burkholderia*	*sp.*	maize roots	Brule, NE
1971	*None*	*None*	transgenic sorghum roots	Mead, NE
2025	*Mucilaginibac-ter*	*rubeus*	transgenic sorghum roots	Mead, NE
2829	*Janthinobact-erium*	*lividium*	maize rhizosphere	Brule, NE
2830	*Janthinobact-erium*	*lividium*	maize rhizosphere	Brule, NE
2987	*Chryseobacte-rium*	*sp.*	maize roots	Brule, NE

Bacterial Isolate ID	**Genus**	**Species**	**Sampled From**	**Collection Site**
4567	*Dyadobacter*	*sp.*	false purple brome extraradical hyphae	Ithaca, NY
4589	*Sphingomonas*	*sp.*	false purple brome extraradical hyphae	Ithaca, NY
1550	*Pseudomonas*	*fluorescens*	transgenic sorghum rhizosphere	Mead, NE
3330	*Streptomyces*	*sp.*	sweet sorghum soil	Mead, NE
571	*Burkholderia*	*sp.*	sweet sorghum roots	Mead, NE
572	*Burkholderia*	*sp.*	sweet sorghum roots	Mead, NE
574	*Burkholderia*	*cenocepacia*	sweet sorghum roots	Mead, NE

Bacterial Isolate ID	**Genus**	**Species**	**Sampled From**	**Collection Site**
606	*Mesorhizobi- um*	*erdmanii*	sweet sorghum roots	Mead, NE
1138	*Ralstonia*	*sp.*	sweet sorghum roots	Florence, SC
1175	*Pantoea*	*dispersa*	sweet sorghum roots	Mead, NE
1204	*Chitinophaga*	*pinensis*	sweet sorghum roots	Mead, NE
1215	*Mucilaginibac- ter*	*rubeus*	sweet sorghum roots	Mead, NE
1474	*Rhizobium*	*sp.*	transgenic sorghum rhizosphere	Mead, NE
2056	*Kosakonia*	*cowanii*	transgenic sorghum roots	Mead, NE
2074	*Agrobacterium*	*larrymoorei*	transgenic sorghum roots	Mead, NE

Bacterial Isolate ID	**Genus**	**Species**	**Sampled From**	**Collection Site**
2083	*Neorhizobium*	*sp.*	transgenic sorghum roots	Mead, NE
3190	*Luteibacter*	*sp.*	sweet sorghum roots	Mead, NE
3198	*Chitinophaga*	*sancti*	sweet sorghum roots	Mead, NE
3215	*Mucilaginibac-ter*	*rubeus*	sweet sorghum soil	Mead, NE
4487	*Comamonas*	*sediminis*	transgenic sorghum roots	Mead, NE
3939	*Streptomyces*	*sp. 1-26*	needle and thread grass roots	Mead, NE
4447	*Pseudomonas*	*frederickbergensis strain Sr4*	Kentucky blue grass roots	Mead, NE
4068	*Streptomyces*	*pratensis strain HQA952*	Red canary grass roots	Mead, NE

Bacterial Isolate ID	Genus	Species	Sampled From	Collection Site
3977	*Streptomyces*	*sp. Strain SKB2.14*	smooth brome grass roots	Mead, NE

6.2 Initial Assay of the 64 Potentially Beneficial Bacterial Isolates

To assess the impact of these isolates on nitrogen-deficient maize growth, the 64 identified bacterial isolates were divided into seven different rounds of experiments with each round containing uninoculated nitrogen-deficient plants (low N control) and uninoculated nitrogen-sufficient plants (high N control). The dry roots and dry shoots were graphed separately. Through one-way ANOVA and Tukey HSD post-hoc tests, the dry shoot weight was analyzed amongst the bacterial isolates and the high and low N controls to assess whether any of the 64 bacterial isolates increased the dry shoot weight compared to the low N control.

In round one, none of the bacterial isolates significantly increased the dry shoot weight of maize compared to the low N control (Figure 4). In round two, none of the bacterial isolates significantly increased the dry shoot weight of maize compared to the low N control (Figure 5). In round 3, none of the bacterial isolates significantly increased the dry shoot weight compared to the low N control (Figure 6). In round 4, bacterial isolates 1611 and 704 significantly decreased the dry shoot weight compared to the low N control (Figure 7). None of the plants inoculated with the bacterial isolates had a significant increase in dry shoot weight compared to the low N control in round 4 (Figure 7). In round 5, none of the plants inoculated with the bacterial isolates had a significant effect on dry shoot weight (grams) compared to the low N control (Figure 8). In round 6, none of the plants inoculated with the bacterial isolates appeared to have a significant increase in dry shoot weight (grams)

compared to the low N control (Figure 9). In round 7, none of the bacterial isolates significantly increased the dry shoot weight (grams) compared to the low N control (Figure 10).

In each of the rounds, none of the bacterial isolates significantly increased the dry shoot weight (grams) in the inoculated plants compared to the low N control plant. To select 15 bacterial isolates that would be further analyzed in both the 2- and 4-week validation experiments, each round of the initial assay was graphed and bacterial isolates that appeared to visually increase dry shoot weight were selected for the further studies. From the results of round 1, bacterial isolates 111 and 726 were selected. From round 2, bacterial isolates 2829, 702, and 726 were selected. No bacterial isolates were selected from round 3. From round 4, bacterial isolates 708, 726, and 730 were selected. In round 5, bacterial isolate 1138 was selected. From round 6, bacterial isolates 1138, 1204, 4487, 4509, 4589, 4606, ,571, 574, and 606 were selected. No bacterial isolates were selected from round 7. The selected bacterial isolates from the 7 rounds were then used for further validation to investigate their plant growth promotion of maize shoot growth under nitrogen deficient conditions.

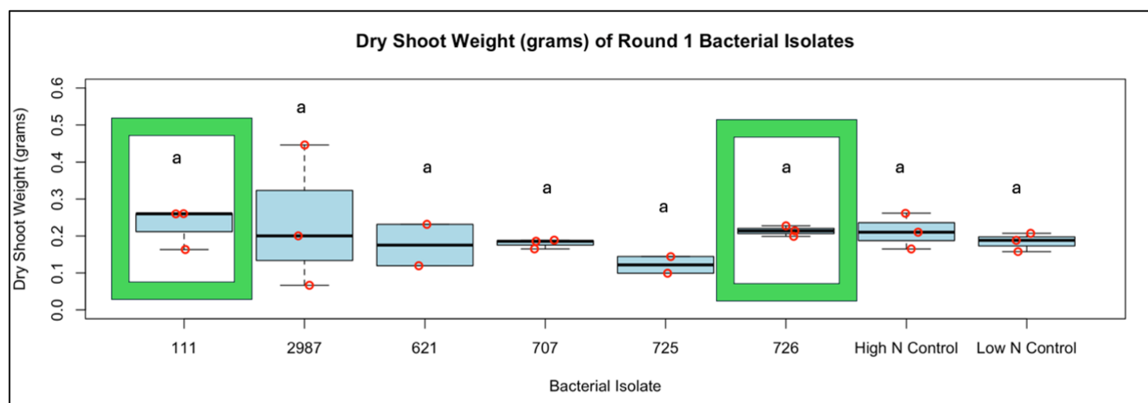

Figure 4: Initial Assay Round 1 Dry Shoot Weight with six Bacterial Isolates. Dry shoot weight (grams) results from round 1 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. Boxed bacterial isolates were selected for the validation studies.

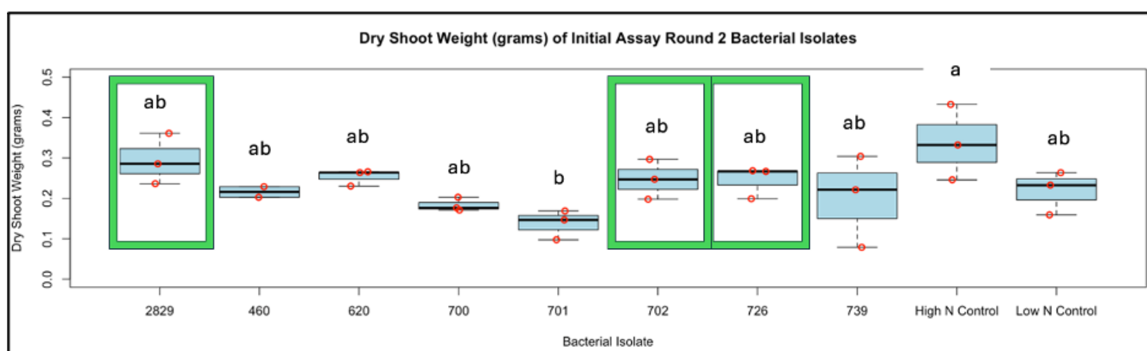

Figure 5: Initial Assay Round 2 Dry Shoot Weight with eight Bacterial Isolates. Dry shoot weight (grams) results from round 2 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. Boxed bacterial isolates were selected for the validation studies.

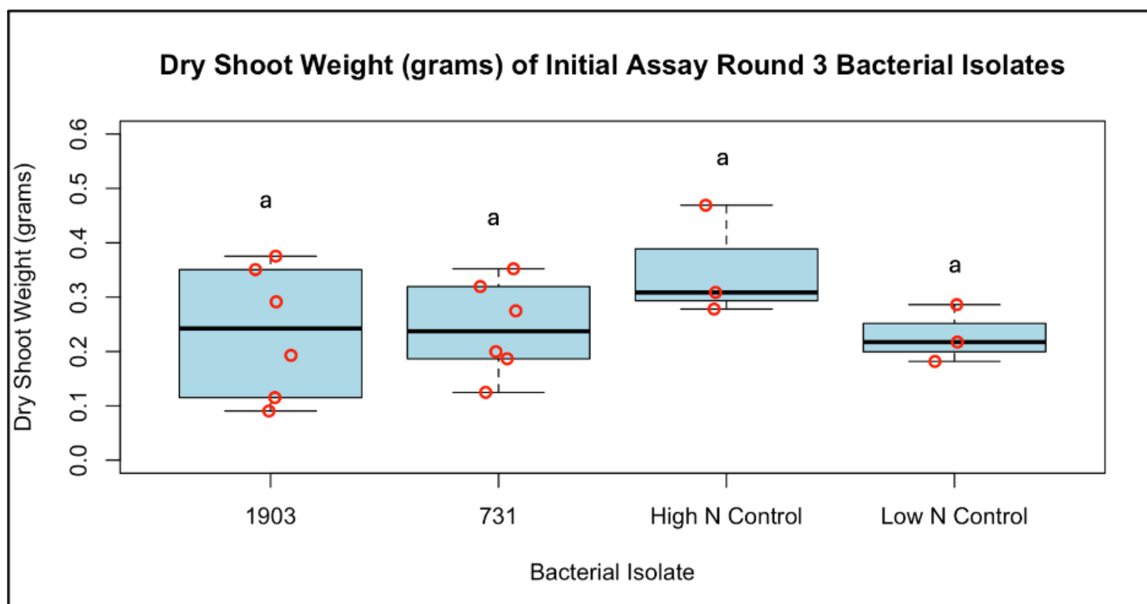

Figure 6: Initial Assay Round 3 Dry Shoot Weight with two Bacterial Isolates. Dry shoot weight (grams) results from round 3 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$.

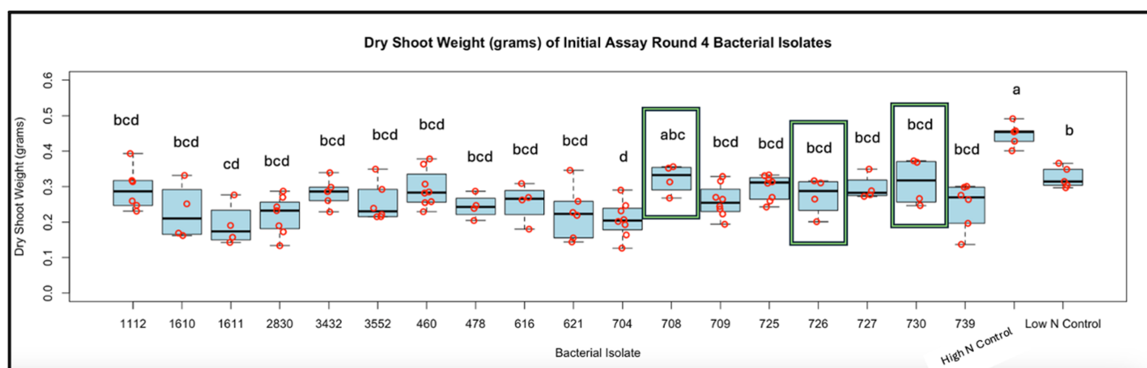

Figure 7: Initial Assay Round 4 Dry Shoot Weight with eighteen Bacterial Isolates. Dry shoot weight (grams) results from round 4 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. Boxed bacterial isolates were selected for the validation studies.

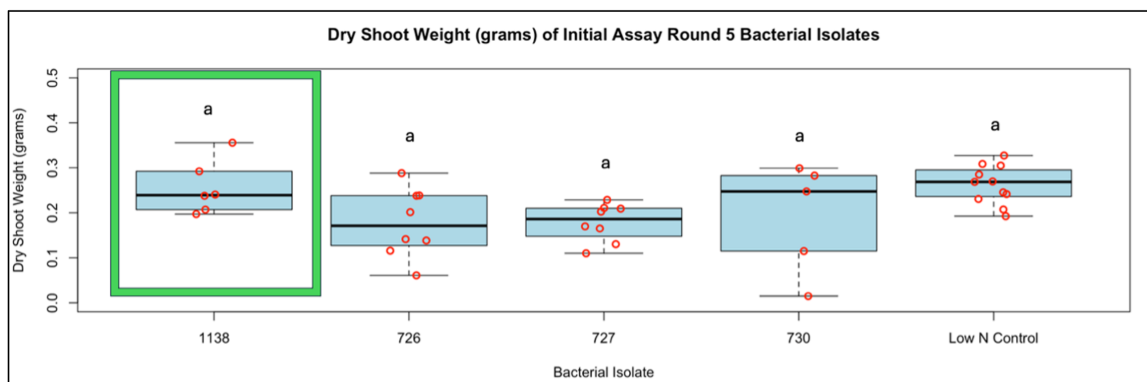

Figure 8: Initial Assay Round 5 Dry Shoot Weight with four Bacterial Isolates. Dry shoot weight (grams) results from round 5 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. Boxed bacterial isolates were selected for the validation studies.

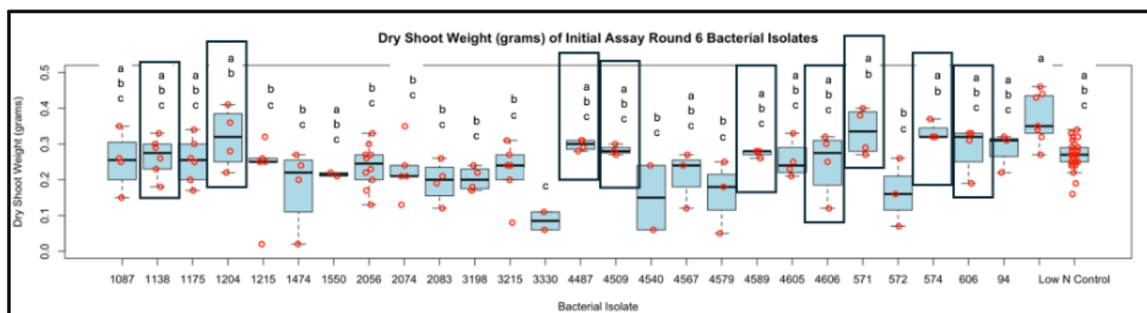

Figure 9: Initial Assay Round 6 Dry Shoot Weight with twenty-six Bacterial Isolates. Dry shoot weight (grams) results from round 6 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$. Boxed bacterial isolates were selected for the validation studies.

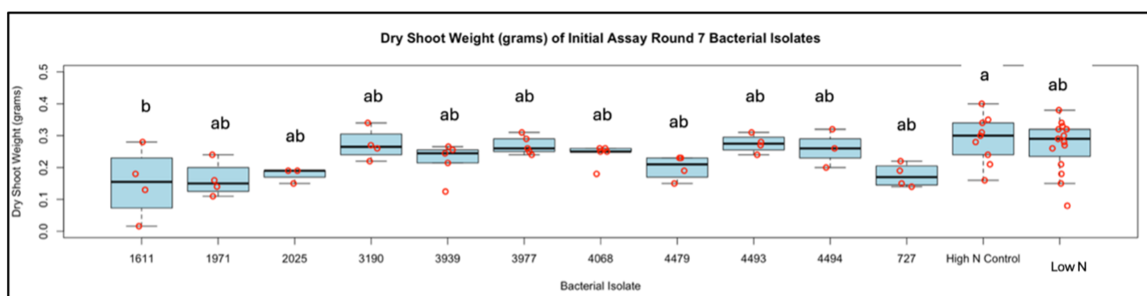

Figure 10: Initial Assay Round 7 Dry Shoot Weight with eleven Bacterial Isolates. Dry shoot weight (grams) results from round 7 of initial assays. Significant differences were determined using ANOVA followed by Tukey's HSD correlation for multiple comparisons. Different letters indicate a significant difference $p < 0.05$.

6.3 Sanger Sequencing

The identities of the 15 selected bacterial isolates from the initial assay were sequenced (Table 6) to confirm the identities listed for them in the Schachtman Lab Culture Collection.

Bacterial Isolate ID	**Isolate Identity**
111	*Arthrobacter sp.*
571	*Burkholderia cepacian*
574	*Burkholderia cepacian*
606	*Mesorhizobi- um erdmanii*
702	*Pseudomonas koreensis*
708	*Leifsonia aquatica*
726	*Pseudomonas* sp. Csya02
730	*Pseudomonas kribbensis*
1138	*Ralstonia picketti*
1204	*Chitinophaga pinensis*
2829	*Janthinobact- erium lividium*
4487	*Comamonas sediminis*
4509	*Dyadobacter fermentans*
4589	*Sphingomonas sp.*
4606	*Acidovorax sp.*

Table 6: List of Bacterial Isolates and their Identities resulting from the Sanger Sequencing

6.4 2-Week Validation Experiments for Selected Bacterial Isolates

To further investigate the potential plant-growth promoting effects of the selected bacteria from the initial assay, a two-week validation experiment was carried out. The two-week validation experiment was carried out with selected bacterial isolates from the initial assay, in two 14-day studies. The replications for the two-week validation experiments were increased to 8 replications per treatment, to improve the statistical power compared to the initial assay, which only had 3-8 replications depending on the round. The selected bacterial isolates were separated into two groups, depending on the bacterial medium they were grown in. Group 1 bacteria were grown in R2A medium while Group 2 bacteria were grown in NLCC

medium (Hegazi et al., 1998).

In the 2-week validation experiment for the Group 1 bacterial isolates, bacterial isolate 111 had a significant positive effect on the dry shoot weight of Mo17 maize compared to the low N control (p-value = 0.018) (Figure 11, Table 7). No other bacterial isolates had a significant growth effect on the dry shoot weight (grams) compared to the low N control.

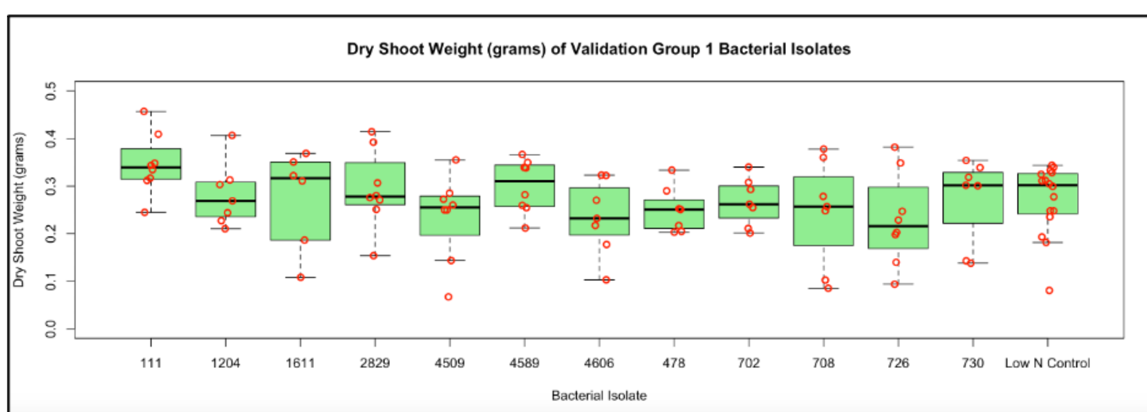

Figure 11: Dry shoot weight (grams) of the Mo17 maize genotype inoculated with Group 1 bacterial isolates in the 2-week validation experiment

In the 2-week validation experiment for the Group 2 bacterial isolates, none of the bacterial isolates had a significant positive growth effect on maize dry shoot weight (grams) compared to the low N control (Figure 12, Table 8). Interestingly, bacterial isolate 571 did have a significant negative growth effect on dry shoot weight compared to the low N control (p-value = 0.0013). Bacterial isolate 574 also had a significant negative growth effect on the dry shoot weight compared to the low N control (p-value = 0.0518) (Table 8).

From the results of the 2-week validation experiments for the Group 1 and 2 bacterial iso-

Bacterial Isolate	Estimate	Standard Error	P-value
111	0.07321	0.03035	0.018
1204	0.01162	0.03189	0.7165
1611	0.009458	0.03381	0.7803
2829	0.02056	0.03035	0.5
4509	-0.03709	0.03035	0.225
4589	0.02761	0.03035	0.3656
4606	-0.03322	0.03187	0.3002
478	-0.01686	0.03187	0.5981
702	-0.01102	0.03187	0.7303
708	-0.02541	0.03187	0.4276
726	-0.04252	0.03035	0.1649
730	0.000391	0.03189	0.9902

Table 7: Differences in dry shoot weight (grams) between Mo17 maize plants inoculated with each of the Group 1 Bacterial Isolates and the Low N Control

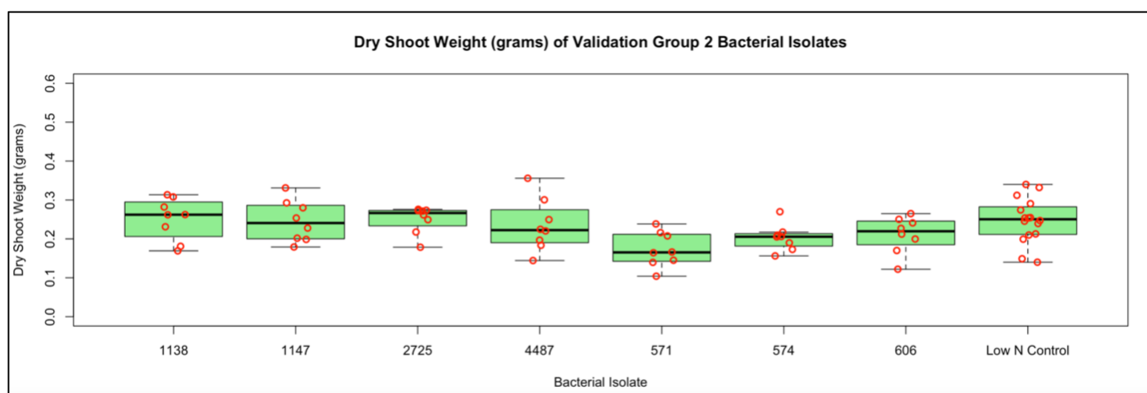

Figure 12: Dry shoot weight (grams) of the Mo17 maize genotype inoculated with Group 2 bacterial isolates in the 2-week validation experiment

lates, three bacterial isolates were selected for further investigation in the 4-week validation experiment, to investigate the effects of these potentially beneficial bacterial isolates in a longer growth period. The three bacterial isolates that were selected were 111, 730, and 4589. Bacterial isolate 111 was selected due to its significant positive growth effect on the dry shoot weight of inoculated Mo17 maize compared to the low N control. Bacterial isolates 730 and 4589 were selected based on the apparent increase in that they imparted to maize dry shoot weight compared to the low N control (Figure 11).

Bacterial Isolate	Estimate	Standard Error	P-value
1138	0.003963	0.02208	0.8582
1147	-0.00156	0.02208	0.9438
2725	0.002925	0.02208	0.895
4487	-0.01271	0.02208	0.5668
571	-0.07447	0.02208	0.0013
574	-0.04376	0.02208	0.0518
606	-0.03625	0.02208	0.1056

Table 8: Differences in dry shoot weight (grams) between Mo17 maize plants inoculated with each of the Group 2 Bacterial Isolates and the Low N Control

6.5 4-week Validation Experiment for Selected Bacterial Isolates

6.5.1 Results from the Soil Water Holding Capacity Experiment

A soil water holding capacity (SWHC) experiment was carried out to determine how much water to provide the plants during the four-week validation experiment. Plants watered to 80 percent SWHC had significantly higher dry shoot weight (grams) than plants watered to 70 percent SWHC (Table 13). Likewise, plants watered to 90 percent SWHC had significantly higher dry shoot weight (grams) compared to the 70 percent SWHC plants. The plants watered at 80 percent and 90 percent SWHC did not differ significantly in their dry shoot weight response. These results suggested that the plants should be kept between 80-90 percent SWHC during the four-week validation experiment.

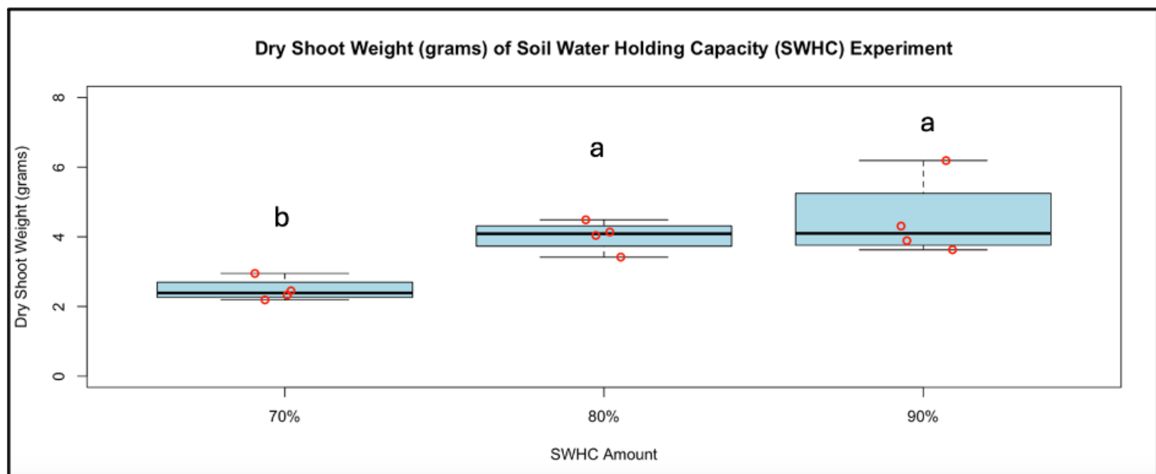

Figure 13: Soil Water Holding Capacity (SWHC) Experiment. Three SWHC amounts were tested to decide which SWHC amount (70, 80, or 90 percent) to water the plants in the four-week validaion experiment at (Different letters indicate a significant difference $p < 0.05$).

6.5.2 Effect of Bacterial Isolates on the Dry Shoot Weight of Inoculated Maize Genotypes

In the four-week validation experiment, bacterial isolates 111, 730, and 4589 were inoculated into 3 different maize genotypes, Mo17, Ames 27065, and NSL 30867, to assess the bacterial isolates' abilities to promote shoot growth in the maize genotypes under low N conditions. The block did not significantly influence the dry shoot weight response (p-value = 0.2473) (Table 9). When investigating each of the factors individually for their effect on the dry shoot weight response, the bacterial isolate alone did not have a significant effect on dry shoot weight (p-value = 0.2431) (Table 9). Conversely, the maize genotype alone did have a significant effect on the dry shoot weight (p-value < 0.0001) (Table 9) under low N conditions. Interestingly, there was a significant interaction effect between the two factors, bacterial isolate and maize genotype (p-value = 0.0092), indicating that the dry shoot weight response is significantly dependent on the combined effect of the bacterial isolate and maize genotype (Table 9).

Effect	**F-value**	**P-value**
Isolate	1.42	0.2431
Genotype	21.61	< 0.0001
Isolate*Genotype	3.09	0.0092
Block	1.32	0.2473

Table 9: 4-Week Validation Experiment - Linear Mixed Model Analysis for the Dry Shoot Weight Response of Each Factor

When analyzing the differences among the maize genotypes alone for the dry shoot weight response (Table 10), the Mo17 maize genotype had a significantly greater effect on dry shoot weight compared to the Ames 27065 maize genotype (p-value < 0.0001). Similarly, Mo17 had a significantly greater effect on dry shoot weight compared to the NSL 30867 maize genotype (p-value <0.0001). The Ames 27065 and NSL 30867 maize genotypes did not have

a significantly different effect on dry shoot weight (p-value = 0.2112).

Maize Genotype	Maize Genotype	Estimate	Standard Error	t Value	P-value
Ames 27065	Mo17	-0.2964	0.04861	-6.1	< 0.0001
Ames 27065	NSL 30867	-0.06045	0.04794	-1.26	0.2112
Mo17	NSL 30867	0.236	0.04606	5.12	< 0.0001

Table 10: 4-Week Validation Experiment - The Significant Differences on Dry Shoot Weight (grams) Between the Three Maize Genotypes

When looking into the differences among maize genotypes on the dry shoot weight response within each bacterial isolate treatment, there was a significant difference between maize genotypes due to bacterial isolate 111 (p-value = 0.0180) (Table 11). There was also a significant difference between maize genotypes due to bacterial isolate 4589 (p-value = 0.0204) and similarly bacterial isolate 730 (p-value < 0.0001). Interestingly, there was no difference among the maize genotypes within the uninoculated low N control (p-value = 0.1146), suggesting that different maize genotypes grown without the presence of bacterial isolates behave similarly in low N conditions.

Isolate	Num DF	F Value	P-value
111	2	4.24	0.018
4589	2	4.1	0.0204
730	2	21.06	< 0.0001
Low N Control	2	2.23	0.1146

Table 11: 4-Week Validation Experiment - Significant Differences on Dry Shoot Weight (grams) Measured Between the Three Maize Genotypes within Each Bacterial Isolate

Due to there being a significant interaction effect between the maize genotype and bacterial isolate (Table 9), the simple effects were analyzed (Table 12). This means that the effect of each bacterial isolate on the dry shoot weight of the inoculated plants was analyzed at each specific maize genotype. Bacterial isolate 730 had a significantly greater effect on dry shoot

weight in the Mo17 maize genotype compared to the low N control (p-value = 0.0010), suggesting a positive growth effect of bacterial isolate 730 on Mo17 maize grown in low nitrogen conditions. Interestingly, the impact of bacterial isolate 730 on Mo17 maize shoot dry weight was also significantly greater than the responses for bacterial isolates 111 (p-value = 0.0034) and 4589 (p-value = 0.0011) grown in Mo17 maize plants (Table 12). Conversely, in the maize genotypes Ames 27065 and NSL 30867, bacterial isolate 730 did not have a significant impact on dry shoot weight compared to the low N control (p-value = 0.3907, p-value = 0.1708), or to bacterial isolate 111 (p-value = 0.2359, p-value = 0.1281) and bacterial isolate 4589 (p-value = 0.1212, p-value = 0.0984) (Figure 15; Figure 16). These results suggest that bacterial isolate 730 has a significant growth effect on the Mo17 maize genotype when grown for 4 weeks in low nitrogen conditions.

When looking into the response of each maize genotype on the dry shoot weight (grams) response at 28 days of growth, the three maize genotypes, Mo17, Ames 27065, and NSL 30867 grown under low nitrogen without bacterial inoculation (low N control), did not differ significantly in their dry shoot weight response (Figure 17). When comparing the maize genotypes inoculated with bacterial isolate 111 and their differences in dry shoot weight at 28 days of growth, the Mo17 maize genotype had a significantly higher dry shoot weight compared to the Ames 27065 maize genotype (Figure 18). Upon investigating the three maize genotypes inoculated with bacterial isolate 730, the Mo17 maize genotype had a significantly greater dry shoot weight compared to the Ames 27065 and NSL 30867 maize genotypes (Figure 19). Between the three maize genotypes inoculated with bacterial isolate 4589, both maize genotypes Mo17 and NSL 30867 had significantly greater dry shoot weight compared to Ames 27065 (Figure 20).

Simple Effect Level	Bacterial Isolate	Bacterial Isolate	Estimate	P-value
Ames 27065	111	4589	0.03792	0.6889
Ames 27065	111	730	-0.1218	0.2359
Ames 27065	111	Low N Control	-0.0311	0.7511
Ames 27065	4589	730	-0.1597	0.1212
Ames 27065	4589	Low N Control	-0.06902	0.482
Ames 27065	730	Low N Control	0.09065	0.3907
Mo17	111	4589	0.03355	0.7232
Mo17	111	730	-0.2846	0.0034
Mo17	111	Low N Control	0.03876	0.6825
Mo17	4589	730	-0.3181	0.0011
Mo17	4589	Low N Control	0.005213	0.9561
Mo17	730	Low N Control	0.3234	0.001
NSL 30867	111	4589	-0.01234	0.8963
NSL 30867	111	730	0.141	0.1281
NSL 30867	111	Low N Control	0.01805	0.8445
NSL 30867	4589	730	0.1534	0.0984
NSL 30867	4589	Low N Control	0.03039	0.7413
NSL 30867	730	Low N Control	-0.123	0.1708

Table 12: 4-Week Validation Experiment - Simple Effect Comparisons Between the Bacterial Isolates and Low N Control at Each Maize Genotype Level

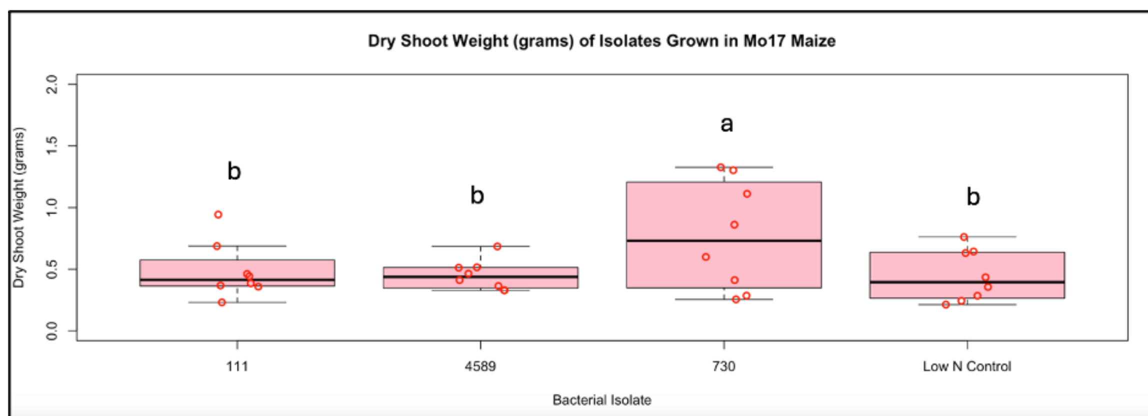

Figure 14: 4-Week Validation Experiment - Dry shoot weight (grams) of the Mo17 maize genotype inoculated with bacterial isolates and the low N control. (Different letters indicate a significant difference $p < 0.05$).

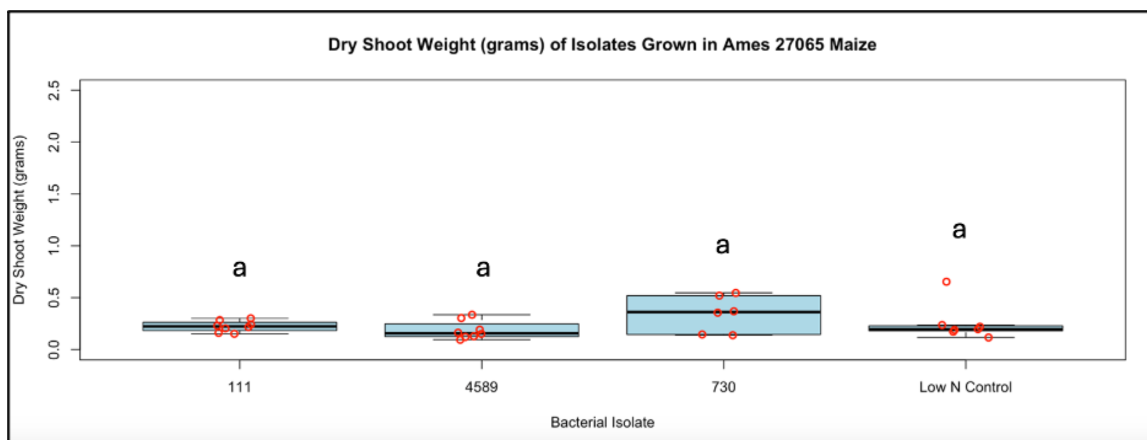

Figure 15: 4-Week Validation Experiment - Dry shoot weight (grams) of the Ames 27065 maize genotype inoculated with bacterial isolates and the low N control. (Different letters indicate a significant difference $p < 0.05$).

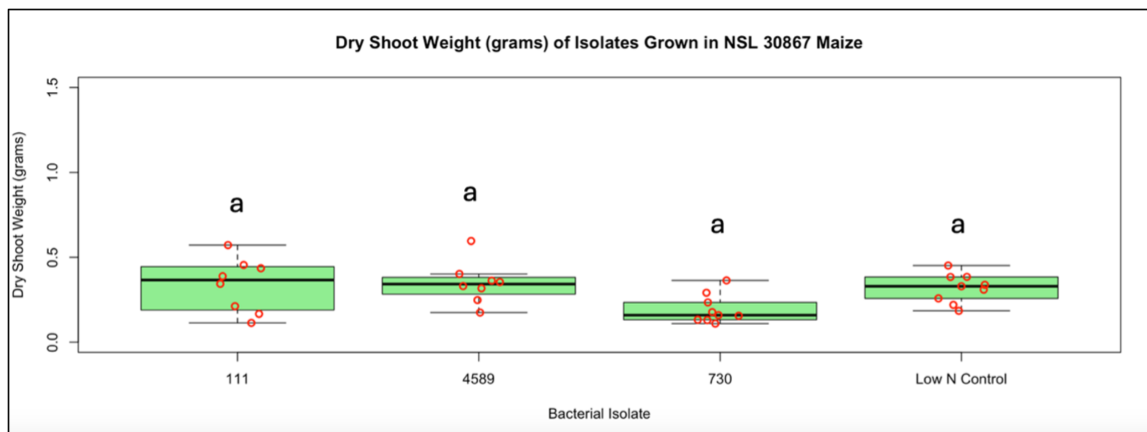

Figure 16: 4-Week Validation Experiment - Dry shoot weight (grams) of the NSL 30867 maize genotype inoculated with bacterial isolates and the low N control. (Different letters indicate a significant difference $p < 0.05$).

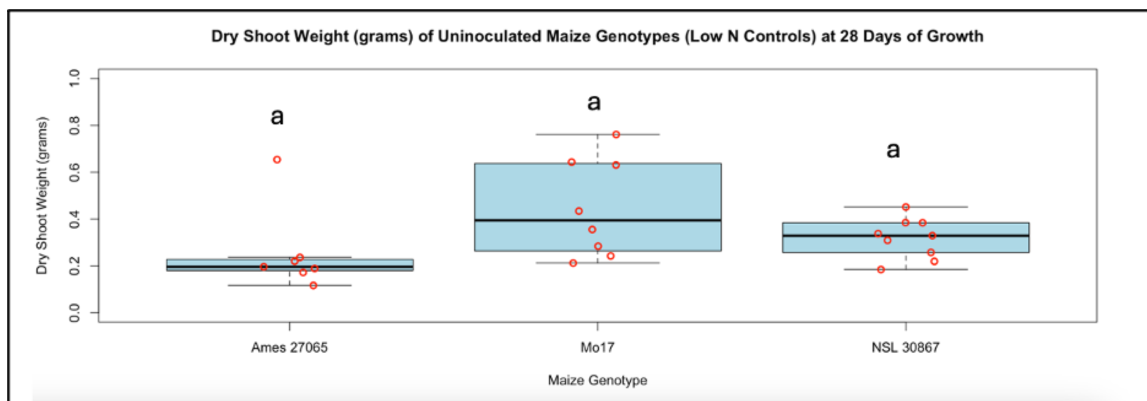

Figure 17: 4-Week Validation Experiment - Dry shoot weight (grams) of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and uninoculated conditions (low N control). (Different letters indicate a significant difference $p < 0.05$).

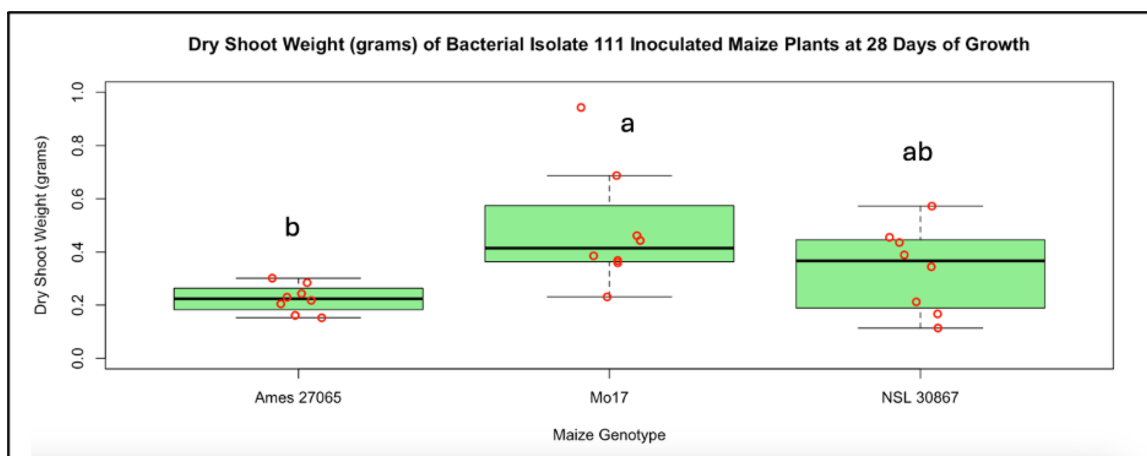

Figure 18: 4-Week Validation Experiment - Dry shoot weight (grams) of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 111. (Different letters indicate a significant difference $p < 0.05$).

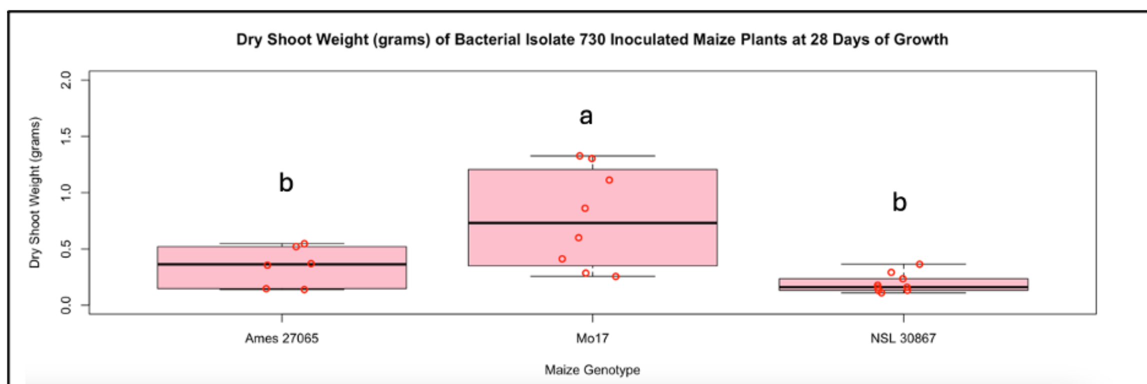

Figure 19: 4-Week Validation Experiment - Dry shoot weight (grams) of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 730. (Different letters indicate a significant difference $p < 0.05$).

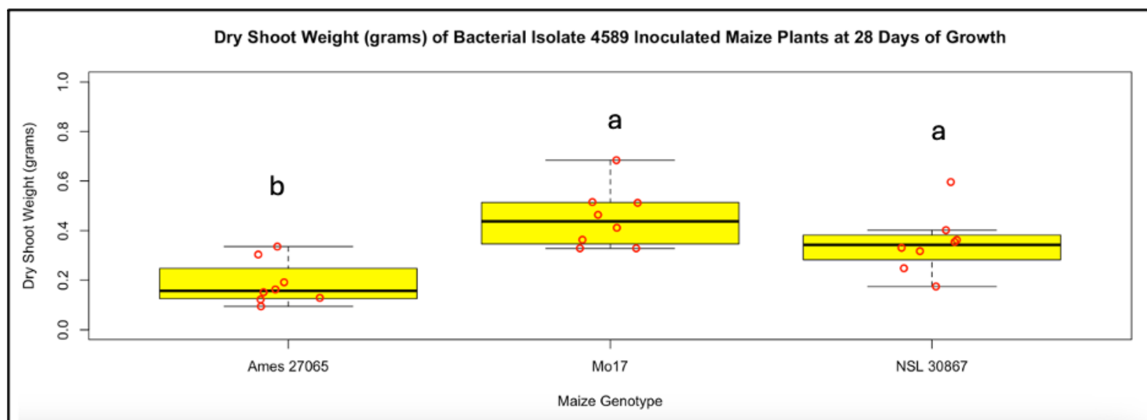

Figure 20: 4-Week Validation Experiment - Dry shoot weight (grams) of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 4589. (Different letters indicate a significant difference $p < 0.05$).

6.5.3 Phenotyping of above ground biomass of maize plants during 4 week experiment

Over the course of the four-week validation experiment, shoot biomass measurements of the plants at the 2-week, 3-week, and 4-week time points were taken through a phenotyping system and analyzed in R. The purpose of this was to capture the dynamics of shoot biomass of the plants inoculated with isolates, 111, 730, and 4589, to determine if significant increases in biomass of the plants compared to the low N control plant, could be detected at 14-, 21-, and 27-days of growth. This was done to augment the destructive harvest data collected at 28 days.

Correlation Analysis To test the accuracy of the biomass measured from the phenotyping system, a correlation was done to access the relationship between the measured dry shoot weight (grams) to the predicted biomass results determined from the imaging system. This correlation was done at the end of the 4-week growth experiment. The correlation value was $r = 0.94$, indicating that the biomass measurements taken from the phenotyping system accurately represented the dry shoot weight measurements taken at the end of the 4-week experiment (Figure 21).

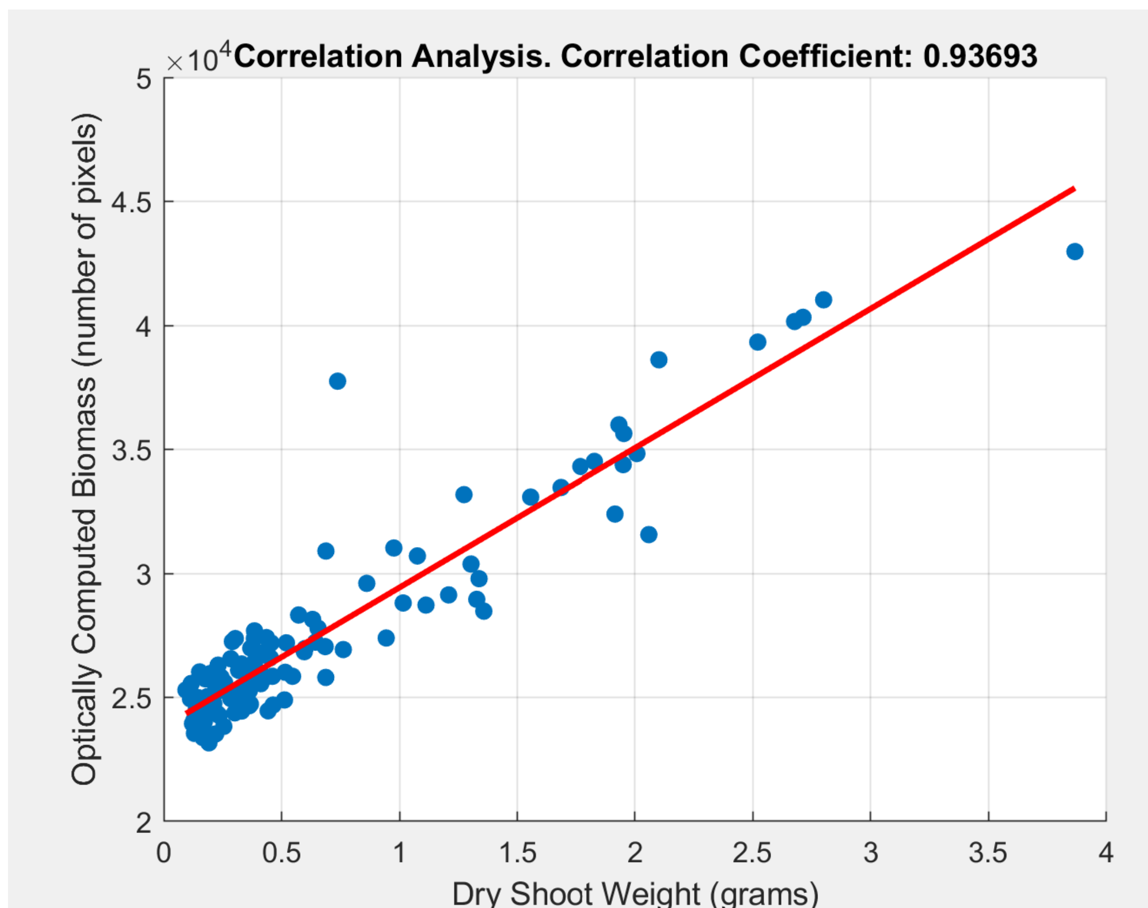

Figure 21: Correlation analysis between the sampled dry shoot weights of the 28-day old plants and the shoot biomass images at 27-days of growth. r = 0.93693

Two-Week Phenotyping Shoot Biomass Measurement Results At 14 days of growth, the plants were imaged and their shoot biomass measurements were predicted to investigate the effects of the 111, 730, and 4589 bacterial inoculations on the above ground growth of the nitrogen-deficient plants (Figure 22). The effect of each of the bacterial inoculations on each of the maize genotypes, Mo17, Ames 27065, and NSL 30867, was also analyzed. When looking into the effect of each individual factor on the shoot biomass (Table 13), the bacterial isolate alone did not have a significant effect on the shoot biomass (p-value = 0.2378). Maize genotype did have a significant effect on the shoot biomass (p-value =

0.0002), indicating that the maize genotype led to differences in above ground growth of the nitrogen-deficient maize at two weeks of growth. The bacterial isolate factor and the genotype did not have a significant interaction effect on the biomass results (p-value = 0.0789). The p-value is still close to significant, so the differences between the dry shoot weight of plants inoculated with each bacterial isolate and the low N control were analyzed at each level of maize genotype. The block did have a significant effect on the plant shoot biomass (p-value = 0.0417), possibly indicating that the area the plants were placed in the growth chamber influenced their growth, possibly due to uneven lighting in the growth chamber.

The maize genotype factor alone had a significant effect on the shoot biomass of the nitrogen-deficient plants at 14 days of growth (p-value = 0.0002), indicating that the different maize genotypes react differently in their response to low nitrogen conditions already at 14 days old. When looking into each of the maize genotypes (Table 14), Mo17 had a significantly greater shoot biomass than Ames 27065 (p-value $<$ 0.0001). In addition, Mo17 had a significantly greater shoot biomass than NSL 30867 (p-value = 0.0292). Interestingly, the NSL 30867 maize genotype had a significantly greater shoot biomass than Ames 27065 (p-value = 0.0207). When investigating the differences of shoot biomass between the three maize genotypes under each bacterial isolate treatment and low N control, none of the maize genotypes were significantly different from one another under the low N control treatment (Figure 26) at 14 days of growth. Likewise, when inoculated with bacterial isolate 111, none of the maize genotypes differed significantly in their influence on the shoot biomass (Figure 27). Maize genotypes inoculated with bacterial isolate 730 did not significantly differ in their shoot biomass response at 14 days of growth (Figure 28). Interestingly, at 14 days of growth, the Ames 27065 maize genotype had a significantly smaller shoot biomass compared to the other maize genotypes when each were inoculated with bacterial isolate 4589 (Figure 29).

The interaction effect between the factor maize genotype and the factor bacterial isolate

was close to significant (p-value = 0.0789), therefore, each of the dry shoot weights of plants inoculated with different bacterial isolates and the low N control were analyzed at each maize genotype level to see how the bacterial isolate influenced the shoot biomass at 14 days of growth in each maize genotype (Table 15). At 14 days of growth, there were no significant differences among the shoot biomass responses between inoculated plants and the low N control in the Mo17 maize genotype (Table 23). At 14 days of growth, Ames 27065 plants inoculated with bacterial isolate 111 had a significantly greater shoot biomass compared to Ames 27065 plants inoculated with bacterial isolate 4589 (p-value = 0.0109). In addition, Ames 27065 plants inoculated with bacterial isolate 4589 had a significant decrease in shoot biomass compared to the low N control Ames 27065 plant (p-value = 0.0119). Similar to the Mo17 maize genotype, the shoot biomass response in the NSL 30867 maize genotype was not significantly different among the different bacterial isolate inoculations and the low N control (Figure 29).

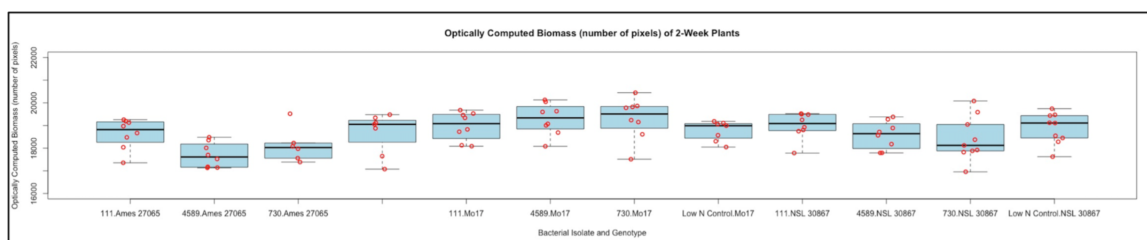

Figure 22: 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the three inoculated maize genotypes with the bacterial isolates 111, 730, and 4589, and the low N control.

Effect	Num DF	P-value
Isolate	3	0.2378
Genotype	2	0.0002
Isolate*Genotype	6	0.0789
Block	8	0.0417

Table 13: 2-Week Biomass Measurements - Linear Mixed Model Analysis for the Dry Shoot Weight Response of Each Factor

Maize Genotype	Maize Genotype	Estimate	Standard Error	P-value
Ames 27065	Mo17	-801.81	180.81	<0.0001
Ames 27065	NSL 30867	-419.27	177.31	0.0207
Mo17	NSL 30867	382.53	171.97	0.0292

Table 14: 2-Week Biomass Measurements - The Significant Differences on Dry Shoot Weight (grams) Between the Three Maize Genotypes

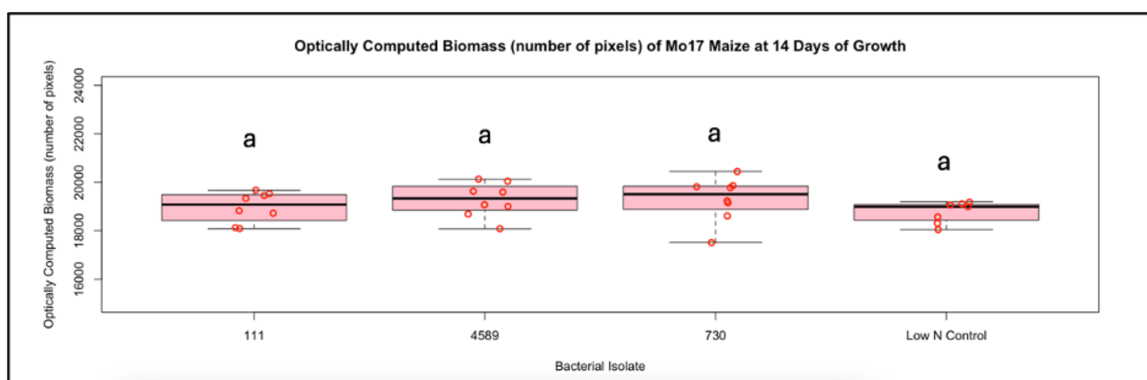

Figure 23: 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Mo17 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control.

Maize Genotype Level	Bacterial Isolate	Bacterial Isolate	Estimate	P-value
Ames 27065	111	4589	945.06	0.0109
Ames 27065	111	730	511.20	0.1954
Ames 27065	111	Low N Control	-20.6004	0.9564
Ames 27065	4589	730	-433.86	0.2712
Ames 27065	4589	Low N Control	-965.66	0.0119
Ames 27065	730	Low N Control	-531.8	0.1911
Mo17	111	4589	-310.98	0.3935
Mo17	111	730	-331.5	0.3635
Mo17	111	Low N Control	217.87	0.5631
Mo17	4589	730	-20.5156	0.9550
Mo17	4589	Low N Control	528.86	0.1626
Mo17	730	Low N Control	549.38	0.1471
NSL 30867	111	4589	572.17	0.1184
NSL 30867	111	730	580.41	0.1033
NSL 30867	111	Low N Control	137.33	0.6977
NSL 30867	4589	730	8.2413	0.9814
NSL 30867	4589	Low N Control	-434.84	0.2207
NSL 30867	730	Low N Control	-443.08	0.1985

Table 15: 2-Week Biomass Measurements - Simple Effect Comparisons Between the Bacterial Isolates and Low N Control at Each Maize Genotype Level

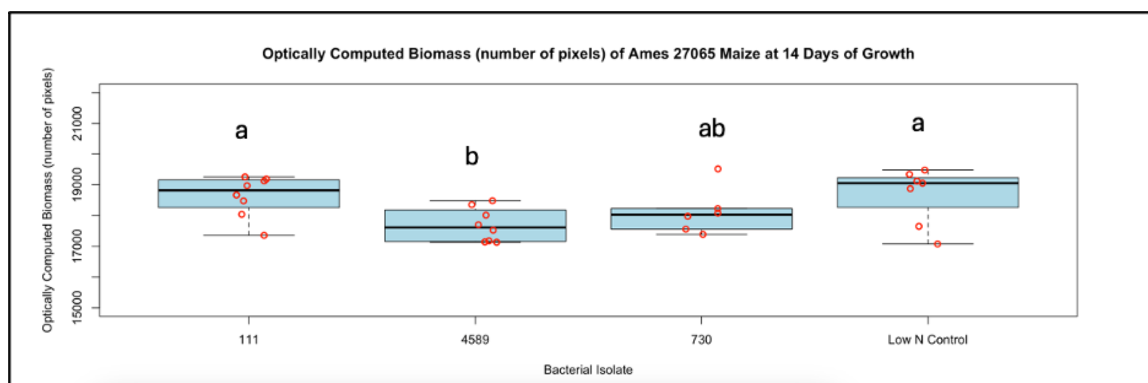

Figure 24: 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Ames 27065 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control.

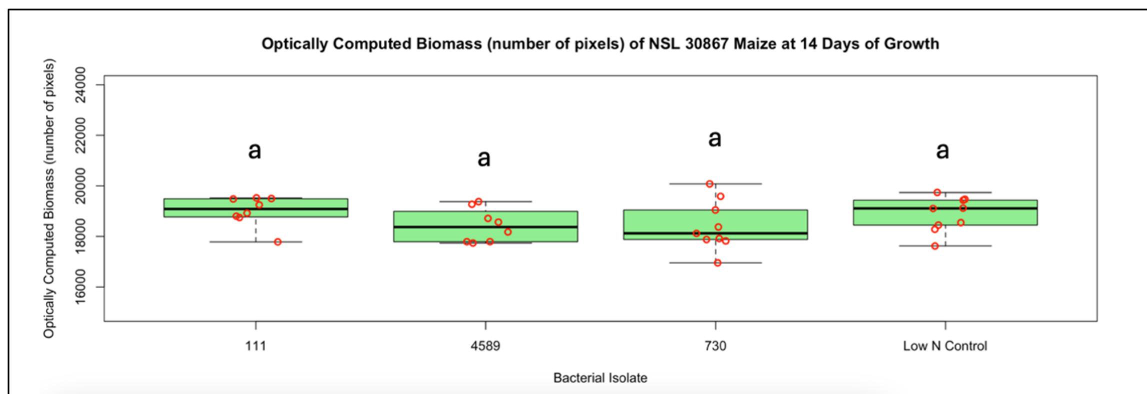

Figure 25: 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the NSL 30867 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control

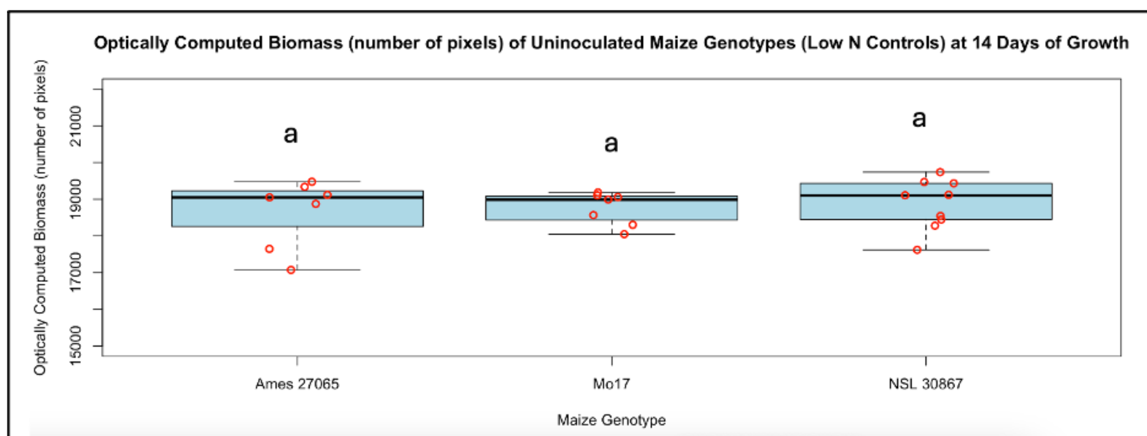

Figure 26: 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and uninoculated conditions (low N control). (Different letters indicate a significant difference $p < 0.05$).

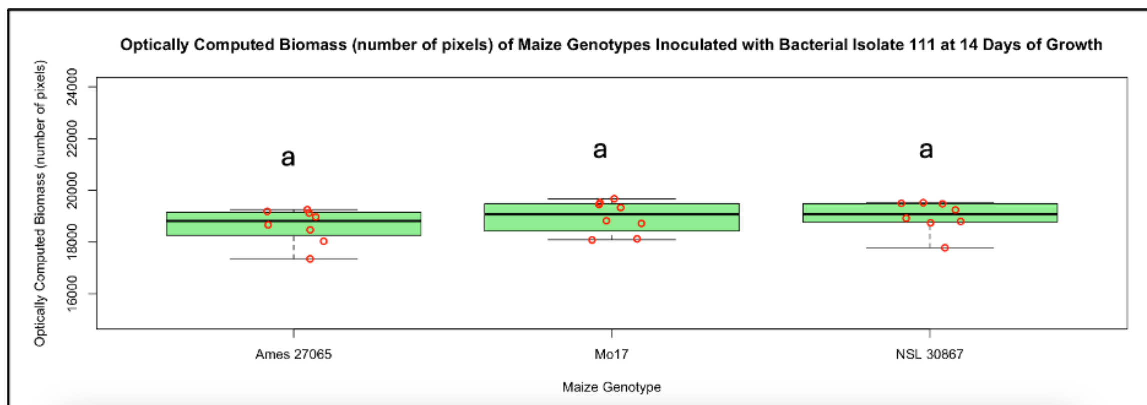

Figure 27: 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 111. (Different letters indicate a significant difference $p < 0.05$).

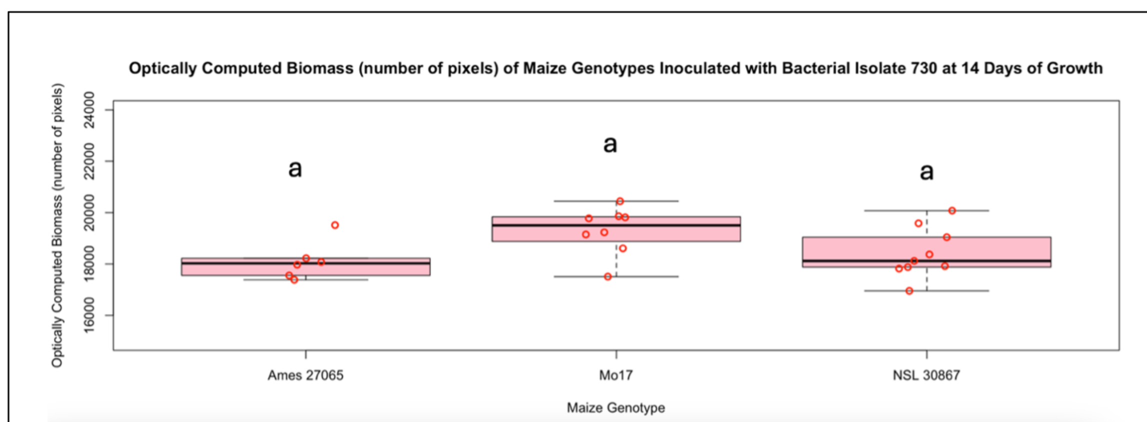

Figure 28: 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 730. (Different letters indicate a significant difference $p < 0.05$).

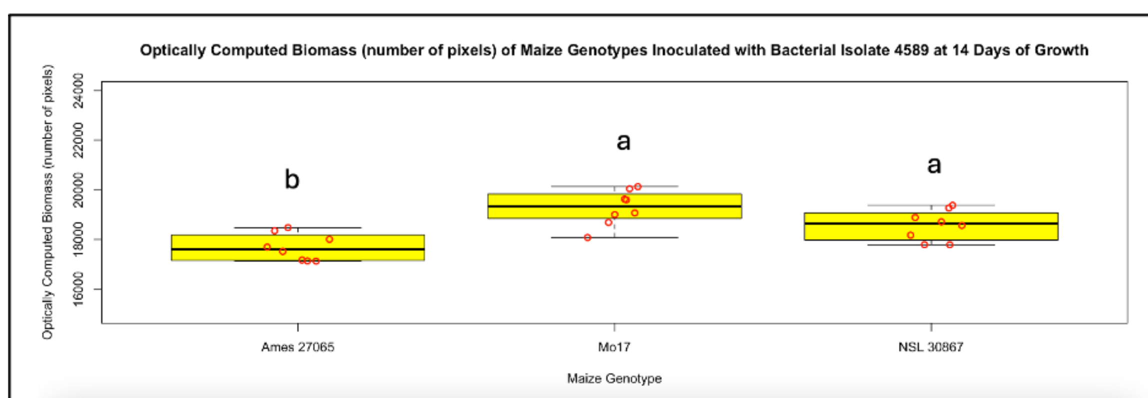

Figure 29: 2-Week Biomass Measurements - Shoot biomass measurements at 14 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 4589. (Different letters indicate a significant difference $p < 0.05$).

Three-Week Phenotyping Shoot Biomass Measurement Results At 21 days of growth, the plants were imaged and their shoot biomass measurements were predicted to investigate the effects of the 111, 730, and 4589 bacterial inoculations on the above ground growth of the nitrogen-deficient plants. The effect of each of the bacterial inoculations on each of the maize genotypes, Mo17, Ames 27065, and NSL 30867, were also analyzed in this analysis (Figure 30). When investigating the effect of each of the factors on the shoot biomass at 21-days of growth (Table 16), the bacterial isolate factor alone did not have a significant effect on the shoot biomass (p-value = 0.4678). Similar to the 2-week results, the maize genotype factor had a significant effect on the shoot biomass (p-value $<$ 0.0001), suggesting the three different maize genotypes were reacting differently to the nitrogen-deficient conditions at 21 days of growth. Interesting, after 21-days of growth, the Isolate x Genotype interaction effect was significant (p-value = 0.0015), suggesting that at this time point, specific bacterial isolates had an influence on the growth of specific maize genotypes.

The maize genotype factor alone had a significant effect on the shoot biomass of the nitrogen-deficient plants at 21 days of growth (p-value $<$ 0.0001), indicating that the different maize genotypes react differently in their response to low nitrogen conditions. When looking into each of the maize genotypes (Table 17), Mo17 had a significantly greater shoot biomass than Ames 27065 (p-value $<$ 0.0001). In addition, Mo17 had a significantly greater shoot biomass than NSL 30867 (p-value $<$ 0.0001). Interestingly, at 21 days of growth, the NSL 30867 and Ames 27065 maize genotypes did not significantly differ from one another in shoot biomass (p-value = 0.3568), indicating that at this time point, the two maize genotypes reacted similarly to low nitrogen conditions. When investigating the different shoot biomass responses between the three maize genotypes at 21 days of growth under the inoculation of the bacterial isolates and the low N control, the maize genotype Ames 27065 had a significantly smaller shoot biomass than the Mo17 maize genotype when grown under low N conditions (Figure 34). When each maize genotype was inoculated with bacterial

isolate 111, none of the maize genotypes significantly differed from one another in their biomass response (Figure 35). Under the inoculation treatment of bacterial isolate 730, the Mo17 maize genotype had a significant increase in shoot biomass compared to both the NSL 30867 maize genotype and the Ames 27065 maize genotype (Figure 36). Similarly, under the inoculation treatment of bacterial isolate 4589, the Mo17 maize genotype had a significant increase in shoot biomass compared to the other two maize genotypes (Figure 37).

Due to the significant interaction effect between maize genotype and bacterial isolate at 21 days of growth, the effects on shoot biomass of each of the bacterial isolates and the low N control were analyzed at each maize genotype level (Table 18). In the maize genotype Mo17, bacterial isolate 730 had a significant effect on the shoot biomass compared to the low N control (p-value = 0.0008). Additionally, at 21 days of growth, bacterial isolate 730 had a significant growth effect on shoot biomass compared to bacterial isolate 111 (p-value = 0.0044) and compared to bacterial isolate 4589 (p-value = 0.0061). In contrast to maize genotype Mo17, bacterial isolate 730 significantly decreased the growth of the maize genotype NSL 30867 compared to bacterial isolate 111 (p-value = 0.0067) and to the low N control (p-value = 0.0268). These results point to the importance of making sure to analyze the effects of a plant growth promoting bacteria in different plant genotypes, as they may react differently.

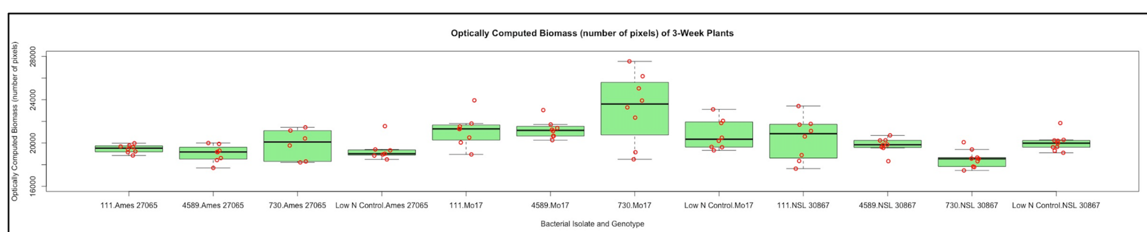

Figure 30: 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the three inoculated maize genotypes with the bacterial isolates 111, 730, and 4589, and the low N control.

Effect	Num DF	P-value
Isolate	3	0.4678
Genotype	2	<0.0001
Isolate*Genotype	6	0.0015
Block	8	0.1539

Table 16: 3-Week Biomass Measurements - Linear Mixed Model Analysis for the Dry Shoot Weight Response of Each Factor

Maize Genotype	Maize Genotype	Estimate	P-value
Ames 27065	Mo17	-2245.64	<0.0001
Ames 27065	NSL 30867	-330.11	0.3568
Mo17	NSL 30867	1915.53	<0.0001

Table 17: 3-Week Biomass Measurements - The Significant Differences on Dry Shoot Weight (grams) Between the Three Maize Genotypes

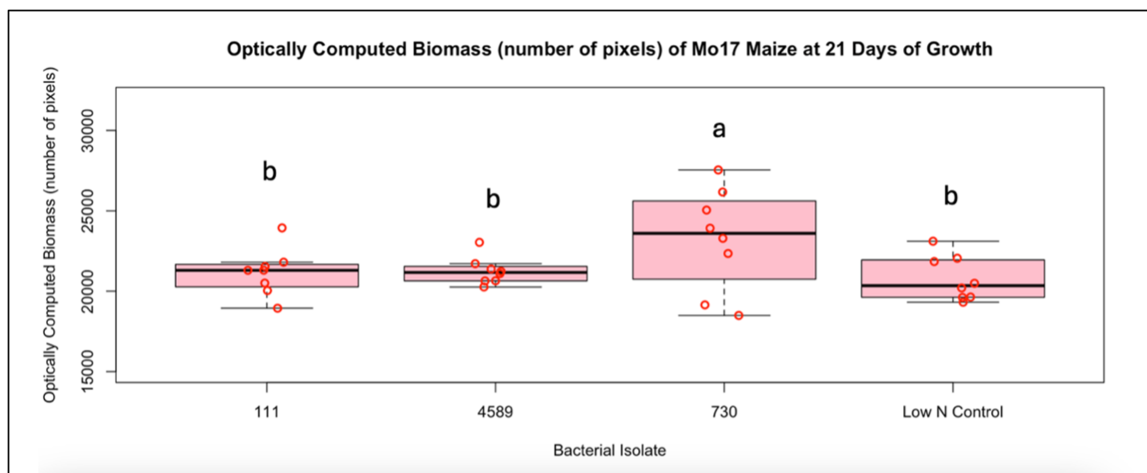

Figure 31: 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Mo17 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control (Different letters indicate a significant difference $p < 0.05$).

Maize Genotype Level	Bacterial Isolate	Bacterial Isolate	Estimate	P-value
Ames 27065	111	4589	428.28	0.5473
Ames 27065	111	730	-418.10	0.5864
Ames 27065	111	Low N Control	99.0915	0.8929
Ames 27065	4589	730	-846.38	0.2721
Ames 27065	4589	Low N Control	-329.19	0.6548
Ames 27065	730	Low N Control	517.19	0.5138
Mo17	111	4589	-80.2188	0.9102
Mo17	111	730	-2073.3	0.0044
Mo17	111	Low N Control	389.14	0.5844
Mo17	4589	730	-1993.08	0.0061
Mo17	4589	Low N Control	469.36	0.5096
Mo17	730	Low N Control	2462.44	0.0008
NSL 30867	111	4589	637.69	0.3709
NSL 30867	111	730	1917	0.0067
NSL 30867	111	Low N Control	410.44	0.5529
NSL 30867	4589	730	1279.31	0.0668
NSL 30867	4589	Low N Control	-227.24	0.7423
NSL 30867	730	Low N Control	-1506.56	0.0268

Table 18: 3-Week Biomass Measurements - Simple Effect Comparisons Between the Bacterial Isolates and Low N Control at Each Maize Genotype Level

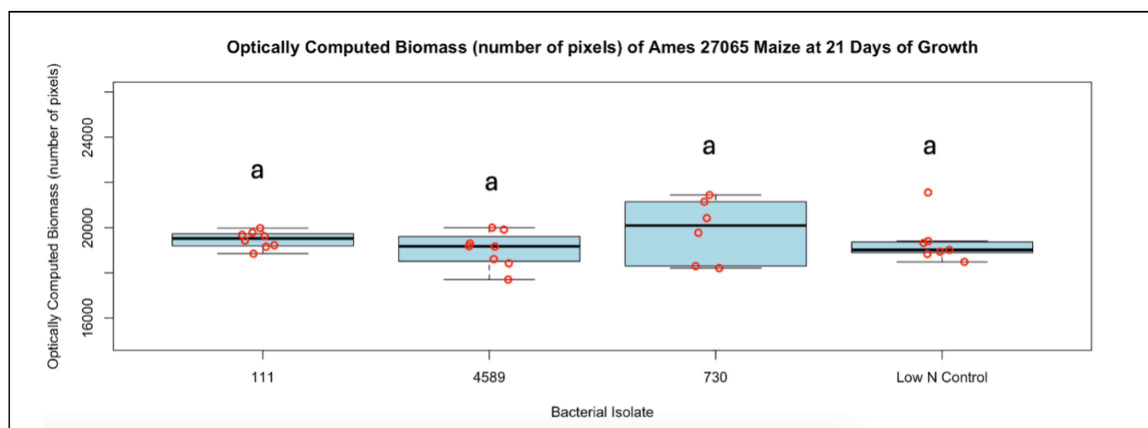

Figure 32: 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Ames 27065 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control.

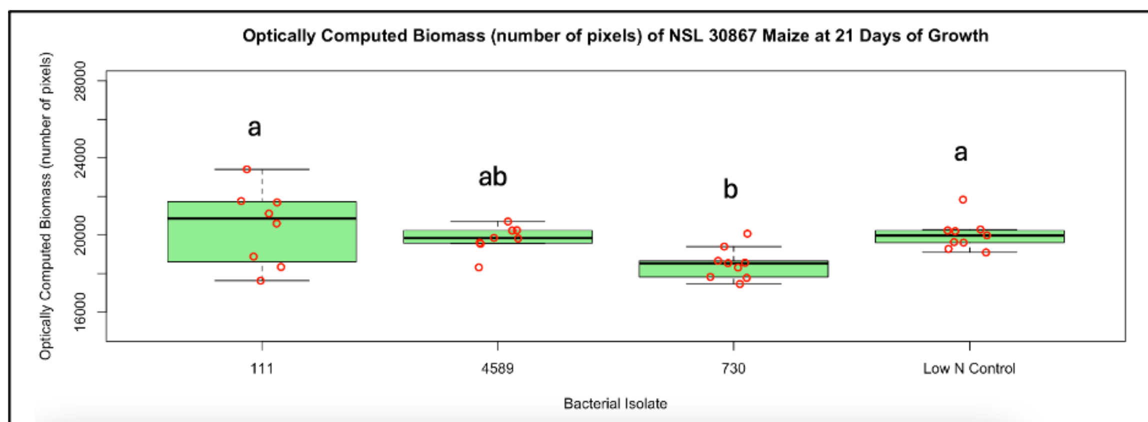

Figure 33: 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the NSL 30867 maize genotype inoculated with the bacterial isolates 111, 730, and 4589, and the low N control (Different letters indicate a significant difference $p < 0.05$).

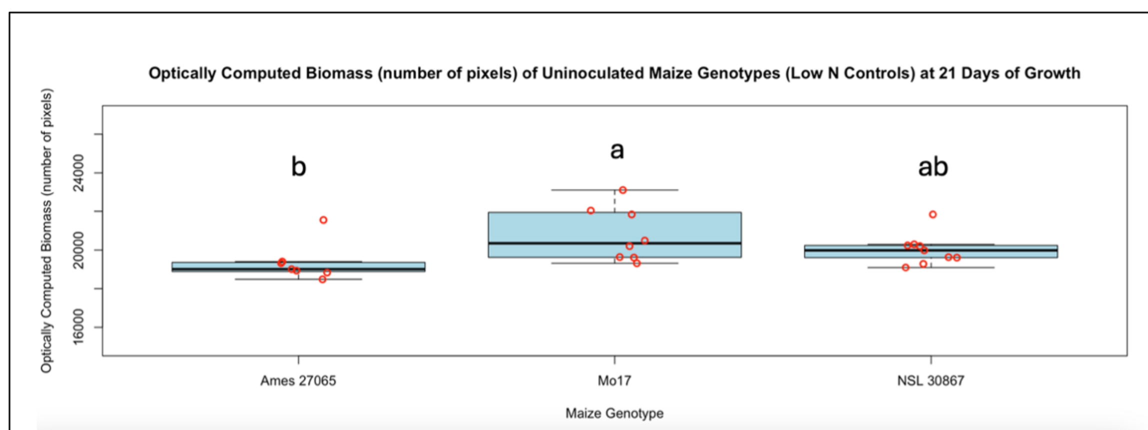

Figure 34: 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and uninoculated conditions (low N control). (Different letters indicate a significant difference $p < 0.05$).

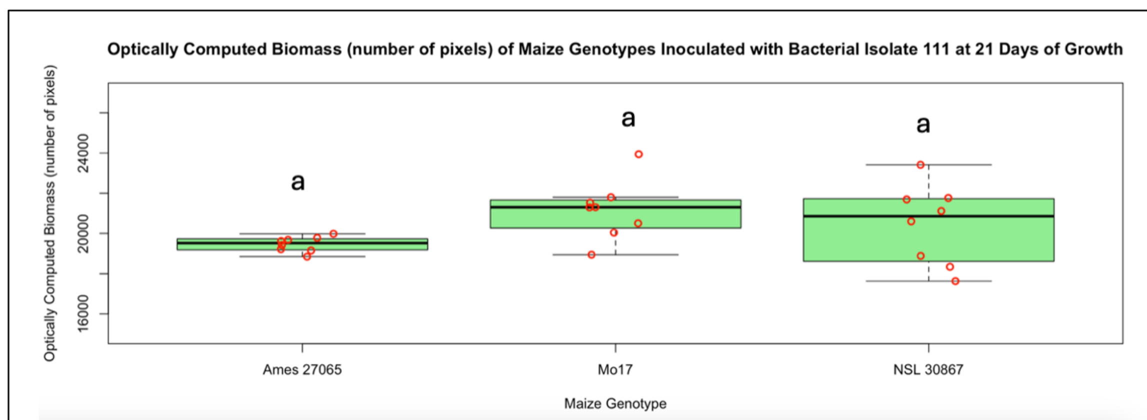

Figure 35: 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 111. (Different letters indicate a significant difference $p < 0.05$).

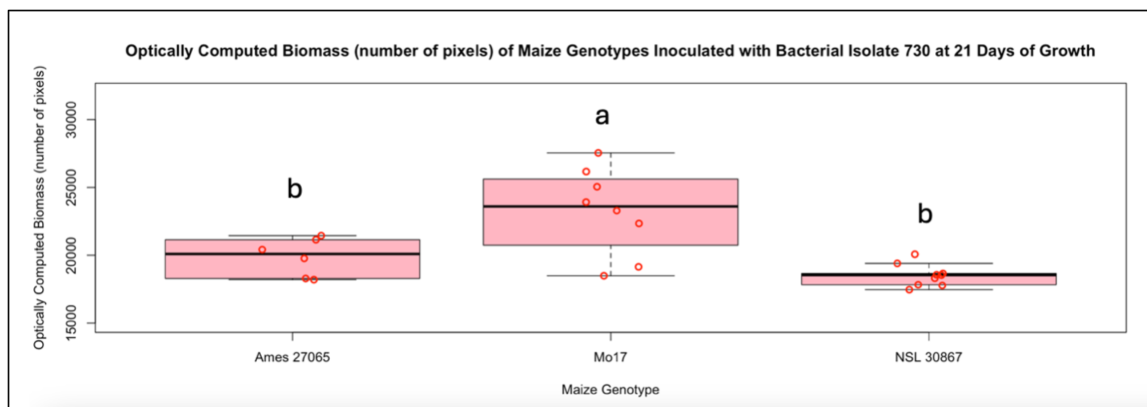

Figure 36: 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 730. (Different letters indicate a significant difference $p < 0.05$).

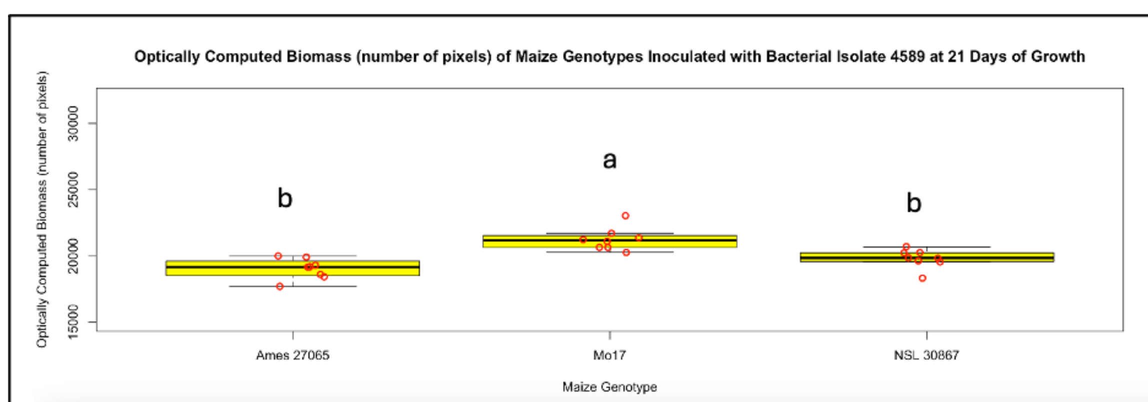

Figure 37: 3-Week Biomass Measurements - Shoot biomass measurements at 21 days of growth of the Mo17, Ames 27065, and NSL 30867 maize genotypes grown under low nitrogen and inoculated with bacterial isolate 4589. (Different letters indicate a significant difference $p < 0.05$).

6.6 Bacterial Genome Analysis of Bacterial Isolates 111 and 730

The following metrics were reported from *Plasmidsaurus* for the whole-genome sequencing, assembly, and annotation of bacterial isolate 111 (*Arthrobacter* sp.) (Figure 38) and bacterial isolate 730 (*Pseudomonas kribbensis*) (Figure 39).

When analyzing the bacterial genome of bacterial isolate 111, a 516-base pair *NifU*-like protein was identified in the first contig of the bacterial isolate at 4,500,855 – 4,501,370 base pairs in the genome, however, no other *nif*-related genes were identified in the bacterial genome of bacterial isolate 111. The absence of the other *nif*-related genes suggests that bacterial isolate 111 does not fix nitrogen via the nitrogenase enzyme. When analyzing the bacterial genome of bacterial isolate 730, no *nif*-related genes were identified, indicating that bacterial isolate 730 does not have nitrogen-fixing capabilities.

To investigate the potential of bacterial isolates 111 and 730 in producing indole-3-acetic acid (IAA) to help promote the growth of the plant in nitrogen deficient maize, essential genes in the indole-3-acetic pathways were searched for in their annotated bacterial genomes. Enzymes in the indole-3-acetamide (IAM) pathway include the enzyme tryptophan-2-monooxygenase (*iaaM* gene) and the IAM hydrolase (*iaaH* gene). In both annotated bacterial genomes for bacterial isolates 111 and 730, the *iaaM* and *iaaH* genes were not present. Enzymes in the IPA pathway include tryptophan transferase, indole-3-pyruvate carboxylase, and indole-3-acetalydehyde oxidase. Upon searching for these enzymes in the annotated genomes, none of them were found in either bacterial isolate 111 or 730. Enzymes in the tryptophan side-chain oxidase (TSO) pathway, including tryptophan side-chain oxidase and indole-3-acetaldehyde dehydrogenase, were not present in either bacterial isolate 111 or 730. Of the enzymes in the tryptamine (TAM) pathway, an amine oxidase and aldehyde dehydrogenase were both present in bacterial isolates 111 and 730. However, the third enzyme, tryptophan decar-

boxylase, was not present in either bacterial genome. The absence of a whole set of essential enzymes for any of the IAA-producing pathways in bacteria suggests that IAA-production is not the plant-growth promoting capability offered to nitrogen-deficient maize plants by either bacterial isolate 111 or 730.

Taxomonic Identification	Phylum	*Actinobacteria*
	Class	*Actinobacteria*
	Order	*Micrococcales*
	Family	*Micrococcaceae*
	Genus	*Arthrobacter*
	Species	*sulfonivorans* strain Ar51 plasmid
Relevant Statistics for the Sequencing Run	Number of Contigs	2 contigs
	Size of Contig 1	4,853,877 bp
	Size of Contig 2	152,808 bp
	N50 (bp)	4,853,877 bp
	Genome size (Mb)	5.0 Mb
	Number of Genes Annotated	4,753 genes
	GC Content	63.10%
	Assembly Completion (%)	99.6% complete
	Assembly Contamination (%)	0.6% contaminated

Figure 38: Genome Sequencing Metrics for Bacterial Isolate 111

Taxonomic Resolution	Phylum	*Proteobacteria*
	Class	*Gammaproteobacteria*
	Order	*Pseudomonadales*
	Family	*Pseudomonadaceae*
	Genus	*Pseudomonas*
	Species	sp. DR 5-09
Relevant Statistics for the Sequencing Run	Number of Contigs	1 contig
	Size of Contig 1	6,305,343 bp
	N50 (bp)	6,305,343 bp
	Genome size (Mb)	6.3 Mb
	Number of Genes Annotated	5,816 genes
	GC Content (%)	60.60%
	Assembly Completion (%)	99.93% complete
	Assembly Contamination (%)	0.05% contaminated

Figure 39: Genome Sequencing Metrics for Bacterial Isolate 730

7 Discussion

A set of 64 potentially beneficial bacterial isolates were sampled from the rhizospheres of maize genotypes grown under nitrogen-deficient conditions (Meier et al. 2022). The reason why these bacterial isolates were selected by the maize genotypes was not explored in Meier et al. 2022, therefore, the beginning of this work started with investigating whether the selected bacterial isolates offered a positive effect on plant traits of maize grown in nitrogen-deficient conditions. Out of the 64 potentially beneficial bacterial isolates, two bacterial isolates appeared to offer a significant plant-growth promoting effect on Mo17 maize plants grown in nitrogen-deficient conditions. The two bacterial isolates were bacterial isolate 111 (*Arthrobacter* sp.) and bacterial isolate 730 (*Pseudomonas kribbensis*). In the two-week validation experiment, nitrogen-deficient Mo17 maize plants inoculated with bacterial isolate

111 had an increase in their dry shoot weight (grams) compared to the low N control plants. In the four-week validation experiment, nitrogen-deficient Mo17 maize plants inoculated with bacterial isolate 730 had an increase in their dry shoot weight (grams) compared to the low N control plants. These results contrasted with the results from the initial assay of the 64 bacterial isolates, where none of the 64 tested bacterial isolates offered a significant effect on the dry shoot weight of nitrogen-deficient plants compared to uninoculated low nitrogen control plants.

A possible explanation for not observing any significant effect by the bacterial isolates in the initial assay is that only 3-8 replicates (depending on the initial assay round) were used for each bacterial isolate. This small number of replicates per bacterial isolate was carried out to test the 64 bacterial isolates quickly. The low number of replicates resulted in a low statistical power, meaning that there may have been a reduced ability to detect a significant effect on dry shoot weight if there had been one. Another possible explanation for this result of not observing a significant growth effect from any of the potentially beneficial bacterial isolates is that the dry shoot weight of the plants was measured after only 14 days of growth. Similarly, in a study investigating the effects of *Bacillus* PGPR-inoculations on maize growth at different vegetative stages, beneficial PGPR-growth effects on traits such as plant height, stem diameter, morphology, and leaf area were observed at the V6 growth stage, while at the V4 growth stage, few beneficial growth effects were detected, compared to uninoculated plants (Lin et al. 2019). In the initial assay of the 64 bacterial isolates, without the presence of a potentially beneficial bacterial inoculation, statistically significant increases in dry shoot weight were sometimes not observed between plants grown in high nitrogen conditions and nitrogen-deficient conditions, suggesting that either 1) more than 14 days of growth may be required to see significant symptoms between a nitrogen-sufficient and nitrogen-deficient plant or 2) the statistical power was too low to detect a significant difference.

Another reason that could possibly explain the nonsignificant results of each of the bacterial isolates in the initial assay is that these bacterial isolates were not recruited one-by-one to the rhizospheres of nitrogen deficient maize (Meier et al. 2022), but instead, were recruited as a rhizospheric bacterial community. Plants release exudates into the soil around their roots to influence the rhizospheric bacterial community members (Bertin et al. 2003). From there, recruited bacterial members interact with one another through cooperation or competition, influencing the overall health of the plant (Chepsergon et al. 2023). Although the 64 bacterial isolates were recruited to nitrogen-deficient maize rhizospheres as a community, each of the 64 bacterial isolates were tested on their own, possibly eliminating the interaction effects among the bacterial species in the community that ultimately may have potentially led to plant growth-promoting effects in the maize in low nitrogen. Similar results in another study showed that a combination of beneficial bacterial species, *Bacillus velezensis* and *Pseudomonas stutzeri*, had a combined beneficial growth effect on plant traits, compared to plants inoculated with only one species (Sun et al. 2021). This was thought to be because the *B. velezensis* stimulated the *P. stutzeri*, forming biofilms on the plant root surface indicating cooperation between the two inoculated species. Conversely, sometimes PGPR may work better at improving plant health alone, as combined bacterial inoculants may outcompete one another or the single PGP-bacterial species inoculated into the plant may work to recruit its own beneficial communities (Tang et al. 2020). One advantage of the growth system utilized for the initial assay was the fully enclosed environment that the maize plant was able to grow inside, as well as, only having to be given water and Hoagland nutrient solution at the time of planting inside the fume hood. Because of this system, the plants were able to be inoculated with only a single bacterial isolate and there was a low chance of contamination from other bacteria in the growth chamber during the two-week growth period.

As stated above, bacterial isolate 111 had a significant positive effect on the dry shoot weight of the 111-inoculated Mo17 maize plants compared to the uninoculated low N control Mo17 maize plants. Isolate 111 was identified as being in the *Arthrobacter* genus. Other studies have found *Arthrobacter* bacterial species to possess plant growth promoting abilities. An *Arthrobacter nicotinovorans* strain isolated from the rhizosphere of *Panax ginseng* was found to significantly increase the shoot weight of inoculated ginseng plants after 15 days of growth (Jiang et al., 2022). The strain was able to produce indole-3-acetic acid, solubilize phosphate, and fix nitrogen. The significant increase in shoot weight after 15 days of growth in the inoculated ginseng plant coincides with the significant increase in dry shoot weight observed in the bacterial isolate 111 (*Arthrobacter* sp.) inoculated Mo17 maize plants after 14 days. When investigating the bacterial genome of bacterial isolate 111, the presence of a *NifU*-like protein was identified in the genome. The four *nif* genes that are required for nitrogen-fixing bacteria to assemble a functional *NifH*, the gene that encodes the iron protein component of nitrogenase, the enzyme required for nitrogen fixation, are *nifH, nifM, nifU*, and *nifS* (Curatti et al., 2014). The *nifU* and *nifS* genes form an [Fe-S] cluster assembly machinery specialized in synthesizing clusters for nitrogenase component proteins. While the bacterial genome analysis revealed the presence of a *nifU*-like protein in bacterial isolate 111, the other genes required to assemble a functional *NifH* for nitrogen fixation, were not present in the genome, suggesting that the nitrogenase enzyme is not present in bacterial isolate 111 (*Arthrobacter sp.*). No genes related to indole-3-acetic acid production were found in the genome of bacterial isolate 111. The bacterial inoculation concentration amount in the ginseng study was also comparable to the bacterial inoculation concentration used in the 2-week validation experiment. The gingseng seeds were inoculated with 3 x 10^8 CFU/mL of *Arthrobacter* bacteria while the maize plants inoculated in this study were inoculated with 10^9 CFU/mL of *Arthrobacter*. In another inoculation experiment, *Arthrobacter terricola* JH1-1 was used to inoculate rice (*Oryza sativa* L.) plants and plant-growth promoting abilities were observed (Chhetri et al., 2022). After 14 days of

growth, the *Arthrobacter* inoculated plants increased the number and length of the lateral roots, as well as stimulated the root hair elongation. Also, after 14 days of growth, the shoot length of the inoculated plants was significantly greater than the control plant. This *Arthrobacter* species tested positive for nitrogen fixation and IAA production. The results in these previous studies point to a positive plant-growth promoting effect of 14–15-day old plants inoculated with an *Arthrobacter* species.

Contrasting results were found in the four-week validation experiment for bacterial isolate 111, though, as it did not offer a significant growth effect on the dry shoot weight in nitrogen-deficient maize in any of the three maize genotypes, Mo17, Ames 27065, and NSL 30867. Experimental conditions may have influenced this difference in results, such as the amount and method of bacterial inoculation (Chai et al., 2022). In the 2-week validation experiment, the bacterial inoculation was applied once at the time of germination, in the form of the seedling priming technique. For the 4-week validation experiment, the seedling priming was used at the time of germination, along with a soil drench method twice every week over the course of the 28 days of growth. This difference in inoculation method may have influenced a different response in plant growth promoting effects from bacterial isolate 111 between the two experiments.

In the four-week validation experiment, bacterial isolate 730 had a significant growth effect starting in week 3 when inoculated in the Mo17 maize genotype, while it did not have a significant growth effect when inoculated in either the Ames 27065 or NSL 30867 maize genotype. The Ames 27065 and NSL 30867 maize genotypes were selected from (Lopes et al., 2022), in which they were selected due to their differences in maize root exudate concentrations. The Ames 27065 maize genotype exhibited high sugar and high jasmonic acid exudation concentrations, while the NSL 30867 maize genotype released low sugar and jasmonic acid exudation concentrations. The differing plant-growth promoting effects of

bacterial isolate 730 on different maize genotypes coincides with other studies investigating the varying responses of different plant genotypes to bacterial inoculants. In one study investigating the responses of 305 different *Arabidopsis thaliana* accessions to *Azoarcus olearius* DQS-4 bacterial inoculation, researchers found that a large portion of the Arabidopsis accessions were nonresponsive to the bacterial inoculation, while other accessions responded positively to some traits and negatively to others (Plucani do Amaral et al. 2023). Within the 305 Arabidopsis accessions, genome-wide association analysis detected highly significant single nucleotide polymorphisms (SNPs) for the traits of primary root length and root fresh weight, suggesting that these plant traits were influenced by the bacterial inoculation. Similarly, in another study, 20 different genotypes of *Brachypodium distachyon* exhibited varying plant trait responses to the inoculation of two plant-growth promoting bacteria, *Azospirillum brasilense* and *Herbaspirillum seropedicae* (Plucani do Amaral et al. 2016). The varying responses of plant genotypes to being influenced by PGPR-inoculation may be explained by candidate genes and loci in the growth-promoted plants having a response to the inoculated PGPR, allowing the beneficial, or negative, effects of the PGPR influence the plant's growth response. For example, when investigating the genetic variation among 302 natural accessions of *Arabidopsis thaliana* plants, the candidate genes associated with the PGPR-mediated changes in shoot and root growth were genes involved in important plant growth-related processes, such as the photosynthesis-related protein Golden2-like 1 (GLK1) (Wintermans et al. 2016). Similarly, in the above study investigating *Azoarcus olearius* DQS-4 bacterial effects on plant growth in 305 different Arabidopsis accessions, eleven loci were identified to be associated with the response of Arabidopsis root fresh weight to *Azoarcus olearius* DQS-4 (Plucani do Amaral et al. 2023). The unresponsiveness of the Ames 27065 and NSL 30867 maize genotypes to bacterial isolate 730 under nitrogen-deficient conditions may indicate that these genotypes do not have candidate genes or loci associated with the PGP-effects of bacterial isolate 730, while the Mo17 maize genotype does. The growth effect starting at 21 days of growth indicates that the plant growth promoting ef-

fects of bacterial isolate 730 may not start to cause an effect until around the 21st day of growth. This coincides with the results seen in the two-week validation experiment, as no bacterial isolates besides 111, offered a significant growth effect, although they appeared to increase dry shoot weight visually when graphed in box plots. This result suggests that other bacterial isolates in the two-week validation experiment may need to be tested in nitrogen deficient maize for a longer growth period, rather than just 14 days, to observe a significant increase in dry shoot weight compared to an uninoculated nitrogen-deficient plant.

Bacterial isolate 730 (*Pseudomonas kribbensis*) belongs to the *Pseudomonas* bacterial genus. Plant growth-promoting abilities of *Pseudomonas* species have been researched and documented, including phytohormone production, nitrogen fixation, siderophore production, and phosphate solubilization (Panpatte et al. 2016; Oteino et al. 2015). The presence of the nitrogenase enzyme, responsible for biological nitrogen fixation (BNF) in certain bacteria, has been documented in *Pseudomonas* species, such as *Pseudomonas stutzeri* strain A1501, a strain isolated from rice roots (Desnoues et al. 2003). In a study investigating the effects of inoculating *Pseudomonas fluorescens* to wheat (*Triticum aestivum* L.) plants in addition to varying levels of nitrogen fertilizer, it was found that the PGP-effects of the *P. fluorescens* inoculant decreased with increasing nitrogen fertilizer rates (Shaharoona et al. 2008), suggesting the PGP-traits of this bacteria, such as IAA production, may be negatively influenced by increased nitrogen amounts in the soil. When investigating the bacterial genome of bacterial isolate 730, no genes related to nitrogen fixation or indole-3-acetic acid production were discovered. Further analysis and experiments for bacterial isolate 730 are necessary to determine the direct plant-growth promoting capability the bacterial isolate offers to a nitrogen-deficient maize plant.

The 64 potentially beneficial bacterial isolates that were identified from the initial screening were selected based on previous results (Meier et al., 2022). The previous work analyzed

3,009 rhizosphere samples collected from 230 maize genotypes grown in both high nitrogen and low nitrogen conditions. From these 3,009 rhizosphere samples, 3,626 reliable amplicon sequence variants (ASVs) were acquired and clustered to 150 microbial groups that spread across 19 major classes of rhizosphere microbiota. Out of the 150 microbial groups, 37 groups were identified as likely under genome selection by the maize plants. When matching the ASVs to the 16s rRNA gene sequences in the Schachtman Lab Culture Collection, 75 percent of the 37 microbial groups were matched at 95 - 100 percent identity. From this matching of ASVs to 16s rRNA gene sequences, 64 potentially beneficial bacterial isolates were identified and selected for the initial screening. When making assumptions on whether a microbial group is under host genetic control and whether it influences plant fitness, it is important to consider the taxonomic resolution at which you are identifying a microbial group, especially when extending the assumptions across similarly related microbial groups. In Meier et al., 2022, there was not a consistent pattern in levels of heritability across closely related groups of bacteria. Due to this, it is necessary to have a high taxonomic resolution when distinguishing between microbial species, as multiple isolates of a bacterial species may act differently when inoculated into a host plant (Gianluigi et al., 2021). For example, in a study comparing the metabolic pathways and genes between 19 different *Pseudomonas fluorescens* strains isolated from either the endosphere or rhizosphere of Eastern cottonwood (*Populus deltoides*) trees, researchers found significant diversity in the genomic makeup and phenotypes of these bacterial strains, even when they had a 99 percent similarity match between their 16s rRNA genes (Timm et al., 2015). While no gene clusters or phenotypic traits were exclusive to either endospheric or rhizospheric *P. fluorescens* strains in *Populus deltoides*, trends were observed such as the endospheric strains having additional genes and pathways relative to the rhizospheric strains, perhaps allowing the entry of endosphere isolates into the roots as compared to the rhizosphere strains living outside the roots. This research study highlights the amount of genotypic and phenotypic diversity that can be found even at the species level between different bacterial strains. The ASVs from Meier et al. 2022 were matched to the

16s rRNA gene sequences in the Schachtman Lab Culture Collection, with a percent match ranging anywhere between 95-100 percent. Any match below 95 percent was not included when identifying the bacterial isolates that would be used for the initial screening. For each ASV that was matched to the 16s rRNA genes in the Schachtman Lab Culture Collection, 1-5 bacterial isolate IDs were identified and matched to the ASV sequence (Supplementary Table 19). For some of the ASVs that matched to multiple bacterial isolate, the genus and species identities were the same. However, for other ASVs that matched to multiple bacterial isolate IDs, the bacterial isolates matched in bacterial genus, while diverged in their bacterial species identity. This variability in the bacterial species matched to the ASVs may have been one disadvantage in this study due to the lower taxonomic resolution that short ASV sequences provide (Timm 2015). Using the 16s rRNA genes for phylogeny determination and identifying bacterial species is widely accepted, however, this molecular method often does not support taxonomic resolution below the genus level (Hartmann et al., 2019). In addition, as seen in Timm et al., 2015, even bacterial isolates that have a 99 percent match of the 16s rRNA gene can possess differences in genotypic and phenotypic traits. To construct more precise phylogenies, at the species and strain level, other more precise molecular genetic methods, such as whole bacterial genome sequencing and metagenomics of uncultured samples, may be more widely utilized in the future, in studies such as this one. Out of the 64 potentially beneficial bacterial isolates that were identified from the 37 microbial groups likely to be under genome selection by maize plants, two bacterial isolates, 111 (*Arthrobacter* sp.) and 730 (*Pseudomonas kribbensis*) promoted the growth of Mo17 maize plants grown under low nitrogen conditions. Bacterial isolate 111 had a 99.658 percent match to its ASV, as well as a match to bacterial isolate 3552 (*Arthrobacter bambusae*). Bacterial isolate 730 also had a 99.658 percent match to its ASV, as well as matches to four other *Pseudomonas* isolates in the collection. Because only a 250 bp region of the 16s rRNA gene was used for sequencing, the taxonomic resolution was only as accurate as the genus level. This explains why most of the ASVs matched to 2-5 different bacterial isolates within the same genus.

Even though bacterial species may be closely related in the same genus, it does not mean that they will behave in similar ways in a plant's rhizosphere, as seen in the variability of heritability across closely related groups in Meier et al., 2022. In addition, bacterial species in the same genus, such as *Pseudomonas*, can play different roles in the health of a plant, whether it is beneficial or pathogenic (Garbeva et al., 2004). The varying degrees of heritability across closely related groups in Meier et al. 2022 further confirm the importance of using a higher taxonomic resolution when identifying bacterial isolates that may offer beneficial plant-growth promoting effects. Two bacterial isolates, 111 and 730, were found to promote Mo17 maize growth under nitrogen-deficient conditions, however, other bacterial isolates that matched to the same ASVs as 111 and 730 did not promote plant growth under nitrogen-deficient conditions. These results highlight the variability in bacterial species when it comes to plant-growth promotion, as well as the need for high taxonomic resolution when identifying bacterial species that may offer a benefit to plants in an agricultural setting.

If these inoculation experiments were to be carried out again, or other experiments similar, there are certain factors that may be beneficial to incorporate for better, more cohesive results in the future. Longer growth experiments, such as the 4-week validation experiment, or ones throughout the whole lifespan of an inoculated plant, may be beneficial in getting to see the full extent of a beneficial bacterial species inoculated into the plant. Sampling and sequencing the microbial members of the rhizosphere is another variable that may be beneficial to see whether or not the inoculated bacterial species is present in the rhizosphere throughout the lifespan of the inoculated plant. Finally, to incorporate and test possible community effects present among bacterial species in the rhizosphere, another addition to these growth experiments may be the inoculation of multiple bacterial isolates into the rhizosphere, instead of just one. In Meier et al., 2022, a supplementary file was included that contained the maize genotypes grown under high and low nitrogen conditions, as well as the bacterial species that were isolated from their rhizospheres. The combination of bacterial

isolates inoculated together into a maize rhizosphere may be selected according to bacterial species that were recruited by the same maize genotype, grown under low-nitrogen conditions, as indicated in the supplementary file in Meier et al., 2022. These additional factors may offer value to future inoculation experiments like the ones carried out in this project.

8 Conclusion

In summary, bacterial isolates *Arthrobacter* sp. and *Pseudomonas kribbensis* offered a plant-growth promoting effect to Mo17 maize grown in nitrogen-deficient conditions. The timing, maize genotype, and bacterial inoculation concentration are all important factors that must be taken into consideration when analyzing the plant-growth promoting effects of beneficial bacterial inoculations on nutrient-deficient crops, as all these factors affected the outcome of the two bacterial isolates' beneficial effects. The direct causes of bacterial isolates 111 and 730's plant-growth promoting capabilities remain to be investigated. This study and others like it bring researchers closer to understanding the interactions between a nutrient-deficient plant and its microbial community members, as well as, harnessing these interactions for producing healthier, more robust crops in the future.

9 References

References

[1] Bertin, C., Yang, X., Weston, L.A. The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil*. 256, 67-83 (2003). Doi:10.1023/A:1026290508166

[2] Brown, C.T., Irber, L. sourmash: a library for MinHash sketching of DNA. *Journal of Open Source Software*. (2016). 1(5). Doi:10.21105/joss.00027

[3] Chepsergon, J., Moleleki, L.N. Rhizosphere bacterial interactions and impact on plant health. *Current Opinion in Microbiology.* (2023). 73. Doi:10.1016/j.mib.2023.102297

[4] Desnoues, N., Lin, M., Guo, X., Ma, L., Carreño-Lopez, RR., Elmerich, C. Nitrogen fixation genetics and regulation in a Pseudomonas stutzeri strain associated with rice. *Microbiology.* (2003). 149, 2251-2262. Doi:10.1099/mic.0.26270-0

[5] Garbeva, P., Antonie van Veen, J., Dirk van Elsas, J. Assessment of the diversity, and antagonism towards Rhizoctonia solani AG3, of Pseudomonas species in soil from different agricultural regimes. *FEMS Microbiology Ecology.* (2004). 47:51-64. Doi:10.1016/S0168-6496(03)00234-4

[6] Gianluigi, G., Bisceglie, F., Pelosi, G., Bonati, B., Cardarelli, M., Antenozio, M.L., Degola, F., Visioli, G. Phyto-Beneficial Traits of Rhizosphere Bacteria: In Vitro Exploration of Plant Growth Promoting and Phytopathogen Biocontrol Ability of Selected Strains Isolated from Harsh Environments. *Plants.* (2022). 11(2):230. Doi:10.3390/plants11020230

[7] Hartmann, A., Fischer, D., Kinzel, L., Chowdhury, S.P., Hofmann, A., Baldani, J.I., Rothballer, M. Assessment of the structural and functional diversities of plant micro-

biota: Achievements and challenges – A review. *Journal of Advanced Research.* (2019). 19:3-13. Doi:10.1016/j.jare.2019.04.007

[8] Hegazi, N., Hamza, M., Ali, S.M., Sedik, M.Z. Modified combined carbon N-deficient medium for isolation, enumeration and biomass production of diazotrophs. *7th International Symposium on Nitrogen Fixation with Non-Legumes.* (1996). 247-253. Doi:10.1007/978-94-011-5232-7-28

[9] Heng, L. Minimap and minasm: fast mapping and de novo assembly for noisy long sequences. *Bioinformatics.* (2016). 32(14):2103-2110. Doi:10.1093/bioinformatics/btw152

[10] Kolmogorov, M., Yuan, J., Lin, Y., Assembly of long, error-prone reads using repeat graphs. *Nat Biotechnol.* (2019). 37:540-546. Doi:10.1038/s41587-019-0072-8

[11] Lin, Y.R., Watts, D.B., Kloepper, J.W., Adesemoye, A.O., Feng, Y.C. Effect of Plant Growth-Promoting Rhizobacteria at Various Nitrogen Rates on Corn Growth. *Agricultural Sciences.* (2019). 10, 1542-1565. Doi:10.4236/as.2019.1012114

[12] MATLAB (Version 2024a) [Software]. (2024). The MathWorks, Inc. Available from https://www.mathworks.com

[13] medaka: Sequence correction provided by ONT Research. https://github.com/nanoporetech/medaka

[14] Meier, M.A., Xu, G., Lopez-Guerrero, M.G., Li, G., Smith, C., Sigmon, B., Herr, J.R., Alfano, J.R., Ge, Y., Schnable, J., Yang, J. Association analyses of host genetics, root-colonizing microbes, and plant phenotypes under different nitrogen conditions in maize. *eLife.* (2022). 11:e75790. Doi:10.7554/eLife.75790

[15] Coordinators, N.R. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* (2018). 4:46(D1):D8-D13. Doi:10.1093/nar/gkx1095.

[16] Ondov, B.D., Starrett, G.J., Sappington, A., Kostic, A., Koren, S., Buck, C.B., Phillippy, A.M. Mash Screen: high-throughput sequence containment estimation for genome discovery. *Genome Biology.* (2019). 20(232). Doi:10.1186/s13059-019-1841-x

[17] Panpatte, D.G., Jhala, Y.K., Shelat, H.N., Vyas, R.V. Pseudomonas fluorescens: A Promising Biocontrol Agent and PGPR for Sustainable Agriculture. *Microbial Inoculants in Sustainable Agricultural Productivity.* (2016). 257-270. Doi:10.1007/978-81-322-2647-5-15

[18] Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research.* (2015). 25:1043-1055. Doi:10.1101/gr.186072.114

[19] Plucani do Amaral, F., Pankievicz, V.C.S., Arisi, A.C.M. Differential growth responses of Brachypodium distachyon genotypes to inoculation with plant growth promoting rhizobacteria. *Plant Mol Biol.* (2016). 90:689-697. Doi:10.1007/s11103-016-0449-8

[20] Plucani do Amaral, F., Wang, J., Williams, J., Tuleski, T.R., Joshi, T., Ferreira, M.A.R., Stacey, G. Mapping Genetic Variation in Arabidopsis in Response to Plant Growth-Promotion Bacterium Azoarcus olearius DQS-4T. *Microorganisms.* (2023). 11, 331. Doi:10.3390/microorganisms11020331

[21] Polypolish: a short-read polishing tool for long-read assemblies. https://github.com/rrwick/Polypolish

[22] Schwengers, O., Jelonek, L., Dieckmann, M.A., Beyvers, S., Blom, J., Goesmann, A. Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. *Microbial Genomics.* (2021). 7(11). Doi:10.1099/mgen.0.000685

[23] Shaharoona, B., Naveed, M., Arshad, M., Zahir, Z.A. Fertilizer-dependent efficiency of Pseudomonads for improving growth, yield, and nutrient use efficiency

of wheat (Triticum aestivum L.). *Appl Microbiol Biotechnol* (2008). 79:147-155. Doi:10.1007/s00253-008-1419-0f

[24] Sun, X., Xu, Z., Xie, J., Hesselberg-Thomsen, V., Tan, T., Zheng, D., Strube, M.L., Dragoš, A., Shen, Q., Zhang, R., Kovács, A.T. Bacillus velezensis stimulates resident rhizosphere Pseudomonas stutzeri for plant health through metabolic interactions. *The ISME Journal.* (2022). 16:774-787. Doi:10.1038/s41396-021-01125-3

[25] Stella, M., Suhaimi, M. Selection of suitable growth medium for free-living diazotrophs isolated from compost. *J. Trp. Agric. and Fd. Sc.*. (2010). 38(2):211-219.

[26] Tang, A., Haruna, A.O., Majid, N.M.A. Jalloh, M.B. Effects of Selected Functional Bacteria on Maize Growth and Nutrient Use Efficiency. *Microorganisms.* 2020. 8(6):854. Doi:10.3390/microorganisms8060854

[27] Timm, C.M., Campbell, A.G., Utturkar, S.M. Jun, S-R., Parales, R.E., Tan, W.A., Robeson, M.S., Lu, T-Y.S., Jawdy, S., Brown, S.D., Ussery, D.W., Schadt, C.W., Tuskan, G.A., Doktycz, M.J., Weston, D.J., Pelletier, D.A. Metabolic functions of Pseudomonas fluorescens strains from Populus deltoides depend on rhizosphere or endosphere isolation compartment. *Front. Microbiol.* (2015). 6:1118. Doi:10.3389/fmicb.2015.01118

[28] Wick R.R. 2017. Filtlong. https://github.com/rrwick/Filtlong

[29] Wick, R.R., Schultz, M.B., Zobel, J., Holt, K.E. Bandage: interactive visualization of de novo genome assemblies. *Bioinformatics.* (2015). 31(20):3350-3352. Doi:10.1093/bioinformatics/btv383

[30] Wintermans, P.C.A., Bakker, P.A.H.M., Pieterse, C.M.J. Natural genetic variation in Arabidopsis for responsiveness to plant growth-promoting bacteria. Plant Molecular Biology. (2016). 90(6):623-634. Doi:10.1007/s11103-016-0442-2

[31] Wu, X., Liu, Y., Shang, Y., Liu, D., Liesack, W., Cui, Z., Peng, J., Zhang, F. Peat-vermiculite alters microbiota composition towards increased soil fertility and crop productivity. *Plant Soil.* (2022). 470:21-34. Doi:10.1007/s11104-021-04851-x

10 Supplementary Tables

ASV	Database Bacterial Isolate IDs	Percent Match	Bacterial Isolate #1 Identity	Bacterial Isolate #2 Identity	Bacterial Isolate #3 Identity	Bacterial Isolate #4 Identity	Bacterial Isolate #5 Identity
asv_000018	1138	100	Ralstonia sp.				
asv_000414	2025; 1215; 3215	100	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000822	1112; 1175	100	Pantoea dispersa	Pantoea dispersa			
asv_004074	731; 707; 726; 702; 730	100	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas kribbensis
asv_004124	2987	100	Chryseobacterium sp.				
asv_004154	1550; 1611; 725	100	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-10		
asv_004174	1087; 606	100	Mesorhizobium erdmanii strain NZP2014	Mesorhizobium erdmanii			
asv_000002	1138	99.658	Ralstonia sp.				
asv_000004	1138	99.658	Ralstonia sp.				
asv_000005	1138	99.658	Ralstonia sp.				
asv_000027	1138	99.658	Ralstonia sp.				
asv_000075	1138	99.658	Ralstonia sp.				
asv_000088	1138	99.658	Ralstonia sp.				
asv_000169	3215; 2025; 1215	99.658	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000198	1215; 2025; 3215	99.658	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000203	1215; 2025; 3215	99.658	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000314	1175; 1112	99.658	Pantoea dispersa	Pantoea dispersa			
asv_000398	1112; 1175	99.658	Pantoea dispersa	Pantoea dispersa			
asv_000420	1175; 1112	99.658	Pantoea dispersa	Pantoea dispersa			
asv_000513	1087; 606	99.658	Mesorhizobium erdmanii strain NZP2014	Mesorhizobium erdmanii			
asv_000728	2025; 1215; 3215	99.658	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		

asv_000826	606; 1087	99.658	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2014			
asv_000856	1087; 606	99.658	Mesorhizobium erdmanii strain NZP2014	Mesorhizobium erdmanii			
asv_000868	1215; 2025; 3215	99.658	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000872	1112; 1175	99.658	Pantoea dispersa	Pantoea dispersa			
asv_001659	1550; 1611; 725	99.658	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-10		
asv_001793	1175; 1112	99.658	Pantoea dispersa	Pantoea dispersa			
asv_001855	478; 3432; 460	99.658	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_001925	707; 731; 730; 702; 726	99.658	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02
asv_002020	478; 3432; 460	99.658	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_002054	1550; 1611; 725	99.658	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-10		
asv_002096	702; 726; 730; 731; 707	99.658	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02
asv_002120	2987	99.658	Chryseobacterium sp.				
asv_002421	707; 731; 730; 702; 726	99.658	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02
asv_002439	3432; 460; 478	99.658	Pseudomonas brassicacearum	Pseudomonas sp.	Pseudomonas sp.		
asv_002457	2056; 1903; 1610	99.658	Kosakonia cowanii	Atlantibacter hermannii	Kosakonia sp.		
asv_002465	2987	99.658	Chryseobacterium sp.				
asv_002467	1087; 606	99.658	Mesorhizobium erdmanii strain NZP2014	Mesorhizobium erdmanii			
asv_002616	2987	99.658	Chryseobacterium sp.				

asv_002624	2056; 1610; 1903	99.658	Kosakonia cowanii	Atlantibacter hermannii	Kosakonia sp.		
asv_002859	2056; 1903; 1610	99.658	Kosakonia cowanii	Atlantibacter hermannii	Kosakonia sp.		
asv_003343	3552; 111	99.658	Arthrobacter bambusae	Arthrobacter sp.			
asv_003659	730; 702; 726; 707; 731	99.658	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02
asv_003958	111; 3552	99.658	Arthrobacter sp.	Arthrobacter bambusae			
asv_004190	4479	99.658	Flavobacterium johnsoniae				
asv_004445	725; 1611; 1550	99.658	Pseudomonas sp. Agri-10	Pseudomonas fluroescens	Pseudomonas fluorescens		
asv_004550	2987	99.658	Chryseobacterium sp.				
asv_004927	111; 3552	99.658	Arthrobacter sp.	Arthrobacter bambusae			
asv_007886	606; 1087	99.658	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2015			
asv_000001	1138	99.315	Ralstonia sp.				
asv_000007	1138	99.315	Ralstonia sp.				
asv_000008	1138	99.315	Ralstonia sp.				
asv_000010	1138	99.315	Ralstonia sp.				
asv_000011	1138	99.315	Ralstonia sp.				
asv_000015	1138	99.315	Ralstonia sp.				
asv_000017	1138	99.315	Ralstonia sp.				
asv_000029	1138	99.315	Ralstonia sp.				
asv_000035	1138	99.315	Ralstonia sp.				
asv_000038	1138	99.315	Ralstonia sp.				
asv_000047	1138	99.315	Ralstonia sp.				
asv_000124	1138	99.315	Ralstonia sp.				
asv_000128	709; 701; 739; 620; 700	99.315	Burkholderia sp. RB142	Burkholderia ambifaria	Burkholderia sp.	Burkholderia sp. CR22	Burkholderia ambifaria
asv_000141	701; 709; 620; 739; 700	99.315	Burkholderia ambifaria	Burkholderia sp. CR26	Burkholderia sp.	Burkholderia ambifaria	Burkholderia sp. RB146
asv_000148	701; 709; 620; 739; 700	99.315	Burkholderia ambifaria	Burkholderia sp. CR27	Burkholderia sp.	Burkholderia ambifaria	Burkholderia sp. RB147
asv_000155	3215; 1215; 2025	99.315	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		

asv_000178	727; 572; 574; 616; 571	99.315	Burkholderia seminalis	Burkholderia sp.	Burkholderia cenocepacia	Burkholderia seminalis	Burkholderia sp.
asv_000232	3215; 1215; 2025	99.315	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000272	1112; 1175	99.315	Pantoea dispersa	Pantoea dispersa			
asv_000279	3215; 2025; 1215	99.315	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000305	1215; 2025; 3215	99.315	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000324	3215; 1215; 2025	99.315	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000339	3215; 2025; 1215	99.315	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000348	1215; 2025; 3215	99.315	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000396	1087; 606	99.315	Mesorhizobium erdmanii strain NZP2015	Mesorhizobium erdmanii			
asv_000409	3215; 1215; 2025	99.315	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000419	3215; 2025; 1215	99.315	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000459	2025; 1215; 3215	99.315	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000509	1175; 1112	99.315	Pantoea dispersa	Pantoea dispersa			
asv_000522	1175; 1112	99.315	Pantoea dispersa	Pantoea dispersa			
asv_000540	1112; 1175	99.315	Pantoea dispersa	Pantoea dispersa			
asv_000709	1175; 1112	99.315	Pantoea dispersa	Pantoea dispersa			
asv_000766	1175; 1112	99.315	Pantoea dispersa	Pantoea dispersa			
asv_000775	1175; 1112	99.315	Pantoea dispersa	Pantoea dispersa			
asv_000982	1175; 1112	99.315	Pantoea dispersa	Pantoea dispersa			
asv_001123	1087; 606	99.315	Mesorhizobium erdmanii strain NZP2016	Mesorhizobium erdmanii			
asv_001348	1087; 606	99.315	Mesorhizobium erdmanii strain NZP2017	Mesorhizobium erdmanii			

asv_001529	707; 731; 730; 702; 726	99.315	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02
asv_001546	606; 1087	99.315	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2016			
asv_001584	460; 3432; 478	99.315	Psuedomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_001619	606; 1087	99.315	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2017			
asv_001689	1087; 606	99.315	Mesorhizobium erdmanii strain NZP2018	Mesorhizobium erdmanii			
asv_001805	460; 3432; 478	99.315	Psuedomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_001821	606; 1087	99.315	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2018			
asv_001888	2987	99.315	Chryseobacterium sp.				
asv_001978	2056; 1903; 1610	99.315	Kosakonia cowanii	Atlantibacter hermannii	Kosakonia sp.		
asv_002108	707; 731; 730; 726; 702	99.315	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02
asv_002149	478; 460; 3432	99.315	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_002240	1175; 1112	99.315	Pantoea dispersa	Pantoea dispersa			
asv_002244	1087; 606	99.315	Mesorhizobium erdmanii strain NZP2019	Mesorhizobium erdmanii			
asv_002252	460; 3432; 478	99.315	Psuedomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_002270	725; 1550; 1611	99.315	Pseudomonas sp. Agri-10	Pseudomonas fluroescens	Pseudomonas fluorescens		
asv_002410	1611; 1550; 725	99.315	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-10		

asv_002472	702; 726; 730; 731; 707	99.315	Pseudomonas kribbensis	Pseudomonas sp. Cysa03	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02
asv_002495	707; 731; 730; 726; 702	99.315	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02
asv_002580	460; 3432; 478	99.315	Psuedomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_002663	730; 726; 702; 707; 731	99.315	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02
asv_002768	1474; 2074; 2083	99.315	Rhizobium sp.	Agrobacterium larrymoorei	Neorhizobium sp.		
asv_002791	2987	99.315	Chryseobacterium sp.				
asv_002807	478; 460; 3432	99.315	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_002838	2987	99.315	Chryseobacterium sp.				
asv_002849	1087; 606	99.315	Mesorhizobium erdmanii strain NZP2020	Mesorhizobium erdmanii			
asv_003033	621; 708	99.315	Dyella yeoguensis	Leifsonia aquatica			
asv_003175	2987	99.315	Chryseobacterium sp.				
asv_003254	1903; 1610; 2056	99.315	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-11		
asv_003367	2987	99.315	Chryseobacterium sp.				
asv_003410	1087; 606	99.315	Mesorhizobium erdmanii strain NZP2021	Mesorhizobium erdmanii			
asv_003503	707; 731; 730; 726; 702	99.315	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa03
asv_003561	478; 3432; 460	99.315	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_003606	111; 3552	99.315	Arthrobacter sp.	Arthrobacter bambusae			
asv_003647	3432; 460; 478	99.315	Pseudomonas brassicacearum	Pseudomonas sp.	Pseudomonas sp.		

asv_003679	707; 731; 730; 702; 726	99.315	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02
asv_003694	1903; 1610; 2056	99.315	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-12		
asv_003697	725; 1550; 1611	99.315	Pseudomonas sp. Agri-10	Pseudomonas fluroescens	Pseudomonas fluorescens		
asv_003700	2056; 1903; 1610	99.315	Kosakonia cowanii	Atlantibacter hermannii	Kosakonia sp.		
asv_003724	3190	99.315	Luteibacter sp.				
asv_003735	2987	99.315	Chryseobacterium sp.				
asv_003743	730; 726; 702; 707; 731	99.315	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02
asv_003758	1550; 1611; 725	99.315	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-10		
asv_003826	4479	99.315	Flavobacterium johnsoniae				
asv_003876	2987	99.315	Chryseobacterium sp.				
asv_003959	2987	99.315	Chryseobacterium sp.				
asv_003969	478; 3432; 460	99.315	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_004360	730; 702; 726; 707; 731	99.315	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02
asv_004460	707; 731; 730; 702; 726	99.315	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02
asv_004604	2987	99.315	Chryseobacterium sp.				
asv_004686	1903; 1610; 2056	99.315	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-13		
asv_004708	478; 3432; 460	99.315	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_004719	2987	99.315	Chryseobacterium sp.				
asv_004794	1903; 1610; 2056	99.315	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-14		
asv_005116	1610; 1903; 2056	99.315	Kosakonia sp.	Atlantibacter hermannii	Kosakonia cowanii		
asv_005487	1474; 2083; 2074	99.315	Rhizobium sp.	Agrobacterium larrymoorei	Neorhizobium sp.		

asv_006231	111; 3552	99.315	Arthrobacter sp.	Arthrobacter bambusae			
asv_006523	707; 731; 730; 726; 702	99.315	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa04
asv_006832	1087; 606	99.315	Mesorhizobium erdmanii strain NZP2022	Mesorhizobium erdmanii			
asv_007398	3552; 111	99.315	Arthrobacter bambusae	Arthrobacter sp.			
asv_008031	2083; 2074; 1474	99.315	Neorhizobium sp.	Agrobacterium larrymoorei	Rhizobium sp.		
asv_008501	3552; 111	99.315	Arthrobacter bambusae	Arthrobacter sp.			
asv_008514	2987	99.315	Chryseobacterium sp.				
asv_015036	700; 620; 739; 701; 709	99.315	Burkholderia ambifaria	Burkholderia sp. CR22	Burkholderia sp.	Burkholderia ambifaria	Burkholderia sp. RB142
asv_015437	701; 709; 700; 620; 739	99.315	Burkholderia ambifaria	Burkholderia sp. CR28	Burkholderia sp.	Burkholderia ambifaria	Burkholderia sp. RB148
asv_015623	620; 739; 700; 701; 709	99.315	Burkholderia sp. CR22	Burkholderia sp.	Burkholderia ambifaria	Burkholderia ambifaria	Burkholderia sp. RB142
asv_000003	1138	98.973	Ralstonia sp.				
asv_000006	1138	98.973	Ralstonia sp.				
asv_000012	1138	98.973	Ralstonia sp.				
asv_000023	1138	98.973	Ralstonia sp.				
asv_000053	1138	98.973	Ralstonia sp.				
asv_000055	1138	98.973	Ralstonia sp.				
asv_000058	1138	98.973	Ralstonia sp.				
asv_000061	1138	98.973	Ralstonia sp.				
asv_000068	1138	98.973	Ralstonia sp.				
asv_000074	1138	98.973	Ralstonia sp.				
asv_000091	1138	98.973	Ralstonia sp.				
asv_000112	700; 620; 739; 709; 701	98.973	Burkholderia ambifaria	Burkholderia sp. CR23	Burkholderia sp.	Burkholderia ambifaria	Burkholderia sp. RB143
asv_000171	701; 709; 700; 739; 620	98.973	Burkholderia ambifaria	Burkholderia sp. CR29	Burkholderia sp.	Burkholderia ambifaria	Burkholderia sp. RB149
asv_000176	700; 739; 620; 709; 701	98.973	Burkholderia ambifaria	Burkholderia sp. CR25	Burkholderia sp.	Burkholderia ambifaria	Burkholderia sp. RB145
asv_000187	709; 701; 620; 739; 700	98.973	Burkholderia sp. RB142	Burkholderia ambifaria	Burkholderia sp. CR22	Burkholderia sp.	Burkholderia ambifaria

asv_000192	2025; 1215; 3215	98.973	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000236	1215; 2025; 3215	98.973	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000242	572; 727; 616; 571; 574	98.973	Burkholderia sp.	Burkholderia seminalis	Burkholderia seminalis	Burkholderia sp.	Burkholderia cenocepacia
asv_000249	574; 616; 571; 727; 572	98.973	Burkholderia cenocepacia	Burkholderia seminalis	Burkholderia sp.	Burkholderia seminalis	Burkholderia sp.
asv_000288	3215; 2025; 1215	98.973	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000310	1215; 2025; 3215	98.973	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000469	3215; 2025; 1215	98.973	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000608	2025; 1215; 3215	98.973	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000626	1175; 1112	98.973	Pantoea dispersa	Pantoea dispersa			
asv_000666	3215; 1215; 2025	98.973	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000668	1175; 1112	98.973	Pantoea dispersa	Pantoea dispersa			
asv_000725	606; 1087	98.973	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2019			
asv_000740	1215; 2025; 3215	98.973	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000922	1087; 606	98.973	Mesorhizobium erdmanii strain NZP2023	Mesorhizobium erdmanii			
asv_001249	606; 1087	98.973	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2020			
asv_001324	1112; 1175	98.973	Pantoea dispersa	Pantoea dispersa			
asv_001416	478; 460; 3432	98.973	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_001628	1175; 1112	98.973	Pantoea dispersa	Pantoea dispersa			
asv_001629	478; 460; 3432	98.973	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		

asv_001719	1175; 1112	98.973	Pantoea dispersa	Pantoea dispersa			
asv_001780	702; 726; 730; 731; 707	98.973	Pseudomonas kribbensis	Pseudomonas sp. Cysa04	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02
asv_001894	707; 731; 730; 702; 726	98.973	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02
asv_002359	2987	98.973	Chryseobacterium sp.				
asv_002634	2987	98.973	Chryseobacterium sp.				
asv_002701	1903; 1610; 2056	98.973	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-15		
asv_002711	606; 1087	98.973	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2021			
asv_002746	478; 3432; 460	98.973	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_002919	726; 702; 730; 731; 707	98.973	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Csya02
asv_003008	1087; 606	98.973	Mesorhizobium erdmanii strain NZP2024	Mesorhizobium erdmanii			
asv_003035	1087; 606	98.973	Mesorhizobium erdmanii strain NZP2025	Mesorhizobium erdmanii			
asv_003043	731; 707; 702; 726; 730	98.973	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas sp. Cysa02	Pseudomonas kribbensis
asv_003147	3432; 460; 478	98.973	Pseudomonas brassicacearum	Pseudomonas sp.	Pseudomonas sp.		
asv_003185	2987	98.973	Chryseobacterium sp.				
asv_003246	1610; 1903; 2056	98.973	Kosakonia sp.	Atlantibacter hermannii	Kosakonia cowanii		
asv_003394	1474; 2083; 2074	98.973	Rhizobium sp.	Agrobacterium larrymoorei	Neorhizobium sp.		
asv_003436	731; 707; 702; 726; 730	98.973	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas sp. Cysa02	Pseudomonas kribbensis
asv_003519	2987	98.973	Chryseobacterium sp.				

asv_003628	606; 1087	98.973	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2022			
asv_003642	726; 702; 730; 731; 707	98.973	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Csya02
asv_003703	2056; 1903; 1610	98.973	Kosakonia cowanii	Atlantibacter hermannii	Kosakonia sp.		
asv_003722	2987	98.973	Chryseobacterium sp.				
asv_003762	606; 1087	98.973	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2023			
asv_003765	3552; 111	98.973	Arthrobacter bambusae	Arthrobacter sp.			
asv_003896	3432; 460; 478	98.973	Pseudomonas brassicacearum	Pseudomonas sp.	Pseudomonas sp.		
asv_004021	708; 621	98.973	Leifsonia aquatica	Dyella yeoguensis			
asv_004056	1903; 1610; 2056	98.973	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-16		
asv_004412	707; 731; 730; 702; 726	98.973	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02
asv_004474	726; 702; 730; 731; 707	98.973	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Csya02
asv_004622	4479	98.973	Flavobacterium johnsoniae				
asv_004680	707; 731; 730; 702; 726	98.973	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa02
asv_004750	478; 3432; 460	98.973	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_005233	2987	98.973	Chryseobacterium sp.				
asv_005372	2987	98.973	Chryseobacterium sp.				
asv_005694	2987	98.973	Chryseobacterium sp.				
asv_005696	1903; 1610; 2056	98.973	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-17		
asv_005710	2074; 2083; 1474	98.973	Agrobacterium larrymoorei	Neorhizobium sp.	Rhizobium sp.		

asv_005749	2987	98.973	Chryseobacterium sp.				
asv_005811	1087; 606	98.973	Mesorhizobium erdmanii strain NZP2026	Mesorhizobium erdmanii			
asv_005902	2987	98.973	Chryseobacterium sp.				
asv_006116	708; 621	98.973	Leifsonia aquatica	Dyella yeoguensis			
asv_006405	731; 707; 702; 726; 730	98.973	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas sp. Cysa02	Pseudomonas kribbensis
asv_006473	1903; 1610; 2056	98.973	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-18		
asv_006643	2987	98.973	Chryseobacterium sp.				
asv_006702	2987	98.973	Chryseobacterium sp.				
asv_006893	3190	98.973	Luteibacter sp.				
asv_006946	111; 3552	98.973	Arthrobacter sp.	Arthrobacter bambusae			
asv_007017	606; 1087	98.973	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2024			
asv_014370	709; 701; 739; 620; 700	98.973	Burkholderia sp. RB142	Burkholderia ambifaria	Burkholderia sp.	Burkholderia sp. CR22	Burkholderia ambifaria
asv_000033	1138	98.63	Ralstonia sp.				
asv_000044	1138	98.63	Ralstonia sp.				
asv_000060	1138	98.63	Ralstonia sp.				
asv_000134	709; 701; 739; 620; 700	98.63	Burkholderia sp. RB142	Burkholderia ambifaria	Burkholderia sp.	Burkholderia sp. CR22	Burkholderia ambifaria
asv_000159	701; 709; 739; 620; 700	98.63	Burkholderia ambifaria	Burkholderia sp. CR30	Burkholderia sp.	Burkholderia ambifaria	Burkholderia sp. RB150
asv_000186	700; 620; 739; 709; 701	98.63	Burkholderia ambifaria	Burkholderia sp. CR24	Burkholderia sp.	Burkholderia ambifaria	Burkholderia sp. RB144
asv_000399	1215; 2025; 3215	98.63	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000482	2025; 1215; 3215	98.63	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		
asv_000593	1215; 2025; 3215	98.63	Mucilaginibacter rubeus	Mucilaginibacter rubeus	Mucilaginibacter rubeus		

asv_000889	1112; 1175	98.63	Pantoea dispersa	Pantoea dispersa			
asv_001049	1175; 1112	98.63	Pantoea dispersa	Pantoea dispersa			
asv_001367	1175; 1112	98.63	Pantoea dispersa	Pantoea dispersa			
asv_001493	606; 1087	98.63	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2025			
asv_001856	2987	98.63	Chryseobacterium sp.				
asv_002043	2987	98.63	Chryseobacterium sp.				
asv_002325	2987	98.63	Chryseobacterium sp.				
asv_002603	606; 1087	98.63	Mesorhizobium erdmanii	Mesorhizobium erdmanii strain NZP2026			
asv_002653	2987	98.63	Chryseobacterium sp.				
asv_002657	478; 3432; 460	98.63	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_003177	460; 3432; 478	98.63	Psuedomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_003231	731; 707; 726; 702; 730	98.63	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas kribbensis
asv_003421	4606	98.63	Acidovorax sp.				
asv_003477	730; 726; 702; 707; 731	98.63	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02
asv_004249	4606	98.63	Acidovorax sp.				
asv_004420	2987	98.63	Chryseobacterium sp.				
asv_004462	2987	98.63	Chryseobacterium sp.				
asv_004540	1087; 606	98.63	Mesorhizobium erdmanii strain NZP2027	Mesorhizobium erdmanii			
asv_004901	4606	98.63	Acidovorax sp.				

asv_004977	478; 3432; 460	98.63	Pseudomonas sp.	Pseudomonas brassicacearum	Pseudomonas sp.		
asv_004983	2987	98.63	Chryseobacterium sp.				
asv_005098	2056; 1610; 1903	98.63	Kosakonia cowanii	Atlantibacter hermannii	Kosakonia sp.		
asv_005350	707; 731; 730; 726; 702	98.63	Pseudomonas sp. Cysa02	Pseudomonas sp. Cysa02	Pseudomonas kribbensis	Pseudomonas koreensis	Pseudomonas sp. Cysa05
asv_005608	726; 702; 730; 731; 707	98.63	Pseudomonas sp. Cysa02	Pseudomonas koreensis	Pseudomonas kribbensis	Pseudomonas sp. Cysa02	Pseudomonas sp. Csya02
asv_005767	1903; 1610; 2056	98.63	Pseudomonas fluorescens	Pseudomonas fluorescens	Pseudomonas sp. Agri-19		
asv_005883	2987	98.63	Chryseobacterium sp.				
asv_006232	2987	98.63	Chryseobacterium sp.				
asv_006604	2083; 2074; 1474	98.63	Neorhizobium sp.	Agrobacterium larrymoorei	Rhizobium sp.		
asv_006662	2056; 1903; 1610	98.63	Kosakonia cowanii	Atlantibacter hermannii	Kosakonia sp.		
asv_007476	3552; 111	98.63	Arthrobacter bambusae	Arthrobacter sp.			
asv_007721	621; 708	98.63	Dyella yeoguensis	Leifsonia aquatica			
asv_015707	701; 709; 739; 620; 700	98.63	Burkholderia ambifaria	Burkholderia sp. CR31	Burkholderia sp.	Burkholderia ambifaria	Burkholderia sp. RB151
asv_000971	94	98.288	Mucilaginibacter sp.				
asv_001231	2987	98.288	Chryseobacterium sp.				
asv_001436	2987	98.288	Chryseobacterium sp.				
asv_001515	2987	98.288	Chryseobacterium sp.				
asv_001649	2987	98.288	Chryseobacterium sp.				
asv_002535	2987	98.288	Chryseobacterium sp.				
asv_002611	2987	98.288	Chryseobacterium sp.				
asv_002640	2987	98.288	Chryseobacterium sp.				
asv_002678	4494; 4567; 4579; 4509; 4493	98.288	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.

asv_002793	2987	98.288	Chryseobacterium sp.				
asv_002841	4606	98.288	Acidovorax sp.				
asv_002877	4567; 4494; 4509; 4493; 4579	98.288	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.	Dyadobacter sp.
asv_003165	4606	98.288	Acidovorax sp.				
asv_003256	2987	98.288	Chryseobacterium sp.				
asv_003442	2987	98.288	Chryseobacterium sp.				
asv_003540	2987	98.288	Chryseobacterium sp.				
asv_003723	94	98.288	Mucilaginibacter sp.				
asv_003749	2987	98.288	Chryseobacterium sp.				
asv_003807	2987	98.288	Chryseobacterium sp.				
asv_004053	2987	98.288	Chryseobacterium sp.				
asv_006212	3330	98.288	Luteibacter sp.				
asv_006327	4606	98.288	Acidovorax sp.				
asv_000301	94	97.945	Mucilaginibacter sp.				
asv_000381	94	97.945	Mucilaginibacter sp.				
asv_000400	94	97.945	Mucilaginibacter sp.				
asv_001021	3330	97.945	Luteibacter sp.				
asv_001141	2987	97.945	Chryseobacterium sp.				
asv_001388	3330	97.945	Luteibacter sp.				
asv_001575	94	97.945	Mucilaginibacter sp.				
asv_001664	94	97.945	Mucilaginibacter sp.				
asv_001716	3330	97.945	Luteibacter sp.				
asv_001798	94	97.945	Mucilaginibacter sp.				
asv_001809	2987	97.945	Chryseobacterium sp.				

asv_002002	2987	97.945	Chryseobacterium sp.				
asv_002014	2987	97.945	Chryseobacterium sp.				
asv_002045	2987	97.945	Chryseobacterium sp.				
asv_002280	2987	97.945	Chryseobacterium sp.				
asv_002309	4567; 4494; 4509; 4493; 4579	97.945	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.	Dyadobacter sp.
asv_002428	2987	97.945	Chryseobacterium sp.				
asv_002464	2987	97.945	Chryseobacterium sp.				
asv_002674	2987	97.945	Chryseobacterium sp.				
asv_002810	2987	97.945	Chryseobacterium sp.				
asv_002874	2987	97.945	Chryseobacterium sp.				
asv_002899	2987	97.945	Chryseobacterium sp.				
asv_002984	2987	97.945	Chryseobacterium sp.				
asv_003004	2987	97.945	Chryseobacterium sp.				
asv_003603	4567; 4494; 4509; 4493; 4579	97.945	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.	Dyadobacter sp.
asv_003863	4509; 4493; 4579; 4567; 4494	97.945	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.
asv_004187	4606	97.945	Acidovorax sp.				
asv_004210	4606	97.945	Acidovorax sp.				
asv_004241	4494; 4567; 4579; 4509; 4493	97.945	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.
asv_004251	2987	97.945	Chryseobacterium sp.				
asv_004291	3330	97.945	Luteibacter sp.				
asv_004344	4567; 4494; 4493; 4509; 4579	97.945	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.
asv_004477	2987	97.945	Chryseobacterium sp.				

asv_004478	2987	97.945	Chryseobacterium sp.				
asv_004507	4606	97.945	Acidovorax sp.				
asv_004572	2987	97.945	Chryseobacterium sp.				
asv_004964	2987	97.945	Chryseobacterium sp.				
asv_005433	4509; 4493; 4579; 4567; 4494	97.945	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.
asv_005932	2987	97.945	Chryseobacterium sp.				
asv_006126	4606	97.945	Acidovorax sp.				
asv_012433	3330	97.945	Luteibacter sp.				
asv_000284	94	97.603	Mucilaginibacter sp.				
asv_000461	94	97.603	Mucilaginibacter sp.				
asv_000594	94	97.603	Mucilaginibacter sp.				
asv_000597	94	97.603	Mucilaginibacter sp.				
asv_000634	94	97.603	Mucilaginibacter sp.				
asv_000676	94	97.603	Mucilaginibacter sp.				
asv_000691	3330	97.603	Luteibacter sp.				
asv_000700	94	97.603	Mucilaginibacter sp.				
asv_000717	94	97.603	Mucilaginibacter sp.				
asv_000797	94	97.603	Mucilaginibacter sp.				
asv_000811	94	97.603	Mucilaginibacter sp.				
asv_000832	94	97.603	Mucilaginibacter sp.				
asv_000842	94	97.603	Mucilaginibacter sp.				
asv_001042	94	97.603	Mucilaginibacter sp.				
asv_001060	4487	97.603	Comamonas sediminis				
asv_001465	2987	97.603	Chryseobacterium sp.				

asv_001839	2987	97.603	Chryseobacterium sp.				
asv_001988	3330	97.603	Luteibacter sp.				
asv_002153	3330	97.603	Luteibacter sp.				
asv_002256	2987	97.603	Chryseobacterium sp.				
asv_002294	3330	97.603	Luteibacter sp.				
asv_002326	2987	97.603	Chryseobacterium sp.				
asv_002452	3330	97.603	Luteibacter sp.				
asv_002606	94	97.603	Mucilaginibacter sp.				
asv_002775	94	97.603	Mucilaginibacter sp.				
asv_002863	3330	97.603	Luteibacter sp.				
asv_002948	3330	97.603	Luteibacter sp.				
asv_003072	4493; 4509; 4579; 4567; 4494	97.603	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.
asv_003213	3330	97.603	Luteibacter sp.				
asv_003297	2987	97.603	Chryseobacterium sp.				
asv_003386	4579; 4493; 4509; 4494; 4567	97.603	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.	Dyadobacter sp.
asv_003516	2987	97.603	Chryseobacterium sp.				
asv_003623	2987	97.603	Chryseobacterium sp.				
asv_003657	3330	97.603	Luteibacter sp.				
asv_003688	2987	97.603	Chryseobacterium sp.				
asv_003692	94	97.603	Mucilaginibacter sp.				
asv_003727	3330	97.603	Luteibacter sp.				
asv_003872	3330	97.603	Luteibacter sp.				
asv_003988	3330	97.603	Luteibacter sp.				

asv_004015	2987	97.603	Chryseobacterium sp.				
asv_004142	4579; 4493; 4509; 4494; 4567	97.603	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.	Dyadobacter sp.
asv_004378	2987	97.603	Chryseobacterium sp.				
asv_004544	4494; 4567; 4579; 4509; 4493	97.603	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.
asv_005100	3330	97.603	Luteibacter sp.				
asv_005123	2987	97.603	Chryseobacterium sp.				
asv_005146	2987	97.603	Chryseobacterium sp.				
asv_006492	4567; 4494; 4493; 4509; 4579	97.603	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.
asv_007161	4579; 4509; 4493; 4494; 4567	97.603	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.
asv_007196	4606	97.603	Acidovorax sp.				
asv_007224	4567; 4494; 4509; 4493; 4579	97.603	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.	Dyadobacter sp.
asv_007312	4606	97.603	Acidovorax sp.				
asv_007589	4606	97.603	Acidovorax sp.				
asv_010213	3330	97.603	Luteibacter sp.				
asv_000300	4487	97.26	Comamonas sediminis				
asv_000388	94	97.26	Mucilaginibacter sp.				
asv_000389	4487	97.26	Comamonas sediminis				
asv_000433	4487	97.26	Comamonas sediminis				
asv_000444	94	97.26	Mucilaginibacter sp.				
asv_000520	94	97.26	Mucilaginibacter sp.				
asv_000543	3330	97.26	Luteibacter sp.				
asv_000582	94	97.26	Mucilaginibacter sp.				

asv_000646	94	97.26	Mucilaginibacter sp.				
asv_000895	94	97.26	Mucilaginibacter sp.				
asv_000908	3330	97.26	Luteibacter sp.				
asv_000957	94	97.26	Mucilaginibacter sp.				
asv_001035	3330	97.26	Luteibacter sp.				
asv_001074	94	97.26	Mucilaginibacter sp.				
asv_001129	94	97.26	Mucilaginibacter sp.				
asv_001164	94	97.26	Mucilaginibacter sp.				
asv_001185	94	97.26	Mucilaginibacter sp.				
asv_001269	94	97.26	Mucilaginibacter sp.				
asv_001308	94	97.26	Mucilaginibacter sp.				
asv_001315	3330	97.26	Luteibacter sp.				
asv_001361	94	97.26	Mucilaginibacter sp.				
asv_001380	94	97.26	Mucilaginibacter sp.				
asv_001381	94	97.26	Mucilaginibacter sp.				
asv_001440	94	97.26	Mucilaginibacter sp.				
asv_001526	704	97.26	Sphingomonas kyeoggiensis				
asv_001540	4487	97.26	Comamonas sediminis				
asv_001556	94	97.26	Mucilaginibacter sp.				
asv_001681	3330	97.26	Luteibacter sp.				
asv_001693	2830; 2829	97.26	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001810	94	97.26	Mucilaginibacter sp.				
asv_001848	94	97.26	Mucilaginibacter sp.				
asv_001927	3330	97.26	Luteibacter sp.				

asv_002119	4487	97.26	Comamonas sediminis				
asv_002765	2987	97.26	Chryseobacterium sp.				
asv_002930	3330	97.26	Luteibacter sp.				
asv_003571	94	97.26	Mucilaginibacter sp.				
asv_003587	2987	97.26	Chryseobacterium sp.				
asv_003942	3330	97.26	Luteibacter sp.				
asv_004132	2987	97.26	Chryseobacterium sp.				
asv_004406	3330	97.26	Luteibacter sp.				
asv_004886	94	97.26	Mucilaginibacter sp.				
asv_005254	3330	97.26	Luteibacter sp.				
asv_005563	4493; 4509; 4579; 4567; 4494	97.26	Dyadobacter sp.	Dyadobacter fermentans	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.
asv_005602	3330	97.26	Luteibacter sp.				
asv_005737	3330	97.26	Luteibacter sp.				
asv_006190	4494; 4567; 4579; 4493; 4509	97.26	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.	Dyadobacter sp.
asv_006750	3330	97.26	Luteibacter sp.				
asv_007305	94	97.26	Mucilaginibacter sp.				
asv_007463	3330	97.26	Luteibacter sp.				
asv_008955	3330	97.26	Luteibacter sp.				
asv_000260	4487	96.918	Comamonas sediminis				
asv_000323	704	96.918	Sphingomonas kyeoggiensis				
asv_000462	704	96.918	Sphingomonas kyeoggiensis				
asv_000473	3330	96.918	Luteibacter sp.				
asv_000493	704	96.918	Sphingomonas kyeoggiensis				

asv_000500	4487	96.918	Comamonas sediminis				
asv_000508	2829; 2830	96.918	Janthinobacterium lividium	Janthinobacterium lividium			
asv_000664	2830; 2829	96.918	Janthinobacterium lividium	Janthinobacterium lividium			
asv_000671	4487	96.918	Comamonas sediminis				
asv_000737	94	96.918	Mucilaginibacter sp.				
asv_000782	2830; 2829	96.918	Janthinobacterium lividium	Janthinobacterium lividium			
asv_000824	4487	96.918	Comamonas sediminis				
asv_000825	94	96.918	Mucilaginibacter sp.				
asv_000844	4487	96.918	Comamonas sediminis				
asv_000848	4487	96.918	Comamonas sediminis				
asv_000900	94	96.918	Mucilaginibacter sp.				
asv_000917	4487	96.918	Comamonas sediminis				
asv_000939	94	96.918	Mucilaginibacter sp.				
asv_000980	4487	96.918	Comamonas sediminis				
asv_001072	94	96.918	Mucilaginibacter sp.				
asv_001092	94	96.918	Mucilaginibacter sp.				
asv_001110	4487	96.918	Comamonas sediminis				
asv_001137	3330	96.918	Luteibacter sp.				
asv_001215	94	96.918	Mucilaginibacter sp.				
asv_001523	3330	96.918	Luteibacter sp.				
asv_001632	2830; 2829	96.918	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001641	94	96.918	Mucilaginibacter sp.				

asv_001774	3330	96.918	Luteibacter sp.				
asv_001782	3330	96.918	Luteibacter sp.				
asv_001937	704	96.918	Sphingomonas kyeoggiensis				
asv_001966	3330	96.918	Luteibacter sp.				
asv_002000	94	96.918	Mucilaginibacter sp.				
asv_002013	3330	96.918	Luteibacter sp.				
asv_002210	94	96.918	Mucilaginibacter sp.				
asv_002296	3330	96.918	Luteibacter sp.				
asv_002386	94	96.918	Mucilaginibacter sp.				
asv_002820	94	96.918	Mucilaginibacter sp.				
asv_002839	3330	96.918	Luteibacter sp.				
asv_002936	3330	96.918	Luteibacter sp.				
asv_003204	3939; 4447; 4068; 3977	96.918	Streptomyces sp. 1-26	Pseudomonas frederick-bergensis strain	Streptomyces paten-sis strain HQA952	Streptomyces sp. Srain SKB2.14	
asv_003304	2830; 2829	96.918	Janthinobacterium lividium	Janthinobacterium lividium			
asv_003391	3330	96.918	Luteibacter sp.				
asv_003591	3330	96.918	Luteibacter sp.				
asv_004395	3330	96.918	Luteibacter sp.				
asv_006842	4447; 3939; 4068; 3977	96.918	Streptomyces sp. 1-27	Pseudomonas frederick-bergensis strain	Streptomyces paten-sis strain HQA953	Streptomyces sp. Srain SKB2.15	
asv_007053	3330	96.918	Luteibacter sp.				
asv_007097	3330	96.918	Luteibacter sp.				
asv_008398	3330	96.918	Luteibacter sp.				
asv_000281	704	96.575	Sphingomonas kyeoggiensis				

asv_000373	4487	96.575	Comamonas sediminis				
asv_000406	2829; 2830	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_000583	4487	96.575	Comamonas sediminis				
asv_000613	704	96.575	Sphingomonas kyeoggiensis				
asv_000727	704	96.575	Sphingomonas kyeoggiensis				
asv_000806	4487	96.575	Comamonas sediminis				
asv_000813	3330	96.575	Luteibacter sp.				
asv_000840	704	96.575	Sphingomonas kyeoggiensis				
asv_000965	704	96.575	Sphingomonas kyeoggiensis				
asv_000973	2830; 2829	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_000991	2829; 2830	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_000998	704	96.575	Sphingomonas kyeoggiensis				
asv_001029	704	96.575	Sphingomonas kyeoggiensis				
asv_001036	3330	96.575	Luteibacter sp.				
asv_001037	4487	96.575	Comamonas sediminis				
asv_001059	704	96.575	Sphingomonas kyeoggiensis				
asv_001089	2829; 2830	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001093	2830; 2829	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001160	2829; 2830	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001340	3330	96.575	Luteibacter sp.				
asv_001373	94	96.575	Mucilaginibacter sp.				

asv_001375	4487	96.575	Comamonas sediminis				
asv_001391	2829; 2830	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001492	704	96.575	Sphingomonas kyeoggiensis				
asv_001563	2829; 2830	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001583	2829; 2830	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001600	4487	96.575	Comamonas sediminis				
asv_001614	2830; 2829	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001708	94	96.575	Mucilaginibacter sp.				
asv_001832	4487	96.575	Comamonas sediminis				
asv_002012	94	96.575	Mucilaginibacter sp.				
asv_002470	3330	96.575	Luteibacter sp.				
asv_002643	4487	96.575	Comamonas sediminis				
asv_002826	3330	96.575	Luteibacter sp.				
asv_002879	3198; 1204	96.575	Chitinophaga sancti	Chitinophaga pinensis			
asv_003778	3330	96.575	Luteibacter sp.				
asv_003795	2829; 2830	96.575	Janthinobacterium lividium	Janthinobacterium lividium			
asv_004017	3330	96.575	Luteibacter sp.				
asv_004156	3330	96.575	Luteibacter sp.				
asv_004629	704	96.575	Sphingomonas kyeoggiensis				
asv_005103	3330	96.575	Luteibacter sp.				
asv_006495	3330	96.575	Luteibacter sp.				
asv_006868	3330	96.575	Luteibacter sp.				

asv_000449	704	96.233	Sphingomonas kyeoggiensis				
asv_000595	2830; 2829	96.233	Janthinobact- erium livid- ium	Janthinobact- erium livid- ium			
asv_000658	704	96.233	Sphingomonas kyeoggiensis				
asv_000677	704	96.233	Sphingomonas kyeoggiensis				
asv_000836	2830; 2829	96.233	Janthinobact- erium livid- ium	Janthinobact- erium livid- ium			
asv_000845	4487	96.233	Comamonas sediminis				
asv_000945	2830; 2829	96.233	Janthinobact- erium livid- ium	Janthinobact- erium livid- ium			
asv_000972	704	96.233	Sphingomonas kyeoggiensis				
asv_001095	4487	96.233	Comamonas sediminis				
asv_001221	2830; 2829	96.233	Janthinobact- erium livid- ium	Janthinobact- erium livid- ium			
asv_001248	704	96.233	Sphingomonas kyeoggiensis				
asv_001435	1204; 3198	96.233	Chitinophaga pinensis	Chitinophaga sancti			
asv_001541	4487	96.233	Comamonas sediminis				
asv_001610	3330	96.233	Luteibacter sp.				
asv_001631	1204; 3198	96.233	Chitinophaga pinensis	Chitinophaga sancti			
asv_001694	704	96.233	Sphingomonas kyeoggiensis				
asv_001718	1204; 3198	96.233	Chitinophaga pinensis	Chitinophaga sancti			
asv_001879	2829; 2830	96.233	Janthinobact- erium livid- ium	Janthinobact- erium livid- ium			
asv_001951	704	96.233	Sphingomonas kyeoggiensis				
asv_002188	2829; 2830	96.233	Janthinobact- erium livid- ium	Janthinobact- erium livid- ium			
asv_002257	2829; 2830	96.233	Janthinobact- erium livid- ium	Janthinobact- erium livid- ium			

asv_002648	3330	96.233	Luteibacter sp.				
asv_002903	2829; 2830	96.233	Janthinobacterium lividium	Janthinobacterium lividium			
asv_002912	2829; 2830	96.233	Janthinobacterium lividium	Janthinobacterium lividium			
asv_003150	3198; 1204	96.233	Chitinophaga sancti	Chitinophaga pinensis			
asv_003347	2830; 2829	96.233	Janthinobacterium lividium	Janthinobacterium lividium			
asv_003825	704	96.233	Sphingomonas kyeoggiensis				
asv_004381	3330	96.233	Luteibacter sp.				
asv_001002	704	95.89	Sphingomonas kyeoggiensis				
asv_001167	1204; 3198	95.89	Chitinophaga pinensis	Chitinophaga sancti			
asv_001264	2830; 2829	95.89	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001274	3330	95.89	Luteibacter sp.				
asv_001447	704	95.89	Sphingomonas kyeoggiensis				
asv_001739	2830; 2829	95.89	Janthinobacterium lividium	Janthinobacterium lividium			
asv_001843	3198; 1204	95.89	Chitinophaga sancti	Chitinophaga pinensis			
asv_001853	3330	95.89	Luteibacter sp.				
asv_001861	704	95.89	Sphingomonas kyeoggiensis				
asv_002028	1204; 3198	95.89	Chitinophaga pinensis	Chitinophaga sancti			
asv_002241	3198; 1204	95.89	Chitinophaga sancti	Chitinophaga pinensis			
asv_002332	2830; 2829	95.89	Janthinobacterium lividium	Janthinobacterium lividium			
asv_002432	3198; 1204	95.89	Chitinophaga sancti	Chitinophaga pinensis			
asv_002764	1204; 3198	95.89	Chitinophaga pinensis	Chitinophaga sancti			

asv_002864	1204; 3198	95.89	Chitinophaga pinensis	Chitinophaga sancti			
asv_002889	3198; 1204	95.89	Chitinophaga sancti	Chitinophaga pinensis			
asv_003294	1204; 3198	95.89	Chitinophaga pinensis	Chitinophaga sancti			
asv_003431	3198; 1204	95.89	Chitinophaga sancti	Chitinophaga pinensis			
asv_003734	1204; 3198	95.89	Chitinophaga pinensis	Chitinophaga sancti			
asv_004835	1204; 3198	95.89	Chitinophaga pinensis	Chitinophaga sancti			
asv_004932	4589; 4605; 4540	95.89	Sphingomonas sp.	Sphingomonas echinoides	Sphingomonas sp.		
asv_000757	4589; 4605; 4540	95.548	Sphingomonas sp.	Sphingomonas echinoides	Sphingomonas sp.		
asv_000883	3330	95.548	Luteibacter sp.				
asv_001056	4605; 4540; 4589	95.548	Sphingomonas echinoides	Sphingomonas sp.	Sphingomonas sp.		
asv_001227	4605; 4540; 4589	95.548	Sphingomonas echinoides	Sphingomonas sp.	Sphingomonas sp.		
asv_001564	1204; 3198	95.548	Chitinophaga pinensis	Chitinophaga sancti			
asv_001775	3198; 1204	95.548	Chitinophaga sancti	Chitinophaga pinensis			
asv_002349	3198; 1204	95.548	Chitinophaga sancti	Chitinophaga pinensis			
asv_002482	3198; 1204	95.548	Chitinophaga sancti	Chitinophaga pinensis			
asv_002565	3330	95.548	Luteibacter sp.				
asv_002743	3198; 1204	95.548	Chitinophaga sancti	Chitinophaga pinensis			
asv_003120	3330	95.548	Luteibacter sp.				
asv_003879	3198; 1204	95.548	Chitinophaga sancti	Chitinophaga pinensis			
asv_003939	3330	95.548	Luteibacter sp.				
asv_004029	1204; 3198	95.548	Chitinophaga pinensis	Chitinophaga sancti			
asv_004263	4605; 4540; 4589	95.548	Sphingomonas echinoides	Sphingomonas sp.	Sphingomonas sp.		
asv_004448	1204; 3198	95.548	Chitinophaga pinensis	Chitinophaga sancti			
asv_004742	1204; 3198	95.548	Chitinophaga pinensis	Chitinophaga sancti			

asv_004914	3330	95.548	Luteibacter sp.				
asv_005427	3198; 1204	95.548	Chitinophaga sancti	Chitinophaga pinensis			
asv_009300	4589; 4605; 4540	95.548	Sphingomonas sp.	Sphingomonas echinoides	Sphingomonas sp.		
asv_000559	4605; 4540; 4589	95.205	Sphingomonas echinoides	Sphingomonas sp.	Sphingomonas sp.		
asv_001048	1971	95.205	None				
asv_001198	1971	95.205	None				
asv_001432	1971	95.205	None				
asv_001481	3330	95.205	Luteibacter sp.				
asv_001519	4540; 4605; 4589	95.205	Sphingomonas sp.	Sphingomonas echinoides	Sphingomonas sp.		
asv_001803	4589; 4540; 4605	95.205	Sphingomonas sp.	Sphingomonas sp.	Sphingomonas echinoides		
asv_001867	4589; 4605; 4540	95.205	Sphingomonas sp.	Sphingomonas echinoides	Sphingomonas sp.		
asv_001969	3330	95.205	Luteibacter sp.				
asv_001973	4605; 4540; 4589	95.205	Sphingomonas echinoides	Sphingomonas sp.	Sphingomonas sp.		
asv_002311	4589; 4540; 4605	95.205	Sphingomonas sp.	Sphingomonas sp.	Sphingomonas echinoides		
asv_002373	4589; 4540; 4605	95.205	Sphingomonas sp.	Sphingomonas sp.	Sphingomonas echinoides		
asv_002381	4589; 4540; 4605	95.205	Sphingomonas sp.	Sphingomonas sp.	Sphingomonas echinoides		
asv_002675	1971	95.205	None				
asv_003134	1204; 3198	95.205	Chitinophaga pinensis	Chitinophaga sancti			
asv_003209	3330	95.205	Luteibacter sp.				
asv_003358	1971	95.205	None				
asv_003566	4540; 4605; 4589	95.205	Sphingomonas sp.	Sphingomonas echinoides	Sphingomonas sp.		
asv_003602	1204; 3198	95.205	Chitinophaga pinensis	Chitinophaga sancti			
asv_003904	4605; 4540; 4589	95.205	Sphingomonas echinoides	Sphingomonas sp.	Sphingomonas sp.		
asv_004155	1971	95.205	None				
asv_004303	1971	95.205	None				
asv_004308	1204; 3198	95.205	Chitinophaga pinensis	Chitinophaga sancti			
asv_004321	1204; 3198	95.205	Chitinophaga pinensis	Chitinophaga sancti			
asv_005718	3198; 1204	95.205	Chitinophaga sancti	Chitinophaga pinensis	Mucilaginibacter rubeus		

asv_006019	1971	95.205	None				
asv_007901	1971	95.205	None				

Table 19: Amplicon Sequence Variants (ASVs) matched to 16s rRNA gene sequences in the Schachtman Lab Culture Collection