IECAG 2021

MDPI

Proceedings

Meeting Agricultural Sustainability through Plant Growth Promoting Bacteria: An Examination of the Mechanisms for Improved Host Uptake of Zinc Nutrient in Maize Using Functional Mutants of *Azospirillum brasilense* †

Stacy L. Wilder [1], Alexandra Bauer Housh [1,2,3], Mary Benoit [4], Stephanie Scott [1,5], Garren Powell [1,5], Michael Schueller [1,2] and Richard A. Ferrieri [1,2,3,4,*]

[1] Missouri Research Reactor Center, University of Missouri, Columbia, MO 65211, USA; wildersl@missouri.edu (S.L.W.); afbkhn@mail.missouri.edu (A.B.H.); srstt9@mail.missouri.edu (S.S.); garren.powell@mail.missouri.edu (G.P.); schuellerm@missouri.edu (M.S.)
[2] Chemistry Department, University of Missouri, Columbia, MO 65211, USA
[3] Interdisciplinary Plant Group, University of Missouri, Columbia, MO 65211, USA
[4] Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA; mvbenoit@mail.missouri.edu
[5] Department of Biochemistry, University of Missouri, Columbia, MO 65211, USA
* Correspondence: ferrierir@missouri.edu; Tel.: 573-882-5211
† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://iecag2021.sciforum.net/.

Abstract: Zinc (Zn) is an essential micronutrient for plant growth and development. Plants can be lacking in Zn since as a trace element it is subject to widespread soil deficiencies. These deficiencies can arise through a lack of diverse agricultural practices, as well as climatic extremes causing excessive soil weathering. It has been estimated that 30% of global arable lands are deficient in Zn resulting in substantial reduction in yield. In recent agricultural practices, plant growth promoting bacteria (PGPB) are finding increased use for reducing crop losses due to conditions of the soil. Among the PGPB, the genus *Azospirillum*—with an emphasis on *A. brasilense*—is probably the most studied microorganism for the mitigation of plant stress. Here we report the investigation of functional mutants HM053, *ipdC* and FP10 of *A. brasilense* to understand how the biological functions of these microorganisms can affect host Zn uptake. HM053 is a *Nif*$^+$ constitutively expressed strain that hyper-fixes N_2 and produces high levels of the plant relevant hormone auxin. FP10 is a *Nif*$^-$ strain that is deficient in N_2-fixation and produces auxin. *ipdC* is a strain that is deficient in auxin production but fixes N_2. Zinc uptake was measured in laboratory-based studies of 3-week-old plants using radioactive Zn-65 ($t_{½}$ = 244 days). Uptake kinetics of the tracer were measured using dedicated radiation detectors. Afterwards, tissues were harvested and counted for levels of Zn-65 radioactivity that had accumulated within the roots and shoots of the plant. Principal Component Analysis using XLSTAT software provided comparisons between microbial biological functions and host Zn-65 accumulation. Results showed that low microbial auxin producing capacity resulted in the greatest accumulation of Zn-65 by the host.

Keywords: plant growth promoting bacteria; zinc nutrient uptake; maize; zinc-65 radiotracer

Citation: Wilder, S.L.; Housh, A.B.; Benoit, M.; Scott, S.; Powell, G.; Schueller, M.; Ferrieri, R.A. Meeting Agricultural Sustainability through Plant Growth Promoting Bacteria: An Examination of the Mechanisms for Improved Host Uptake of Zinc Nutrient in Maize Using Functional Mutants of *Azospirillum brasilense*. **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

Zinc has been implicated with a broad spectrum of growth characteristic in higher plants. For example, in apple, visible symptoms of Zn deficiency in dicotyledons were rosetting and decreases leaf size [1]. This later trait, coined "little leaf" syndrome, was observed as a common growth characteristic in many fruit tree species

subjected to Zn deficiency [2]. Zinc also plays important roles in many biochemical functions within plants. It is an essential component of over 300 enzymes [3]. It also plays a role in DNA and RNA metabolism, cell division, and protein synthesis [4]. A lack of sufficient Zn during plant growth can decrease yield and crop quality as a consequence of the disruption in these normal metabolic functions [5–7]. Today, approximately 30% of global crop production is lost due to essential nutrient deficiencies caused by climatic extremes that result in excessive soil weathering and by a lack of diverse agricultural practices that deplete nutrient levels in soil. Additionally, foods produced from Zn-deficient crops can result in human Zn deficiency, which can in turn have an impact on human well-being by reducing the body immune functions and increasing the risk of growth stunting in children or the risk of adverse pregnancy outcomes in women [5,8]. Plant growth promoting bacteria (PGPB), which can help their host weather difficult conditions, are finding increased use in agriculture. These organisms can activate physiological and biochemical responses within their host for mutual benefit to build natural tolerances to environmental stresses and thereby reduce losses in the field [9–14].

2. Materials and Methods

Plant Growth: Maize kernels from Elk Mound Seed Co. (Elk Mound, WI, USA) (Hybrid 8100) were dark germinated at room temperature for two days on sterilized paper towels wetted with sterile water in a petri-dish. Seeds were inoculated with bacteria culture as appropriate and transplanted to plastic seed germination pouches (Phytotc, Inc., Beijing, China) wetted with sterile Hoagland's basal salt solution for approximately one week. They were then transferred to individual 600 mL hydroponics cells that were continuously aerated and fill with Hoagland's nutrient (pH 6.0). Nutrient was exchanged on a five-day cycle. Growth conditions consisted of 12-h photoperiods, 500 μmol m^{-2} s^{-1} light intensity, and temperatures of 25 °C/20 °C (light/dark) with humidity at 70–80%.

Bacteria Growth: Functional mutants were grown in liquid NFbHP-lactate medium following published procedures [9]. Cultures were washed with sterile water and diluted to approximately 10^6 to 10^8 colony forming units per milliliter (CFU mL^{-1}). Root inoculation involved adding the inoculum to a petri dish of 10–20 maize seedlings and rocking in the shaking incubator for two hours. Seedlings were placed into germination pouches for five days before transplanting to hydroponics.

Zinc-65 Studies: Zinc-65 ($t_{½}$ = 244 days) was purchased from Perkin-Elmer Life Sciences (Akron, OH USA). One hour before administration of radiotracer, plants were removed from their hydroponics cells and suspended in 600-mL beakers consisting of 100 mL of deionized water. Plants were maintained at the same daytime light and temperature conditions as that used to maintain their growth. An aqueous solution of zinc-65 radiotracer at 0.74 MBq was injected into the beaker of water in which the roots were immersed. A radiation detector (Eckler & Ziegler, Inc., Valencia, CA, USA, 1-inch Na-PMT detector) affixed to the plant 8 cm above the base of the stem provided dynamic feedback on Zn-65 transport from roots to shoots. Data was acquired at 1Hz sampling rate using 0–1 V analog input into an acquisition box (SRI, Inc., McLean, VA, USA). After three hours of acquisition, roots were cut from the shoots, thoroughly washed in water, blotted dry. Both root and shoot tissues were weighed and then counted in a 3-inch NaI-PMT gamma well-type detector for quantifying amount of Zn-65 radioactivity.

Statistical Analysis: Data was subjected to the Shapiro-Wilk Normality Test to identify outliers so all data groups reflected normal distributions. Data was analyzed using the Student's *t*-test for pair-wise comparisons made between non-inoculated controls and bacteria treatment. Statistical significance was set at $p < 0.05$. The Zn-65 uptake and allocation data was analyzed by Principal Component Analysis (PCA) using XI.STAT software version 2020.3 (Addinsoft Inc., New York, NY, USA).

3. Results and Discussion

Figure 1 results demonstrated different rates for Zn-65 transport as a function of *A. brasilense* inoculation showing that *ipdC* > HM053 > FP10. FP10 was most like non-inoculated control plants. Tissue distribution measurements using 'cut and count' techniques revealed a similar dissimilarity between *ipdC* bacteria and the other inoculants (Figure 2). Systematic trends defining uptake and in *planta* translocation of Zn-65 become apparent in the PCA biplot (Figure 3). Here, the information included in our uptake versus allocation measurements were represented by feature vectors (Fl and F2) representing 71.89% and 28.11% of the information embedded in the data respectively. As displayed, each of the treatments clustered together, indicating behavior within a treatment-type that is distinct from other treatments. It shows that FP10 and non-inoculated maize are similar in overall zinc uptake and shoot allocation behavior. HM053 inoculated maize exhibit a slight elevation in allocation patterns relative to control and FP10. *ipdC* is most unique in its uptake and allocation patterns than other treatments in the *X*- and *Y*-axis direction.

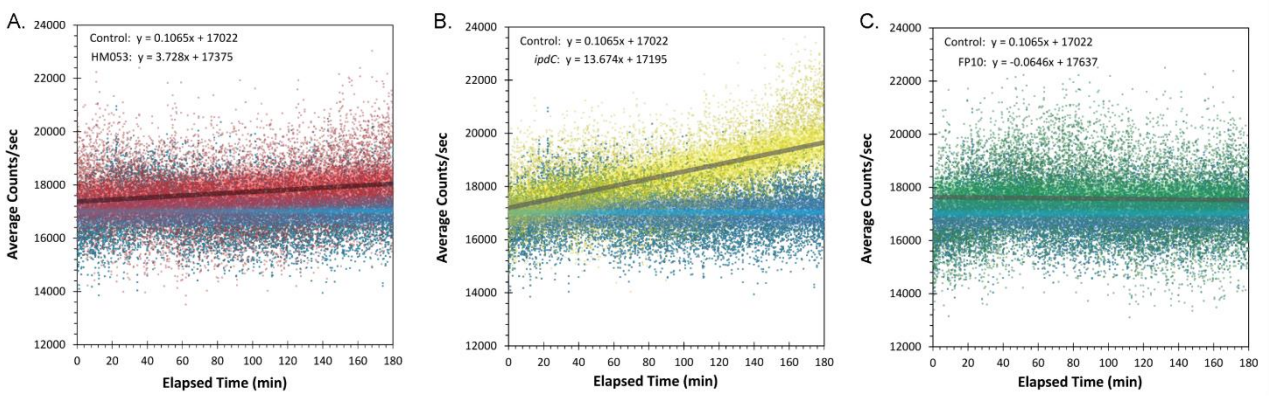

Figure 1. Dynamic Zn-65 transport over 3-h of acquisition. Each data point reflects N = 5–6 biological replicates at 1 Hz sampling frequency.

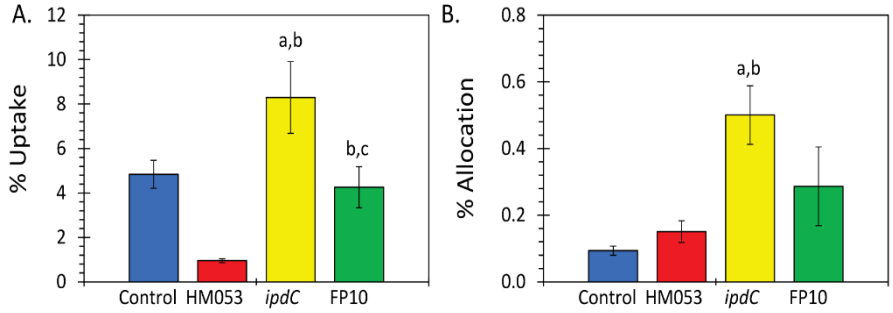

Figure 2. (Panel **A**); 'Cut and count' measurements yielded information on plant uptake of Zn-65 presented as the percent of the Zn-65 dose administered to the beaker that was assimilated by the plant over 3-hours. (Panel **B**); root-to-shoot allocation of Zn-65 is presented as the percent of the administered dose of radiotracer. Date reflects means for N = 5–6 replicates (±SE). Statistical significance $p < 0.05$ was designated by 'a' in a comparison of treatment to control, 'b' in a comparison of *ipdC* or FP10 to HM053 and 'c' in a comparison of FP10 to *ipdC* treatment.

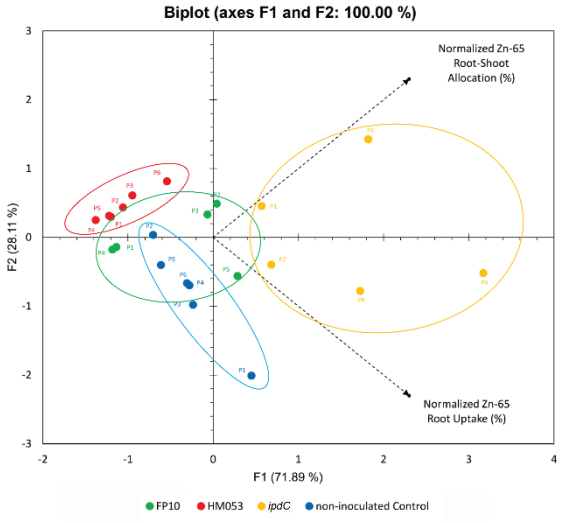

Figure 3. Principal component analysis correlates Zn-65 uptake and root-shoot allocation to the biological functions of the beneficial microbes.

What distinguishes *ipdC* from the other microbial inoculants examined in this study is its deficiency in producing auxin (indole-3-acetic acid), an important plant hormone. Our past studies showed that the HM053 mutant exhibited the highest level of auxin biosynthesis, being 2-times that of FP10 and 13-times that of *ipdC* [15]. Furthermore, auxin biosynthesis in plants and Zn levels have been positively correlated. With tryptophan being the principal intermediate in auxin biosynthesis, withholding Zn was shown to lower plant tryptophan levels [16], and auxin levels [17], while exogenous treatment with Zn increased tryptophan levels [18]. We suspect that the mechanism for promoting plant Zn-65 uptake in the present study has to do with the auxin producing capacity of the microorganism. We note that while *ipdC* lacks the ability to biosynthesize auxin, it still possesses the molecular machinery to produce indole—a key precursor to tryptophan biosynthesis [15]. In fact, maize root indole emissions with *ipdC* inoculation were nearly 2-times that of HM053 inoculated plants, and 1.5-times that of FP10 inoculated plants. We suspect this behavior may be due to bacteria-root indole trafficking which could elevate the endogenous pool of plant tryptophan causing an elevation in Zn uptake. To the best of our knowledge, no one has examined whether tryptophan treatments will elevate endogenous levels of plant Zn.

4. Conclusions

The present work shows evidence that certain biological traits of root-associating microorganisms can have beneficial effects on the host plant in promoting Zn uptake. While these actions could benefit over the long term in improved crop yield, we know little about their effects on crop nutrition value. Most particularly, we ask whether prolonged Zn accumulation can result in a higher Zn content in the corn kernel. Future studies using ion chromatography will examine this feature.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Acknowledgments: This research was supported by Agriculture and Food Research Initiative Award No. 2017-67013-26216 from the USDA National Institute of Food and Agriculture and through a startup award from the University of Missouri administered through University of Missouri Research Reactor Center. We would also like to thank Prof. Emanuel de Souza (U. Paraná, Brazil) whose institution provided the HM053 functional mutant of *A. brasilense* in this work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; Academic Press Pub.: New York, NY, USA, 1995; p. 559.
2. Swietlik, D. Zinc nutrition of fruit crops. *Hort. Technol*. **2002**, *12*, 45–50.
3. Fox, T.L.; Guerimot, M.L. Molecular biology of cation transport in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol*. **1998**, *49*, 669–696.
4. Dickinson, K.; O'Brieo, J.; Edwards, T. Metalosate "zinc in plant nutrition". *Albion* **2003**, *4*, 1–4.
5. Alloway, B.J. Soil factors associated with zinc deficiency in crops and humans. *Environ. Geochem. Health* **2009**, *31*, 537–548.
6. Cakmak, I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil* **2008**, *302*, 1–17.
7. Cavagnaro, T.R. The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: A review. *Plant Soil*. **2008**, *304*, 315–325.
8. Hess, S.Y. National Risk of zinc deficiency as estimated by National Surveys. *Food Nutr. Bull*. **2017**, *38*, 3–17.
9. Goswami, D.; Thakker, J.N.; Dhandhukia, P.C. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. *Null* **2016**, *2*, 1127500.
10. Yang, J.; Kloepper, J.W.; Ryu, C.-M. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* **2009**, *14*, 1–4.
11. Sarma, B.K.; Yadav, S.K.; Singh, D.P.; and Singh, H.B. Rhizobacteria mediated induced systemic tolerance in plants: Prospects for abiotic stress management. In *Bacteria in Agrobiology: Stress Management*; Springer: Berlin/Heidelberg, Germany, 2012; pp. 225–238.
12. Pérez-Montaño, F.; Alías-Villegas, C.; Bellogín, R.A.; del Cerro, P.; Espuny, M.R.; Jiménez-Guerrero, I. Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiol. Res.* **2014**, *169*, 325–336.
13. Pankievicz, V.C.S.; do Amaral, F.P.; Santos, K.F.D.N.; Agtuca, B.; Xu, Y.; Schueller, M.J. Robust biological nitrogen fixation in a model grass-bacterial association. *Plant J.* **2015**, *81*, 907–919.
14. Backer, R.; Rokem, J.S.; Ilangumaran, G.; Lamont, J.; Praslickova, D.; Ricci, E.; Subramanian, S.; Smith, D.L. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Front. Plant Sci.* **2018**, *9*, 1473.
15. Housh, A.B.; Powell, G.; Scott, S.; Anstaett, A.; Gerheart, A.; Benoit, M.; Powell, A.; Waller, S.; Guthrie, J.M.; Higgins, B.; et al. Functional mutants of *Azospirillum brasilense* bacterium elicit beneficial physiological and metabolic responses in *Zea mays* that contribute to increased host iron assimilation. *ISME J.* **2021**, doi:10.1038/s41396-020-00866-x.
16. Hanafy, A.A.H.; Khalil, M.K.; Abd El-Rahman, A.M.; Nadia, A.M.H. Effect of zinc, tryptophan and indole acetic acid on growth, yield and chemical composition of valencia orange trees. *J. Appl. Sci. Res*. **2012**, *8*, 901–914.
17. Price, C.A. *Molecular Approaches to Plant Physiology*; McGraw Hill: Philadelphia, PA, USA, 1970; p. 398.
18. Singh, M. Effect of zinc, phosphorus and nitrogen on tryptophan concentration in rice grains grown on limited and unlimited soil. *Plant Soil* **1981**, *62*, 305–308.